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A FEASIBLE ECONOMIC AND NATURAL APPROACH OF FEATHER DEGRADATION USING KERATINOLYTIC BACTERIA FROM BARSHI REGION, MAHARASHTRA.

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Abstract: In past few years, the world has experienced a high rise in the market of poultry industry. This industry has added a non degradable feather waste to the environment on very large scale. This feather is even not degraded by the enzymes such as Trypsin, Pepsin and has become a global issue. Use of microorganisms in feather degradation is a trending and eco-friendly solution. The ultimate aim of the current study was to degrade feather using keratinolytic bacteria. Bacteria were isolated and screened from the soil samples collected from the dumping yards of chicken shops, poultry farm and feather waste collected areas. Among them isolate C1B is gram positive, rod shaped, endospore forming bacterial species, morphological and biochemical identification showed that isolate C1B was *Bacillus species*. It was found that the bacterium had the efficiency of degrading 84.5% of feather within 72hrs. The bacteria used feather keratin as a principal source of carbon, nitrogen, sulphur and energy. The present study would highlight the degradation of feather waste using bacteria.

Keywords: Poultry Industries, Feather Waste, Feather degrading Bacteria, Keratin, Keratinase assay.

1. INTRODUCTION:

Poultry is a highly upright incorporated food industry in India and matches the competence level of many western countries. Throughout the year 2016-17 the poultry meat production in the nation has recorded to almost 3.46 million tons (www.mofpi.nic.in). worldwide about 2 million tons of feathers are expel out per annum as a waste product from poultry industry (Verma *et.al*., 2017) Due to uncared for management, poultry waste especially feathers have become one of the problematic pollutants due to their recalcitrant nature (Khardenavis *et al*., 2009).

Chicken feathers include nutrients around 91% keratin protein, 1% lipids, and 8% water. The amino acid principally composed of Cystine, Glutamine, Proline and Serine. 16% Serine is the most abundantly present amino acid. However almost lysine, tryptophan, histidine, glutamic acid and glycine are absent (Kannappan and Bharathi, 2012)

The most common methods used for chicken feather discarding is burning and dissolution by chemical treatments (Cai *et.al*, 2008). Traditional feather deprivation methods such as hydrolysis by using alkali and cooking by steam pressure consume large amount of energy and reduces the quality of proteins (Papadopoulos *et.al*., 1986 and Yamamura *et.al*., 2002). These methods demolish vital amino acids such as lysine, Tryptophan,

Methionine and produces low nutritive amino acids such as lanthionine, lysinoalanine etc (Latshaw *et.al.*,1994; and Wang X and Parsons 1997).

Feathers mainly constitute with Keratin protein which is a extensively cross-linked by disulphide bonds, hydrogen bonding and hydrophobic interaction, fibrous and insoluble structural protein and thus becomes mechanically stable and extremely resistant to biodegradation by common proteases enzymes (Ramnani *et al*., 2005; Thys *et al*.,2004).

Keratinases enzymes are proteolytic in nature and plays very important role for hydrolyzing keratin containing substrate such as feathers, hair and collagen (Godfrey and West, 1996). Many bacteria and fungi have been reported for process of keratin degradation. These keratinolytic micro-organisms include *Bacillus sp*, *Micrococcus sp*, *Actinomycetes* sp; *Clostridium sp* etc present in different ecological conditions and solubilize keratin containing substrates on their own preferences. Feather Keratin degrading fungi include many dermatophytic and non-dermatophytic fungi (Kushwaha 1983 and Ulfig *et al*., 1996).

Bacterial strains produce enzymes which degrade the beta-keratin in specifically found in feathers. The enzymes make it feasible for the bacteria to obtain carbon, nitrogen sulfur, and energy for their growth and proper maintenance from the degradation of beta-keratin. Employment of keratinolytic microorganisms for feather degradation is an inexpensive, environmentally safe alternative. Keratinases produced from bacteria convert the insoluble chicken feather keratin to soluble digestible product could be a tremendous material for preparation of fertilizer, animal feed and natural gas (Joshi *et al*., 2007)

The present study is focused on the isolation and identification of a feather degrading bacteria from poultry chicken feather waste contaminated soil around the Barshi region Maharashtra, India. Therefore suggesting substitute method for the disposal of the chicken feather waste.

2. MATERIALS AND METHODS:

2.1 Collection of soil samples:

The soil samples were collected from different locations of a regular poultry feather waste dumping site near Chicken Shops from Barshi town region. The samples were kept under in sampling bags and carried immediately to the laboratory and subjected for further study.

2.2 Enrichment:

Whole feather broth medium that composed of (Feather-5, K_2HPO_4 - 0.3, KH_2PO_4 - 0.4, NaCl - 0.5, pH 7.5 (g/l). The medium was sterilized by autoclaving and inoculated with 1ml inoculums from 10^{-5} dilutions in aseptic condition. Incubated for 7days at 120 rpm agitation conditions at 37°C (Shih and Michael 1992). Feather degradation in culture broth was determined by visual observation.

2.3 Screening on Skim milk agar:

Skim milk agar medium was sterilized at 121°C for 15 min and prepared Petri plates aseptically. From each dilution 0.1ml of sample was spread using L-rod in sterile conditions over Skimmed Milk agar plate and incubated at 37°C for a period of 24 hrs (Ramya, 2014). The clear zone formed around the colonies due to proteolytic enzyme were subculture by growing the bacteria in nutrient broth medium at 37°C at 120 rpm for 24 hrs.

2.4 Secondary Screening and selection of keratinolytic feather degrading isolate:

Skim milk agar plates were prepared for primary screening of bacteria that produced protease enzyme. The selected protease producing bacterial 24 hrs old culture were subsequently grown in whole feather broth medium in which the feather functions as principal source of carbon and nitrogen (Lin *et al*., 1995) at 120 rpm at 37°C incubated for 7 day. Keratinolytic bacterial strains which degrade feathers were selected.

2.5 Identification of feather degrading bacteria by biochemical Analysis:

The bacteria which degrade the feathers were identified based on morphological cultural and biochemical characteristics. Bergey's Manual of Determinative Bacteriology, 8th edition (Brenner *et al*., 2004).The culture which showed maximum feather degradation was selected for further study.

2.6 Keratinase production by feather degrading isolate:

Feather degradation by the isolate was carried out in 100ml whole-feather broth medium prepared. Sterilized at 121°C for 15 min. 1% v/v of 24 hrs old inoculums was inoculated in aseptic condition and incubated at 37°C on shaker at 120 rpm for 144 hrs. At 24 hrs of intervals, the enzyme was extracted for measuring the keratinase activity (Vidya and palaniswamy 2013 and Chaudhari *et al*., 2013) and few modification.

2.7 Assay for Keratinolytic activity:

Keratinase activity was determined as described by Sivakumar T. *et al*., 2012; Korkmaz H.2004; Cheng-gang CAI *et al*., 2008 and Poopathi S. *et al*., 2014 with a few modifications. Keratinase activity was determined with Keratin azure (K8500, Sigma-Aldrich, USA) as substrate. Keratin azure was the hair like substance it was ground into a fine powder using liquid nitrogen in mortar and pestle. To initiate the keratinase assay process o.4% (w/v) of fine powder of keratin azure was mix with 0.8 ml of 50mMpotassium phosphate buffer pH7.5 in 1.5ml Eppendorf tube. This mixture was agitated at 37°C for 1 hr with constant agitation at 120 rpm. A 0.2 ml of crude enzyme was added to keratin mixture. The enzyme and substrate mixture incubated at 50°C for 1 hr with 200rpm shaking. 0.2 ml of 10% TCA was added to stop the enzyme substrate reaction and placed at room temperature for 30 minute. Then centrifuged at 15000rpm for 15min. The absorbance of supernatant was measured spectrophotometrically at 595 nm.

The control was treated in the same way, except that TCA was added before the addition of enzyme solution. One unit (U) keratinase activity was defined as the amount of enzyme cause 0.01 absorbance raise between the sample relative to control at 595 nm under the given conditions.

2.8 Percentage of feather degradation:

Percentage of feather degradation was determined from the remaining degraded part of feather after degradation process by the isolates. Dry feather weight taken in whole feather broth medium for fermentation process consider as the initial feather weight. Final feather dry weight obtained by the filtering degraded granulated form of feather after degradation through Whatman Filter Paper-3 and dried at 50°C until weight of harvested residual feathers stabilized to constant value. The isolate showing significant feather degradation were selected as promising isolate for further study.

The percent feather degradation was determined using the following formula:

Feather degradation (%) = (initial feather weight – final feather weight)/ initial feather weight ×100.

(Shabaan *et al*., 2014 and Huang *et al*., 2015).

3. RESULTS :

Table1: Biochemical test of isolate C1B.

Sr No.	Test	Result
1	Gram nature	Positive
2	Shape and arrangement	Short rod in chain
3	Glucose	AG
4	Fructose	AG
5	Lactose	AG
6	Maltose	AG
7	Manitol	AG
8	Indole production	Negative
9	Methyl red test	Positive
10	Voges-Proskauer test.	Positive
11	Citrate utilization	Positive
12	Catalase activity	Positive
13	Starch hydrolysis test.	negative
14	Hydrogen sulphide test	Negative
15	Urease Activity	Negative
16	Gelatin hydrolysis test	Positive
17	Oxidase	Negative
18	Nitrate reduction	Negative
19	Endospore Formation.	Positive

Note: 'AG'= Acid and gas production.

Table2: Keratinase production by Isolate C1B.

Times in hrs	Keratinase Activity in U/ml.
24	10
48	26
72	76
96	24
120	7
144	0

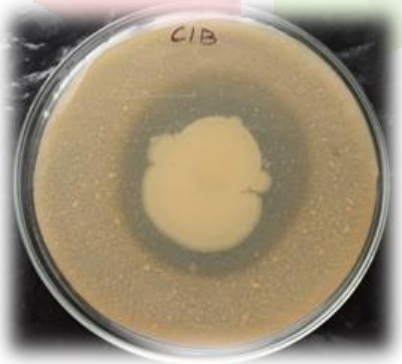

Fig.1: Production of clear zone on Skim Milk Agar by the isolate C1B

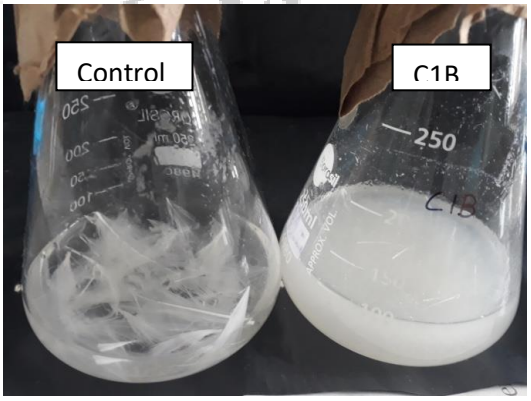

Fig.2: Feather degradation by the isolate C1B.

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3.1 Screening and isolation of feather degrading isolate:

A feather degrading bacteria were isolated from chicken feather waste dumping site near to Safa chicken shop center Barshi region. The enriched soil sample was screened on skim milk agar plate, the clear zone showing colony (fig1) was cultivated in whole feather broth medium. The whole feather broth medium shows the degradation of feather by the isolates (fig2). Identification and characterization of feather degrading isolates were

done. The morphological, biochemical tests showed that the isolate C1B bacterium was *Bacillus species*. It was rod shaped gram positive, motile, positive for catalase, but negative for oxidase activity. It was positive for citrate, methyl red, Voges- Proskauer but negative for Indole test and nitrate reduction test (Table1).

3.2 Keratinase production by the feather degrading bacteria:

The isolate *Bacillus cereus C1B* was utilizes the feathers keratin as sole source of carbon and nitrogen and energy for their growth purpose. The visual observation of biodegradation process was further confirmed by measuring the keratinase activity using keratin azure as substrate. Change in absorbance at 595 nm was measured with interval of 24 hrs of time period after inoculation, and the maximum keratinase activity was noted as 76 U/ml after 72 hrs (Table2).

3.3 Percentage of feather degradation:

Bacillus cereus C1B exhibited the 84.5% feather degradation after 72 hrs of cultivation.

4. DISCUSSIONS:

The bacterium isolated from the contaminated site of feather waste has been shown the feather degrading potential through the action of keratinase enzyme.(Joshi *et al*.,2007) Microorganisms utilizes feather keratin as a source of carbon and energy for growth and survival. The presence of keratinous substrates usually induces keratinase enzyme (Mazotto *et al*., 2010). Feather meal was used for isolation of keratinase producer by many scientists (Tapia and Simoes. 2008; Ramya *et al*., 2014; Mousavi *et al*., 2013; Govarthanan *et al*., 2011; Shah, 2015; Fakhfakh-Zouari *et al*., 2010). Several *Bacillus* sp. including *Bacillus licheniformis, Bacillus amyloliquefaceins, Brevibacillus brevis* US575, *Bacillus pumilus, Bacillus cereus, Bacillus subtilis, Bacillus halodurans* have been documented to be potential sources of keratinases (Jaouadi *et al*., 2013; Lin *et al*., 1992; Cortezi *et al*., 2008; Kumar *et al*., 2010; Adiguzel *et al*., 2009; Mazotto *et al*., 2010; Prakash *et al*., 2010). Keratin degradation process have been reported many *Bacillus* species such *B. licheniformis, B. pumilus, B. subtilis, B. cereus*, and *B. megaterium*. (Kim *et al*., 2004; Riffel and Brandelli, 2006). Mehta *et al*., (2014) reported complete degradation of feather waste within 7days by the *Bacillus sonorensis* strain NRRLB-23154 isolated from poultry farm.

5. CONCLUSIONS:

A promising feather keratin degrading bacteria was isolated from poultry chicken feather waste dumping site area of chicken shop, Barshi, Maharashtra. This isolates degrade the whole feathers for the uptake of nutrition for their growth. The16S rRNA identification confirms that a gram positive isolate was *Bacillus cereus*, designated as *Bacillus cereus* C1B.The *Bacillus cereus* C1B was Degraded the 84.5% feather and report 76 U/ml maximum activities after 72 hrs. This was the potential member for poultry chicken feather degradation.

6. REFERENCES:

1. Adıguzel Arife Candaş Zengin, Behzat Oral Bitlisli1, Niels Thomas Eriksen. 2009. Potential Usage of Keratinolytic Enzymes from *Bacillus cereus* in the Leather Industry. Abstract from XXX Congress of the International Union of Leather Technologists & Chemists Societies, Beijing, China.
2. Brandelli A, Riffel A.2005. Production of an extracellular keratinase from *Chryseobacterium sp*. growing on raw feathers. Electronic Journal of Biotechnology. 8(1):35-42.
3. Brenner DJ, Krieg NR, Staley JT. Bergey's manual of systematic bacteriology (2nd Ed) Part B Springer, New York. 2004; 2:323-358.
4. Cai CG, Lou BG, Zheng XD. 2008. Keratinase production and keratin degradation by a mutant strain of Bacillus subtilis. J Zheijang Unive; 9:60–7.
5. Chaudhari PN, Chaudhari BL, Chincholkar SB. 2013.Iron containing keratinolytic metallo-protease Produced by *Chryseobacterium gleum*. Process Biochem; 48:144–51.
6. Cheng-gang CAI, Ji-shuang CHEN3, Jiong-jiong QI2, Yun YIN2, Xiao-dong ZHENG. 2008. Purification and characterization of keratinase from a new *Bacillus subtilis* strain. Journal of Zhejiang University SCIENCE B 9(9):713-720.
7. Cortezi M, Contiero J, Lima CJB De.2008. Characterization of a Feather Degrading by *Bacillus amyloliquefaciens* Protease : A New Strain. World Journal of Agricultural Sciences. 4(5):648-656.
8. Fakhfakh-Zouari N, Hmidet N, Haddar A, Kanoun S, Nasri M. 2010. A novel serine metallokeratinase from a newly isolated *Bacillus pumilus* A1 grown on chicken feather meal: biochemical and molecular characterization. Appl. Biochem. Biotechnol. 162, 329-344
9. Godfrey, T. 1996. Protease in waste treatment, pp. 315-316. In T. Godfrey and S. Wes (eds.) Industrial Enzymology. Macmillan Press, London.

10. Govarthanan M, Selvankumar T, Arunprakash S. 2011. Production of keratinolytic enzyme by a newly isolated feather degrading *Bacillus Sp*. from chick feather waste. International Journal of Pharma and Bio Sciences. 2(3):259-265.
11. Huang Y, Busk PK, Lange L. 2015. Production and Characterization of Keratinolytic Proteases Produced by *Onygena corvina*. Fungal Genomics Biol. 4(1):119. doi:10.4172/2165-8056.1000119.
12. Jaouadi NZ, Rekik H, Badis A, *et al.*, 2013. Biochemical and Molecular Characterization of a Serine Keratinase from *Brevibacillus brevis* US575 with Promising Keratin-Biodegradation and Hide-Dehairing Activities. *PLoS One*. 8(10):1-18. doi:10.1371/journal.pone.0076722.
13. Joshi S.G, Tejashwini M.M, Revati, N. Sridevi R. Roma. D. 2007. Isolation, Identification and Characterization of a Feather Degrading Bacterium International Journal of Poultry Science 6 (9): 689-693, ISSN 1682-8356
14. Kannapan Saravanan, Bharathi Dhurai. 2012. Exploration on amino acid content and morphological structure in chicken feather fiber, JTATM 7:3.
15. Khardenavis AA, Kapley A, Purohit H. 2009. Processing of poultry feathers by alkaline keratin hydrolyzing enzyme from *Serratia sp*.HPC 1383. Waste Manag. 29:1409–15.
16. Kim J.M, Lim W. J, Suh H. J. 2001. Feather-degrading *Bacillus species* from poultry waste. Process Biochemistry. 37: 287–291.
17. Korkmaz H, Hur H, Dincer S. 2004. Characterization of alkaline keratinase of Bacillus licheniformis strain HK-1 from poultry waste. *Annals of Microbiology*. 54 (2): 201- 211.
18. Kumar R, Balaji S, Uma TS, Mandal a B, Sehgal PK. 2010. Optimization of influential parameters for extracellular keratinase production by *Bacillus subtilis* (MTCC9102) in solid state fermentation using Horn meal-a biowaste management. Appl Biochem Biotechnol. 160(1):30-39. Doi: 10.1007/s12010-008-8452-4.
19. Kushwaha R.K.S. 1983. The in-vitro degradation of peacock feathers by some fungi. Mykosen. (26): 324-326.
20. Latshaw J.D, Musharaf N, Retrum R.1994. Processing of feather meal to maximize its nutritional value for poultry. Anim. Feed Sci.Technol. 47: 179–188.
21. Lin, X, D.W. kelemen, E.S. Miller and J.C.H. Shih. 1995. Nucleotide sequences and expression of kerA, the gene encoding a keratinolytic proteases of *bacillus licheniformis* PW-1. Applied Environ. Microbiol. 61:1469-1474.
22. Mazotto AM, Lage Cedrola SM, Lins U, 2010. Keratinolytic activity of *Bacillus subtilis* from native feather- degrading *Streptomyces albus.* International Journal of Development Research. 3(8):034-039.
23. Mehta RS, Jholapara RJ, Sawant CS. 2014. Isolation of A Novel Feather-Degrading Bacterium and Optimization of It's of Its Cultural Conditions for Enzyme Production. International Journal of Pharmacy and Pharmaceutical Sciences. *6(1):194-201.*
24. Mousavi S, Salouti M, Shapoury R, Heidari Z. 2013. Optimization of keratinase production for feather degradation by *Bacillus subtilis.* Jundishapur J Microbiol. 6(8):6-10. doi:10.5812/jjm.7160.
25. Papadopoulos M.C, El Boushy A.R, Roodbeen A.E, Ketelaars EH. 1986. Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal. Animal Feed Sci Technol. 14:279–90.
26. Poopathi S, Thirugnanasambantham K, Mani C, Lakshmi P.V. and Ragul K. 2014.Purification and characterization of keratinase from feather degrading bacterium useful for mosquito control – A new report Tropical Biomedicine 31(1): 97–109
27. Prakash P, Jayalakshmi SK, Sreeramulu K. 2010.Purification and characterization of extreme alkaline, thermostable keratinase, and keratin disulfide reductase produced by *Bacillus halodurans* PPKS-2. Appl Microbiol Biotechnol. 87, 625-633.
28. Ramnani P, Singh R, Gupta R. 2005. Keratinolytic potential of Bacillus licheniformis RG1: structural and biochemical mechanism of feather degradation. Can J Microbiol. 51(3):191-196
29. Ramya K, Deepak V. M. M and Anju Tankachi. 2014. Isolation, Optimization Of Production Conditions, Characterisation And Partial Purification Of Keratinase Enzyme From *Bacillus Sp*. International Journal of Current Research. 6 (01): 4413-4419.
30. Riffel A, and Brandelli A. 2006. Keratinolytic bacteria isolated from feather waste. Braz. J. Microbiol., 37: 395-399.
31. Shabaan M. T, Attia M, Sabhaa M, El-Sabagh and Amany A. M. Ahmed. 2014. Isolation, Screening and Selection of Efficient Feather Degrading Bacteria. Current Science International 3(4): 488-498.
32. Shah M, 2015. A novel feather degrading *Acinetobacter* sp. PD 12 isolated from feather waste dumping site in Mumbai Introduction : European Academic Research; III(1):757-773.

33. Sivakumar T, Shankar T, Thangapandian V, Ramasubramanian V. 2013.Optimization of Cultural Condition for Keratinase Production Using *Bacillus cereus* TS1. Insight Microbiology, 3(1): 1-8.
34. Tapia D.M.T. and Simoes M.L.G. 2008. Production and partial characterization of keratinase produced by a microorganism isolated from poultry processing plant waste water. Afr. J. Biotechnol. 7:296-300.
35. Thys R.C.S, Lucas F.S, Riffel A, Heeb P. and Brandelli A. 2004. Characterization of a protease of a feather-degrading *Microbacterium species*. Lett. appl. Microbiol. 39: 181-186. https://doi. org/10.1111/j.1472-765X.2004.01558.x
36. Ulfig K, Terakowski M, Płaza G, Kosarewicz O.1996. Keratinolytic fungi in sewage sludge. Mycopathologia. 136(1): 41-46.
37. Verma A, Singh H, Anwar S, Chattopadhyay A. 2017. Microbial keratinases: industrial enzymes with waste management potential. Crit Rev Biotechnol ; 37:476–91.
38. Vidhya D, Palaniswamy M. (2013). Identification and characterization of a local bacterial strain with high keratinolytic activity from chicken feathers. IJPBS. 3(3):308-316.
39. Vidhya D. and Palaniswamy M. 2013. Isolation and optimization of a bacterial keratinase isolated from poultry feather wastes. Indo. Am. J. Pharmaceutical Res., 3: 7217-7224.
40. Wang X, Parsons CM. 1997.Effect of processing systems on protein quality Feather meals and hog hair meals. Poult Sci; 76:491–6.
41. www.mofpi.nic.in
42. Yamamura S, Morita Y, Hasan Q, Rao S.R, Murakami Y, Yokoyama K, Tamiya E. 2002. Characterization of a new keratin-degrading bacterium isolated from deer fur. J. Biosci. Bioeng.93: 595-600.