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SCREENING OF NATIVE BACTERIAL ISOLATES FOR KERATINASE PRODUCTION AND ITS APPLICATION IN DETERGENT FORMULATION AND WASH PERFORMANCE

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Microbial keratinase mediated biodegradation of keratin rich wastes such as chicken feathers, nails, hairs and wool, is an environmentally-friendly process, which converts profuse waste into low-cost, nutrient-rich materials such as biofertilizer and animal feed. In the present paper soil samples collected from different poultry farms located in Bikaner were screened for keratinase production. The investigation resulted in isolation of 96 bacterial isolates, out of which 15 were confirmed keratianse producers as determined by their hydrolytic activity on Keratin Powder (KP) agar medium. On the basis of higher keratinase activity (11.6 U/ml) bacterial isolate S-5 was selected and evaluated for detergent stability and wash performance. The bacterium was identified as a strain of *Bacillus tequilensis*. This keratinase was stable in different detergents and exhibited potential to remove coffee and blood stains from cotton fabrics when used alone. However, better stain removal was observed when it was used along with 0.2 % detergent solution.

Key Words: - Detergent stability, Keratinase, Native Bacterial Isolates, Wash performance.

Introduction

Keratinases is a sub-class of proteolytic enzymes produced by microorganisms. Keratinases can be obtained from bacteria, fungi and actinomycetes. Keratinases are extracellular microbial proteases produced in an environment rich in keratinous substrates such as feathers, hairs, nails etc[1]. Microbial keratinase mediated biodegradation of keratin rich wastes is an eco-friendly biotechnological process, which converts profuse waste into low-cost, nutrient-rich materials[2]. Keratinase mediated hydrolysis of feathers significantly enhances the level of essential amino acids in the product. High protein content of keratin waste can be used as a good source of protein and amino acids by systemic recycling[3]. This provides a cheap and alternative protein (amino acid) feed stuff for animal and for many other purposes[3]. However, poor digestibility of keratin is a problem in recycling. Bikaner district and its nearby area have good population of people who are non-vegetarians. There are a good number of poultry farms in and around the city, generating lot of feather wastes on daily basis. These feathers dumped in the dumping areas cause foul smell and health hazard. So, converting this waste in to a useful product would preserve the good health of the environment and would also help in achieving the goal of "waste to wealth". In this process keratinophillic microorganism consume the keratin wastes and clean the environment. Besides waste remediation, keratinases have applications in traditional industrial sectors including feed, detergent, medicine, cosmetics and leather manufacture[1,3,4]. Crude enzyme

can also act as a nutraceutical product, leading to significant improvement and prominently in chicken breed performance[1,3]. They also find application in more recent field such as prion killing for treatment of the dreaded mad cow disease, biodegradable plastic manufacture and feather meal production[5-7].

Materials and Methods

Media and reagents:

Skim Milk Powder (SMP) agar medium (Himedia catalog no. -M763), Keratin Powder (KP) agar medium (Composition g/l: Keratin powder- 5.0, Peptone- 5.0, Yeast extract- 3.0, Agar-Agar powder- 20.0, pH-7.4, Double Distilled water- to make the final volume to 1000ml), Nutrient Broth (NA) (Composition g/l: Peptone- 5.0, Sodium chloride-5.0, Meat Extract-1.5, Yeast Extract-1.5, pH-7.4, Double Distilled water- to make the final volume to 1000ml) Nutrient agar (NA)- NA was prepared by adding agar-agar at a concentration of 1.5% to NB. Production Medium-I was prepared as per the modified method of[8]. (Composition g/l: NaCl – 0.5, KH_2PO_4-0.7, K_2HPO_4-1.4, $MgSO_4$-0.1, Yeast Extract-2.0, Keratin Powder-1.0 (Prepared from chicken feather as described in section-Preparation of Keratin powder), pH-7.0, Distilled water- 50 ml, Production medium-II (Composition g/l: Peptone-0.12, Yeast extract-0.075, Keratin powder – 0.25, pH-7.0, Distilled Water-25ml. Acetone, Dimethyl sulphoxide (DMSO), Trichloro acetic acid (TCA). Keratin powder - prepared as per the method of[8].

Preparation of Keratin powder:

Collection of Chicken Feathers

Chicken feathers were collected from Poultry farm of RAJUVAS, Bikaner. The feathers were washed with tap water for 2-3 times. These were dried in sun light or oven for 2-3 days. After drying the feathers were cut into small pieces and ground with the help of mixer grinder. Ground feathers were dipped into DMSO solution and heated at 100º C for 2 h. After heating the solution was cooled at room temperature.

Precipitation of Keratin Powder:

Feathers were first washed to clean them using either distilled water or chloroform & methanol mixture or distilled water and detergent. The washed feathers were used for keratin extraction as follows:

To 100 ml of feather containing solution 50 ml of chilled acetone was added and mixed well. This resulted in precipitation of keratin. The precipitation was removed by passing the solution through Whatman filter no. 4. The precipitate was washed with distilled water for 2-3 times. It was then dried in an oven at 50º C for 30 minutes. After drying the keratin precipitate was stored in refrigerator for further use. Three types of feather powder were prepared based on the preparation method :-

(A) Keratin Powder 1:- Feathers were washed with distilled water.

(B) Keratin Powder 2 :- Feathers were washed with Chloroform and Methanol.

(C) Keratin Powder 3:- Feathers were washed with distilled water and Detergent.

Screening for Keratinase Producing Microorganisms:

Sample collection

Soil sample were collected from different poultry farms of Bikaner district using sterile plastic bags and sterile spatula. The soil samples were brought to the laboratory of the Department and stored in refrigerator until further use.

Isolation of Bacteria:

Appropriate dilutions (10^{-1} to 10^{-7}) of soil samples were prepared sterile normal saline solution and 0.1 ml of each dilution was plated on to nutrient agar plates. The plates were then incubated at 37 ºC for 24 h and were observed for bacterial colonies.

Screening of Keratinolytic Bacteria:

Qualitative screening:

Qualitative screening was performed on two different media, that is SMP and KA. The bacterial isolates were streaked onto SMP plates. The streaked SMP plates were

incubated at 37ºC for 24h and then observed for hydrolytic zones. The bacteria which showed hydrolytic zones on SMP plates were streaked on KA plates to confirm their keratinolytic activity.

Quantitative Screening:

In order the select a potential Keratinase producing bacterial isolate on the basis of high titre of keratinase activity. The keratinase positive bacterial isolates were grown in fermentation broth for determining the titre value of extracellular keratinase produced by them. The detailed methodology is given below:-

(i) Preparation of Seed Culture

Seed culture of a bacterial isolates was prepared by inoculating a single colony of the isolate to NB. The inoculated NB was incubated at 37º C till the OD_{280nm} reached between 0.8 to 1.0.

(ii) Fermentative Production of Keratinases by Bacterial Isolates

Inoculation of a bacterial culture from seed medium was performed by adding 1 ml of it into 50 ml of the production medium contained in 250 ml Erlenmeyer flask. The inoculated flasks were incubated at 37º C & 150 rpm for 48h.

(iii) Recovery of Crude Keratinase Enzyme by Centrifugation

For determination of keratinase production profile in the fermentation medium, broth Samples from the fermentation broth were taken at 24 h and 48 h. The broth samples and were centrifuged at 10000 rpm for 10 min. After centrifugation the supernatant having the extracellular Keratinase was taken in another vial for determination of enzyme activity and further experimental works.

Quantitative Assay of Keratinase:

Quantitative assay of extracellular keratinase was performed using the assay method given by Gradisar et al.[9].

Assay method

The keratinolytic activity assay was performed as per the method described by [9].

Enzyme Unit

One unit (U) of keratinolytic activity is defined as an increase of corrected A_{280} for 0.100 under the conditions described.

Application of Keratinase in Stain Removal from Fabrics:

Keratinase being a protease enzyme has the ability to remove proteinaceous stains from fabrics. Keeping this in mind the crude extracellular keratinase was used for removal of different type of stains from cloths. As proteases are generally added to detergents as washing-aids to clean protein based stains, the *B. Tequilensis* S-5 keratinase was evaluated for stability in different detergents.

Evaluation of Stability of keratinase in Different Detergents:

In order to determine the stability of keratinase in different detergents, it was incubated with different detergents comprised of 1 ml keratinase (supernatant) + 1 ml of 0.2 % detergent*. 100 µl of enzyme + detergent solution was added to respective wells on KA plates with the help of Micropipette. The plates were then incubated at room temperature for overnight. Next day spray 10% TCA (Tricholoro acetic acid) on Plate.

*Detergents used- Nirma, Tide, Wheel and Arial.

Wash performance experiment

For wash performance enzyme was used to remove blood and coffee stains from the cotton fabrics as described below:

Blood and coffee were used separately to stain cotton fabric pieces of 30×30 mm dimension. The stained fabric pieces were dried at 40ºC for overnight. The dried fabrics were then washed using methods as described in A,B,C and D.

A) 10 ml of Tap water
B) 8 ml of Tap water + 2 ml of keratinase (crude fermentation broth without cells)
C) 10 ml of 0.2% detergent solution
D) 8 ml of 0.2 % detergent solution+ 2 ml keratinase (crude fermentation broth without cells)

Figure 1 :-Soil Sample Collection Site 1

Figure 2:-Soil Sample Collection Site 2

Figure 3:- Feathers collection site

Figure 4:- Feather collection site

Figure 5:- Feather Drying in Oven

Figure 6:- Feather Drying in Sun Light

Figure 7:- Keratin Powder Extraction Unit (Soxhlet Extraction Unit)

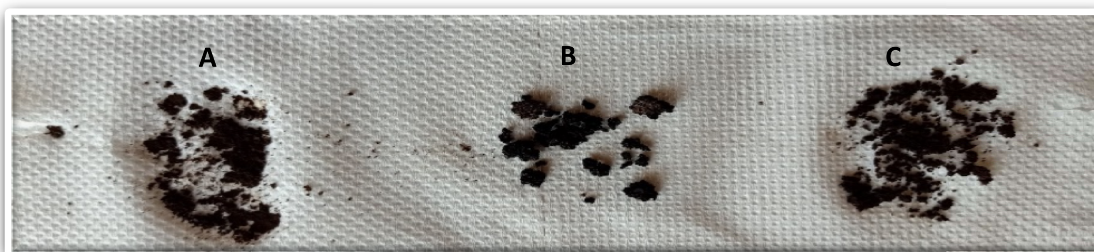

Figure 8: Keratin precipitates obtained using different methods: (A) feathers were washed with distilled water (B) feathers were washed with Chloroform and Methanol (1:1) (C) feathers were washed with distilled water and 1% Detergent solution (1:1)

Results and Discussion

Qualitative and Quantitative Screening for Keratinase Producing Bacteria:

Dilution plating of soil samples on NA plates resulted in a total of 96 bacterial isolates. Out of these 96 bacterial isolates, 44 bacterial isolates were positive for protease activity as they exhibited hydrolytic zones on SMP agar plates. However, out of these 44 bacterial isolates only 15 were positive for keratinase activity as they produced hydrolytic zones on KA medium also. The results of qualitative screening on these two media are presented in Table 1.

Table 1:- Result of Qualitative and Quantitative Screening for Bacterial Keratinase Producers

S.No.	Bacterial Isolate no.	Qualitative Screening		Quantitative Screening
		Zone of Hydrolysis on KP agar medium (mm)	Zone of Hydrolysis on SMP agar medium (mm)	Keratinase activity (U/ml)
1.	Isolate no.1	0.6	0.7	2.5
2.	Isolate no.2	0.7	0.7	1.0
3.	Isolate no.3	0.6	0.8	2.6
4.	Isolate no.4	0.7	0.8	8.9
5.	Isolate no.5	0.8	0.9	11.6
6.	Isolate no.31	0.7	0.7	3.1
7.	Isolate no.42	0.6	0.7	1.0
8.	Isolate no.44	0.6	0.7	3.0
9.	Isolate no.48	0.6	0.6	2.4
10.	Isolate no.49	0.6	0.7	1.4
11.	Isolate no.53	0.5	0.6	1.1
12.	Isolate no.65	0.6	0.7	1.3
13.	Isolate no.82	0.4	0.6	0.8
14.	Isolate no.86	0.5	0.6	1.7
15.	Isolate no.92	0.5	0.6	1.2

Screening results presented in Table 1 shows that out of 96 total bacterial isolates obtained from different soil samples, 15 were confirmed keratinase producers as they hydrolyzed the keratin substrate present in the KP agar medium and exhibited hydrolytic zones around their colonies as shown in Fig. 1. These isolates were further subjected to quantitative screening to quantify the keratinase produced by them in terms of enzyme titre values (U/ml) of the fermentation broth. The results showed that isolate S-5 exhibited maximum keratinase activity i.e. 11.6 U/ml. This isolate was therefore selected for further investigations. Next highest keratinase producer was bacterial isolate S-4, which produced 8.9 U/ml of keratinase. Many of the previous researchers have used milk or casein as a substrate for screening of microbial keratinase activity[1,6,10]. However, milk or casein are generic substrate used for determining proteolytic activity. In order to ascertain the keratinolytic activity of any sample the screening medium should include either pure keratin or any source of keratin like feathers, hairs or wools etc. In this regard some researchers[11,12] have used Azokeratin/Keratin azure as a substrate for determining keratinase activity in bacterial samples. Whereas, others[10] have used raw feathers as substrate for determination of keratinase activity in different fungal samples.

Culture Characterization and Identification:

The bacterial isolate S-5 was subjected to various morphological and biochemical tests in order to identify it. The morphological and biochemical tests are presented in Table 2.

CHARACTERISTICS	OBSERVATION
Physiological characteristics	
Sporulation	Positive
Motility	Motile
Morphological characteristics	
Shape of bacteria	Rod shaped
Colony shape	Round with undulations
Colony surface	Smooth & shiny
Colony margin	Undulating
Colony appearance	Whitish
Colony elevation	Convex
Colony opacity	Opaque
Biochemical characteristics	
Glucose catabolism	Positive
Catalase	Positive
Citrate	Positive
Flagella	Flagellated
Gas	Negative
Gelatin Hydrolysis	Positive
Indole	Negative

Table 2: Results of morphological, physiological, cultural and biochemical characteristics of bacterial isolate S-5

16S rRNA Identification of the Bacterial Isolate S-5:

16S rDNA sequence of the culture:
GGGAGCTTGCTCCCTGATGTTAGCG
GCGGACGGGTGAGTAACACGTGGG
TAACCTGCCTGTAAGACTGGGATAA
CTCCGGGAAACCGGGGCTAATACCG
GATGGTTGTTTGAACCGCATGGTTC
AAACATAAAAGGTGGCTTCGGCTAC
CACTTACAGATGGACCCGCGGCGCA
TTAGCTAGTTGGTGAGGTAATGGCT
CACCAAGGCAACGATGCGTAGCCG
ACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTC
CTACGGGAGGCAGCAGTAGGGAAT
CTTCCGCAATGGACGAAAGTCTGAC
GGAGCAACGCCGCGTGAGTGATGA
AGGTTTTCGGATCGTAAAGCTCTGT
TGTTAGGGAAGAACAAGTACCGTTC
GAATAGGGCGGTACCTTGACGGTAC
CTAACCAGAAAGCCACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGAATTA
TTGGGCGTAAAGGGCTCGCAGGCG
GTTTCTTAAGTCTGATGTGAAAGCC
CCCGGCTCAACCGGGGAGGGTCATT
GGAAACTGGGGAACTTGAGTGCAG
AAGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCG
CCCCTTAGTGCTGCAGCTAACGCAT
TAAGCACTCCGCCTGGGGAGTACGG
TCGCAAGACTGAAACTCAAAGGAA
TTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCA
ACGCGAAGAACCTTACCAGGTCTTG
ACATCCTCTGACAATCCTAGAGATA
GGACGTCCCCTTCGGGGGCAGAGTG
ACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTGA
TCTTAGTTGCCAGCATTCAGTTGGG
CACTCTAAGGTGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACG
TCAAATCATCATGCCCCTTATGACC
TGGGCTACACACGTGCTACAATGGA
CAGAACAAAGGGCAGCGAAACCGC
GAGGTTAAGCCAATCCCACAAATCT
GTTCTCAGTTCGGATCGCAGTCTGC
AACTCGACTGCGTGAAGCTGGAATC
GCTAGTAATCGCGGATCAGCATGCC
GCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCACGAG
AGTT

On the basis of biochemical characteristics and 16S rRNA analysis the selected bacterial isolate was identified as a strain of *Bacillus tequilensis.*

Bacterial keratinases have been reported from different bacterial genera such as *Fervidobacterium*, *Pseudomonas*, *Microbacterium*, *Proteus, Streptomyces* , *Bacillus* and others [1,2.13]. However, the most prominent bacterial genus for keratinase production is *Bacillus,* with many species reported for keratinase production, such as *Bacillus licheniformis*[14] , *Bacillus pumilus*[15] *Bacillus subtilis*[16,17], *Bacillus amyloliquefacien*[18], *Bacillus cereus*[19] and *Bacillus halodurans*[20]. The bacterial isolate reported in this paper has been identified as *Bacillus tequilensis*.

Figure 9:- Keratin Positive Bacterial isolates showing hydrolytic zone around the streak and isolated colonies on SMP agar plates

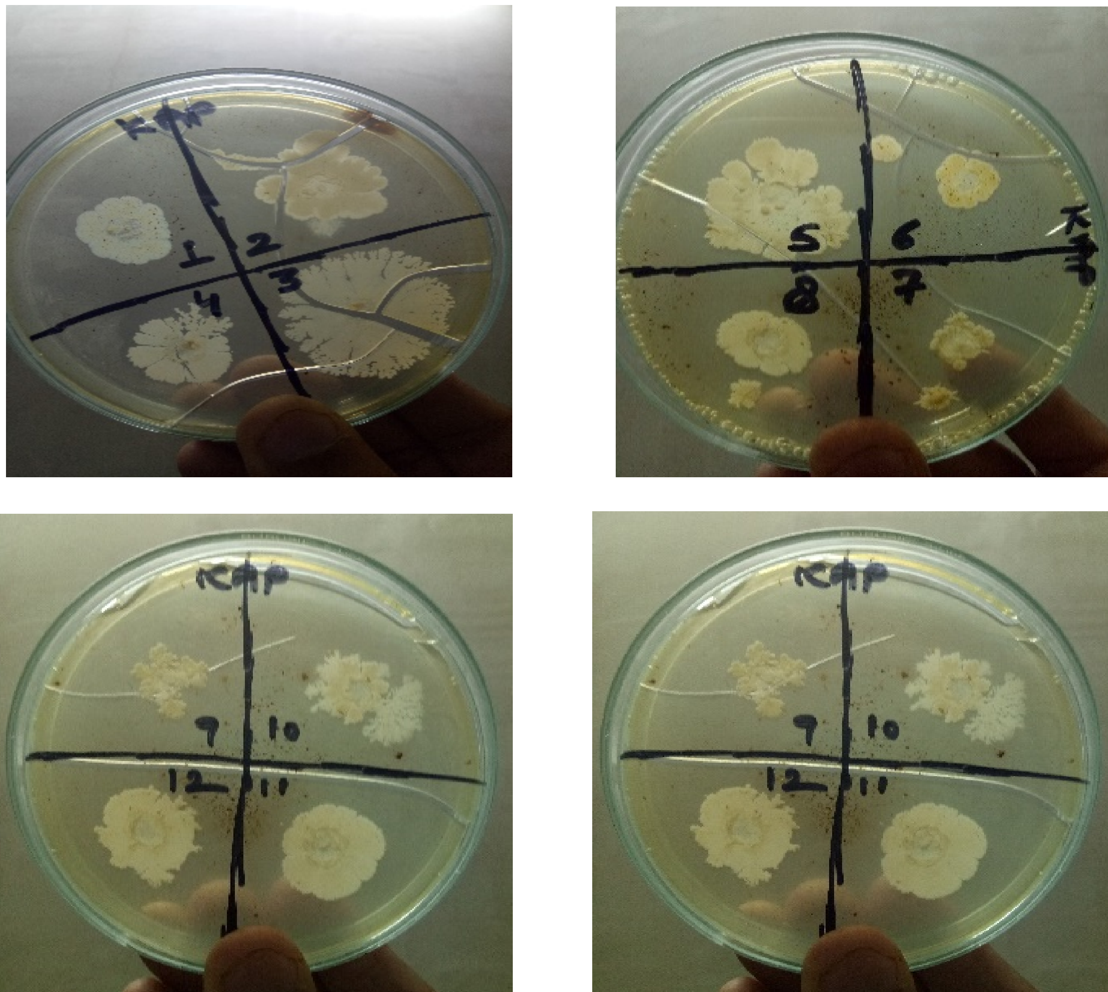

Figure 10: Confirmation of keratinase activity on Keratin Powder (KP) agar plates

Evaluation of Bacillus tequilensis S-5 keratinase for stability in detergents

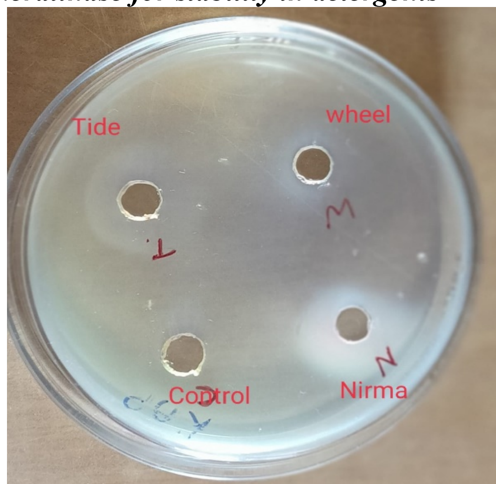

Fig. 11:- Detergent stability of S-5 keratinase in Nirma,Tide, Wheel and Arial.

From Fig. 11 it is clear that the keratinase of *Bacillus tequilensis* S-5 exhibited higher stability in Nirma compared to other detergents. So the combination of S-5 keratinase with Nirma was used for wash performance experiments. Keratinase activity of S-5 was measured as zone diameter on KP agar plates (Fig 11) after 24 h of incubation.

The result of wash performance experiment as observed from Fig12. shows that the keratinase from *Bacillus techlensis* (S-5) culture completely removed coffee and blood stains.

From Fig 12 it can be observed that the *Bacillus tequilensis* S-5 keratinase was able to remove both blood and coffee stains from the cotton fabrics. Further better stain removal was observed when enzyme was used in combination with the detergent solution as compared to when detergent was used alone or when the enzyme was used alone. However, when only water was used for washing, no reduction in stain intensity could be observed. Application of keratinase in washing of cloths has been reported by earlier researchers also[1,4,21].

However, majority of applications of keratinases have remained confined to two

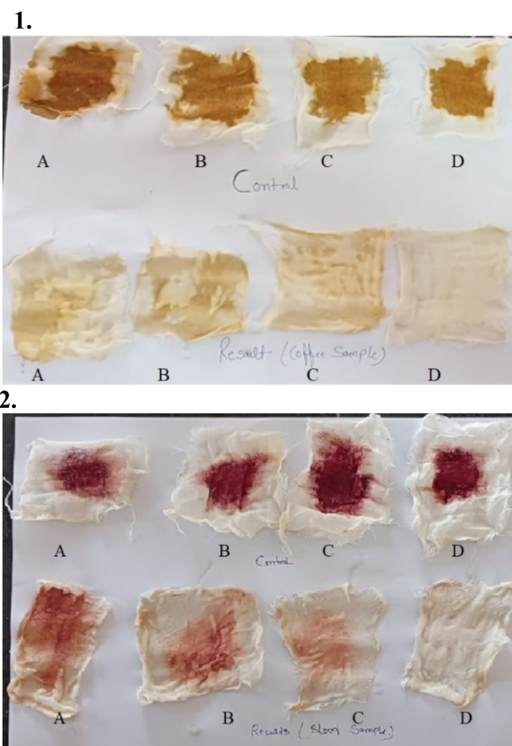

Fig. 12:- Wash performance: 1. Coffee stained cloths: (1A) 10ml of Tap water (1B) 8 ml of Tap water + 2ml of keratinase (1C) 10ml of 0.2 % Detergent (1D) 8 ml of 0.2 % Detergent + 2ml of keratinase. 2. Blood stained cloths: (2A) 10ml of Tap water (2B) 8 ml of Tap water + 2ml of keratinase (2C) 10ml of 0.2 % Detergent (2D) 8 ml of 0.2 % Detergent + 2ml of keratinase

major areas i.e. degradation of feathers and/or dehairing of hides and animal skins[2,13,22,23,24] . The reason behind the use of keratinases for these two particular applications is obvious as this is the natural function of keratinases. However, their usage in other non conventional sectors like in detergent formulations would increase their industrial potential and sustainability.

Conclusion:-

The present investigation resulted in isolation of *Bacillus tequilensis* S-5 capable of producing a detergent stable keratinase enzyme with potential to remove blood and coffee stains from cotton fabric. The enzyme also has the ability to dissolve raw feathers into digested slurry, which could be used as

biofertilizer as well as animal meal as it is rich in important amino acids (data not shown here).

References:-

1. Baid S, Vashishtha A, Ahmed A, Verma S & Meghwanshi G K 2016, Bacterial Keratinase catalysed bioremediation of keratin rich wastes for potential agricultural and other applications. *J. Phytol. Res*. 29(1 & 2): 17-30.

3 Lakshmi PJ, Chitturi CH, Lakshmi MK 2013, Efficient degradation of feather by keratinase producing Bacillus sp. *Int. J. Microbiol*., Vol. 2013, Article Id 608321, 7 . Sharma I & Kango N 2021, Production and characterization of keratinase by *Ochrobactrum intermedium* for feather keratin utilization. *Int. J. Biol. Macromol.* 166: 1046-1056.

4 Cavello IA, Hours RA & Cavalitto SF 2012, Bioprocessing of "hair waste" by *Paecilomyces lilacinus* as a source of a bleach-stable, alkaline, and thermostable keratinase with potential application as a laundry detergent additive: characterization and wash performance analysis. *Biotechnol. Res Int.* 1–12.

5 Bressollier P, Letoumeau F, Urdaci M & Vemcuil B 1999, Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*. *J.Appl. Environ. Microbiol.* 65: 2570-2576.

6 Prasad VH, Kumar G, Kartik KL & Rao KVB 2010, Screening of extracellular Keratinase producing bacteria from feather processing area. *J. sci. res*. 2(3): 559-565.

7 Qiu J, Wilkens C, Barrett K & Meyer AS 2020, Microbial Enzymes Catalyzing Keratin Degradation, Classification, Structure, Function. *Biotechnol Adv*. 44: 107607. Doi:10.1016/J.Biotechadv.2020.107607.

8 Thanaa HA, Nadia HA & Latifa AM 2011, Production, Purification and Some Properties of Extracellular Keratinase from Feathers-Degradation by *Aspergillus oryzae* NRRL-447. *J. of Appl. Sci. Environ. Sanit.* 6(2): 123-136.

9 Gradisar H, Kern S, Friedrich J 2000, Keratinase of *Doratomyce microspores*. *Appl. Microbiol. Biotechnol*. 53:196-200.

10 Kumar J & Kushwaha RKS 2014, Screening of fungi efficient in feather degradation and keratinase production. *Arch. Appl. Sci. Res.,* 6(1):73-78.

11 Sangali S, & Brandelli A 2000, Feather keratin hydrolysis by a *Vibrio Sp.* Strain Kr2. *J Appl. Microbiol.* 89(5): 735-43.

12 Bihari Z, Vidéki D, Mihalik E, Szvetnik A, Szabó Z, Balázs M, Kesseru P & Kiss I 2010, Degradation of native feathers by a novel keratinase-producing, thermophilic isolate, *Brevibacillus thermoruber* T1e. Z Naturforsch C. *J. Biosci*. 65(1-2): 134-40.

13 Kerouaz B, Jaouadi B, Brans A, Saoudi B, Habbeche A, Haberra S, Belghith H, Gargroui A & Ladjama A 2021, Purification and biochemical characterization of two novel extracellular keratinases with feather-degradation and hide-dehairing potential. *Process Biochem., 106*: 137-148.

14 Williams CM, Richter CS, Mackenzie JM, & Shih JC 1990, Isolation, identification, and characterization of a feather-degrading bacterium. *Appl. Environ. Microbiol.* 56: 1509 – 1515.

15 El-Refai HA, Abdelnaby MA, Gaballa A, El-Araby MH & Fattah A 2005, Improvement of the newly isolated *Bacillus pumilus* Fh9 keratinolytic activity. *Process Biochem*. 40: 2325-2332.

16 Macedo A, Dasilva W, Gava R, Driemeier D, Henriques J & Termignoni C 2005, Novel keratinase from *Bacillus subtilis* S14 exhibiting remarkable dehairing capabilities. *Appl. Environ. Microbiol*.71(1): 594-596.

17 Cai C, Lou B & Zheng X 2008, Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis*. *J Zhejiang Univ Sci B* 9(1): 60-7doi: 10.1631/jzus.B061620.

18 Ghosh A, Chakrabarti K & Chattopadhyay D 2009, Cloning of feather-degrading minor extracellular protease from *Bacillus cereus* DCUW:

dissection of the structural domains. *Microbiol.-SGM.* 155: 2049–2057.

19 Yang L, Wang H, Lv Y, Bai Y, Luo H, Shi P, Huang, H & Yao B 2016, Construction of a rapid feather-degrading bacterium by overexpression of a highly efficient alkaline keratinase in its parent strain *Bacillus amyloliquefaciens* K11. *J. Agric. Food Chem.* 64: 78–84.

20 Shrinivas D & Naik GR 2011, Characterization of alkaline thermostable keratinolytic protease from thermo alkalophilic *Bacillus halodurans* Jb 99 Exhibiting Dehairing Activity. *Int. Biodeterior. Biodegrad.* 65: 29–35.

21 El-Ghonemy DH & Ali TH 2021, Effective bioconversion of feather-waste keratin by thermo-surfactant stable alkaline keratinase produced from *Aspergillus sp.* DHE7 with promising biotechnological application in detergent formulations. *Biocatal. Agric. Biotechnol*., *35*: 102052. Doi: 10.1016/J.Bcab.2021.102052 Biocatal. Agric. Biotechnol.,

22 Hamiche S, Mechri S, Khelouia L, Annane R, El-Hattab M, Badis A & Jaouadi B 2019, Purification and biochemical characterization of two keratinases from Bacillus amyloliquefaciens S13 isolated from marine brown algae *Zonaria tournefortii* with potential keratin-biodegradation and hide-unhairing activities. *Int. J. Biol. Macromol.* 122: 758-769.

23 Gupta S, & Singh R 2014, Hydrolyzing proficiency of keratinases in feather degradation. *Indian. J. Microbiol. 54*(4): 466–470.

24 Azza MA, Mamdouh SE, Ismail SA, Emran MA & Hashem AM 2018, Biodegradation of feather waste by keratinase produced from newly isolated *Bacillus licheniformis* Alw1, *J. Gent. Eng. and Biotechnol*. 16(2): 311-318.