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Isolation, Identification and Characterization of Keratinolytic Bacteria from Poultry Waste

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Abstract: ***The poultry sector is an important sector for all the economies of the world but this sector also adds to a bulk of poultry wastes. The study will enhance the understanding of decomposition process of poultry waste and mitigation strategy. This research will provide a platform for the scholars to concentrate on the keratolytic property of the isolated microbes which helps in the decomposition of feathers, litter, and other poultry wastes. Further chemical and morphological characteristics of these isolated microbes are analysed to understand the biological process of breaking the keratinous enzymes contained in the poultry wastes and their scientific utilization for producing other useful products such as cattle fodder or renewable energy generation etc. Soil samples were collected from Poultry farm of Dausa and Jaipur district in Rajasthan. Isolation and identification of bacterial pathogens were carried out based on their growth pattern and colony characteristics on selective and differential media. Gramstaining, Catalase, Oxidase, Urease and motility tests were carried out to confirm the findings. We identified four separate types of keratinolytic bacteria in this study. The isolates were obtained, they were cultivated on feather broth to determine keratinase activity. Keratinase being found in microbial culture of keratin - rich wastes, which were found to be applicability for enzyme production & waste treatment and providing value to these agro - industrial leftovers.***

Keywords: Keratin, Degradation, Feather Meal Broth, Feather Meal Agar

1. Introduction

Around 24 billion chickens are slaughtered annually worldwide, resulting in about 8.5 billion tons of feather, 150 tons of litter and billions of tones of other wastes. The waste of this sector is a great cause of concern owing to health and air pollution as they produce very serious microbe - infection, offensive odours, it enhances the breeding of flies, rodents and other infection causing insect. The waste from this sector comprises of feathers, litter, unusable departed chickens, blood, bones, damaged eggs, hatcheries, broiler and layers. The waste generated by this industry is hazardous and is not easily degradable. Non availability of the waste processing units for this sector is a cause of great concern as in absence of such processing; disposal of a wastes is very difficult. Feathers comprise approximately ten percent of the body weight of whole chicken and are toughest in decomposition process as they have highest proportion of pure keratin protein which is hard to decompose. After decomposition of keratin by keratinolytic bacteria's, it can be chemically processed and added as directly supplement to the cattle fodder. This study was performed to estimate the keratolytic property of isolated bacteria's and to identify it morphologically and biochemically and to select those with high potential of poultry waste degradation. The process of keratolysis utilizes keratinase which are very specific protease that degrade keratin specifically and are produced by Dermatophyte, Saprophytic Fungi & certain Bacillus species.99% of the surviving bacterial species are not capable of being treated under "bacteria culture process" because they are non - culturable. Though these feathers can be processed on an extremely high temperature and pressure and then grounded to make cattle fodder, but food supplement produced in the process is very costly and is time consuming. Some developing countries are also using, feather waste in fertilizers and land – filling, fertilizer, cattle feed, fuel etc. Chicken feathers compose of approximately 91 percent of protein (majorly keratin), 8 percent water, and 1 percent lipids. The sequencing of amino - acid is similar in all feathers including chicken feathers. Keratin consists of polypeptide - chains that are connected by peptide - bonds and contains amino - acids. Keratin is the name given to a large family of homologous proteins that have a filamentous (fibrous) structure. These proteins are expressed in epithelial cells and in epidermal cells where they are assembled forming cytoskeletal structures within the cell and epidermal derivatives such as hair, nail and horn. Keratin consists of polypeptide - chains that are connected by peptide - bonds and contains amino - acids. Keratins are fibrous proteins present in certain fish, reptiles, and vertebrates inside the integument. In structure of keratin there are several bonds like hydrogen bond, hydrophobic bond and disulphide bridges. Keratin is characterised by high content of amino acids such as glycine, alanine, serine and valine. Also, keratin contains in its structure minor amounts of methionine, lysine and tryptophane. Most eminent components (7÷12%) are cystine and cysteine, which are sulphur - containing amino acids. Keratin enzymatic degradation is a multi - stage task that needs the following steps: (i) keratinase adsorption to the surface of a macromolecule by means of hydrophobic and electrostatic interactions, accompanied by catalytic action. (ii) the multi - stage keratin deterioration method entails two primary mechanisms: disulphide bond elimination (sulfitolysis), and proteolysis. Sulfitolysis may take place only in the existence of reduction agents like dithiothreitol (DTT), sodium sulphide, glutathione, disulfide reductase, mercaptoethanol, thioglycolic acid, or cysteine functioning in the degradation of keratin molecules in combination with keratinases.

2. Material and Method

Sample collection

Sample were collected from mentioned eight areas; Site1: Behind police station Bandikui. Site2: Near railway station. Bus stand, Sikandra road Bandikui. Site3: Bharat Singh

agriculture farm near shyosinghpura, village, Akoda, Rajasthan. Site4: Simran farm Ltd, SMS Colony, Hajiwala, Muhana, Rajasthan. Site5**:** Poultry farm (Shrawan Ji) Bhootawali. Site6: Gram, Bhutawali Rd, Post, Bindayak, Jaipur, Rajasthan**.** Site7: Vikram poultry farm (Mokhampura - Akoda Rd, palbas, Rajasthan. Site8: Desi murgi farm meeno ki dhani, Lakhana, Sanganer, Jaipur, Rajasthan.

Screening of Bacteria's

Bacterial isolation pre - enrichment was done by transferring 10 g of soil sample into 90 mL Feather Meal Broth (FMB) (0.3 g L - 1 K2HPO4, 0.5 g L - 1 sodium chloride, 10 g/L cut feathers, and 0.4 g/L KH2PO4) and afterwards incubated for 48 hrs at 37 ºC. Serial dilution of the sample was then done at 10 - 3, 10 - 5 and 10 - 7 dilutions and plated into Feather Meal Agar (FMA) (FMB + 15g/L agar). The plates were then incubated for five days at 98.6 ºF. Separate colonies were streaked into FMA and incubated for two days. Cultural characteristics of isolates were observed. Well isolated colonies were streaked into Luria Bertani (LB) Agar (5g/L yeast extract, 10g/L tryptone, 15 g/L agar and 10g/L NaCl slants) and incubated at 37 ºC for 24 hours. These served as culture stocks for the succeeding steps. Isolates were Gram stained and observed under light microscope to check for purity and to determine morphological characteristics. Two types of screening were done: Preliminary screening, Confirmatory Screening. Gelatinase assay, Keratinase activity assay, Catalase test, Spore staining was also performed.

DNA Isolation

The isolates were inoculated into five mL LB broth and then incubated for 18 - 24 hours. Cells were collected by transferring 1.5 mL of cell cultures into 1.5 mL sterile Eppendorf tubes and then centrifuging them at room temperature for 5 minutes at 5, 000 rpm speed. The process was done repeatedly in the same tubes until all cells in the culture broths were collected. Cells were washed twice with sterile distilled water and recentrifuged at the same conditions and then resuspended in 200 µL Tris - EDTA (TE) buffer and mixed briefly. Tubes were first placed into boiling water bath for five minutes and then placed in freezer for 15 minutes. This freeze - thaw cycle was repeated three times. Resulting suspension was centrifuged at 5, 000 rpm for five minutes. Supernatant was transferred to new sterile Eppendorf tubes and labelled accordingly**.**

Characterization of Keratinolytic bacterial isolates

Different morphological, physical, physiological, and biochemical characterizations molecular were conducted in order to classify the keratinolytic strains of bacteria extracted by enrichment procedure. That are Cell morphology, Colony morphology, Gram reaction (Staining/non staining method), Motility, Endospore test, Temperature and pH, Catalase activity, Casein hydrolysis, H2S production, Citrate utilization, Hydrolysis of gelatin, Indole production, MR - VP (Methyl Red - Voges Proskauer) test, Growth on McConkey agar, Urea hydrolysis and Starch hydrolysis.

Identification of keratinolytic bacterial isolates

Lysis/homogenization: Cells grown in monolayer should be lysed by suspend 1 - 3 colonies aseptically and mixed with 450 µl of "B Cube" lysis buffer in a 2 ml micro centrifuge tube and lyse the cells by repeated pipetting. Add 4 µl of RNAse A and 250 µl of "B Cube" neutralization buffer. Vortex the content and incubate the tubes for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules, mix DNA solutions by inversion. Centrifuge the tubes for 15 minutes at 14, 000 rpm at 10 ˚C. Following centrifugation, transfer the resulting viscous supernatant into a fresh 2 ml micro centrifuge tube without disturbing the pellet. Add 600 µl of "B Cube" binding buffer to the content and mix thoroughly by pipetting and incubate the content at room temperature for 5 minutes. Transfer 600 µl of the contents to a spin column placed in 2 ml collection tube. Centrifuge for 2 minutes at 14, 000 rpm and discard flow - through. Reassemble the spin column and the collection tube then transfer the remaining 600 µl of the lysate. Centrifuge for 2 minutes at 14, 000 rpm and discard flow - through. Add 500 µL "B Cube" washing buffer I to the spin column. Centrifuge at 14, 000 rpm for 2 mins and discard flow - through. Reassemble the spin column and add 500 µl "B Cube" washing buffer II and Centrifuge at 14, 000 rpm for 2 mins and discard flow - through. Transfer the spin column to a sterile 1.5 - ml microcentrifuge tube 14. Add 100 µl of "B Cube" Elution buffer at the middle of spin column. Care should be taken to avoid touch with the filter. Incubate the tubes for 5 minutes at room temperature and Centrifuge at 6000 rpm for 1 min. Repeat the above - mentioned step 14 and 15 for complete elution. The buffer in the microcentrifuge tube contains the DNA. DNA concentrations were measured by running aliquots on 1% agarose gel. The DNA samples were stored at - 20°C until further use.

3. Result

In this study, 2 isolates were extracted from the poultry waste sample. All isolates were distinguished on the basis of bacterial development and culture on feather broth and their standard morphological, physical, and biochemical characterization. Out of the 2 isolates identified, one was gram - negative, and one was gram - positive. These isolates were recognized by various biochemical tests like MR - VP, Indole, Oxidase, Citrate, Nitrate decrease test, Catalase, Starch hydrolysis, and Gelatine hydrolysis test. Soil with degrading feathers was used as microbial source to increase the chances of isolating bacteria with keratinase activity. Furthermore, samples were pre - enriched in FMB for two days to magnify the population of the target microorganisms from the soil samples. Initial isolation yielded 2 bacterial isolates that were capable of thriving in FMA. Preliminary screening was done to verify the proteolytic activity. The protease assay was used, and the no. of isolates were narrowed down and then evaluated. The isolates were grown on milk agar. Four isolates were able to form clearing zones indicative of proteolytic activity. These isolates were recovered and were subjected for further testing.

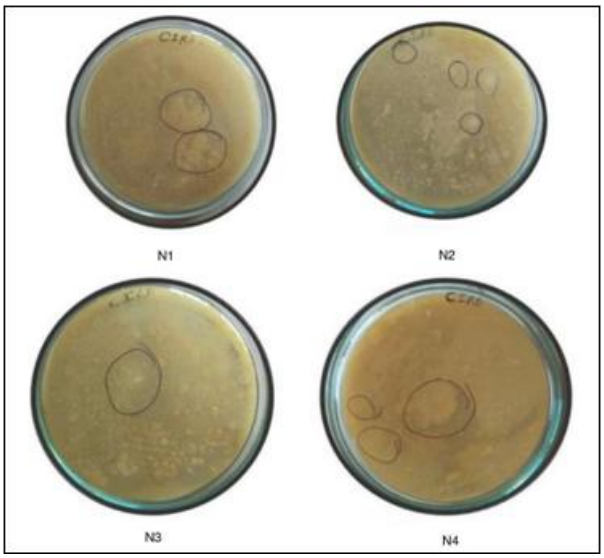
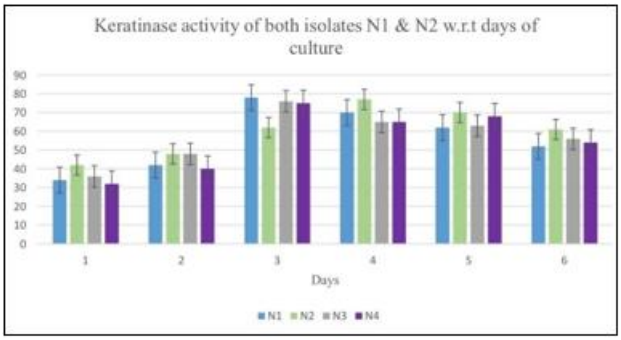


Figure 1: Showing isolates with clear zones surrounding it

Isolates with highest keratinase activity were identified by further interrogating their capacity to degrade feathers. This was done by using feather broth. It is a medium that consists of feathers and is a sole source of nitrogen and carbon. Whole feathers were supplemented in the media in order to visually inspect feather degradation. This was further supported by the observed highest turbidity in these isolates' indicative of feather utilization for growth. These four isolates (N1, N2, N3 and N4) were recovered and identified (Figure - 1).

Table 1: Keratinase activity w.r.t days of culture

	Relative Activity			
Days	Starin 1	Starin 2	Starin 3	Starin 4
1	34	42	36	32
2	42	48	48	40
3	78	62	76	75
4	70	77	65	65
5	62	70	63	68
6	52	61	56	54



Graph 1: Depicting keratinase activity of both isolates

The keratinase activity of both the isolates were calculated by cultivating them on a feather broth. The percentage of degradation of feather in the culture media was recorded as the keratin digesting activity of the bacteria. The maximum activity of N1, N3 & N4 was seen on 3rd day of culture (Graph - 1). The maximum activity of N2 was seen at 4th day of culture.

Isolates Extracted

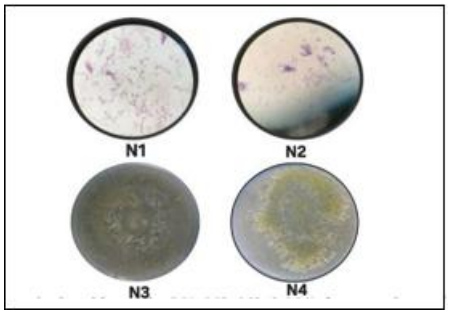


Figure 2: Four isolated bacteria (N1, N2, N3 & N4) from poultry soil sample

After isolation, the process of gram staining was done. The pink coloured bacteria were rod shaped and it was Gram negative bacteria. The non - stained bacteria were found to be Gram - positive.

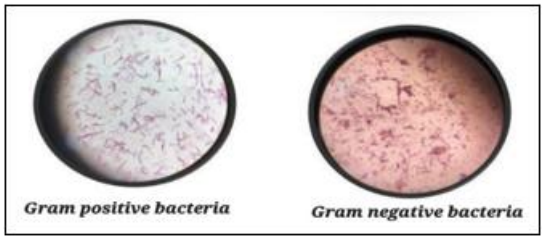


Figure 3: Results after gram staining of isolates

Growth on the MacConkey agar

The isolates obtained were cultured on the MacConkey agar plate to confirm the presence of any gram - negative bacteria if present. MacConkey agar is a bacteria specific and differential culture source. It has made to isolate Gram - negative and enteric bacilli (those that live in the intestine) and distinguish them based on lactose fermentation.1st Lactose fermenters shift colour on MacConkey agar to red or pink, whereas non - fermenters do not. With crystal violet and bile salts, the media inhibits Gram - positive organisms' development, allowing for the collection and extraction of gram - negative bacteria. It includes bile salts (that suppress most Gram - positive bacteria), crystal violet dye (which inhibits some Gram - positive bacteria as well), and phenol red dye (that inhibits some Gram - positive bacteria) (which turns pink if the microbes are fermenting lactose) (Figure3).
The four isolates grown on MacConkey agar. The isolate N1 was found to be unable to grow on the agar plate whereas the N2, N3 & N4 isolate formed the colony. This led to differentiation between the four isolates as N1 being the gram - positive bacteria and N2, N3 & N4 the gram - negative bacteria (Figure - 3).

Comparative results of all four isolates

Preliminary morphological and biochemical parameters were used to classify all of the keratinolytic bacterial strains. The comparative morphological, physiological, and biochemical characters of the selected strains were depicted in Table 2.

Table 2 Comparative results of both the isolates

No.	Tests performed	Isolate 1 (N1)	Isolate 2 (N2)	Isolate 3 (N3)	Isolate 4 (N4)
Morphological observation					
1.	Colour	Yellow	Creamy	Creamy shiny	White
2.	Colony shape	Round to obvate	Pinpoint	Round	Irregular shaped
3.	Cell shape	Short rods	Rod	Circular	Rod shaped
4.	Motility	Positive	Negative	Positive	positive
Biochemical tests					
1.	Catalase test	Positive	Positive	Positive	Positive
2.	Starch test	Positive	Negative	Negative	Positive
3.	Gelatin test	Positive	Negative	Negative	Positive
4.	H2S test	Positive	Negative	Negative	Positive
5.	Indole test	Negative	Positive	Negative	Negative
6.	Citrate test	Positive	Negative	Negative	Negative
7.	Urea test	Positive	Negative	Positive	Positive
8.	Methyl red test	Negative	Negative	Negative	Negative
9.	Voges-Proskauer test	Positive	Negative	Negative	Negative

When grown on broth comprising native feather, the experimental feather - degrading microorganisms generated a strong keratinolytic activity, indicating that they were isolated from poultry waste. During harvest, the feathers became fully degraded. The bacteria have the ability to be used in biotechnological keratin hydrolysis processes. The identification of isolated strains was completed by analysing segregates with various biochemicals. Isolates were also subjected to biochemical tests such as the MR - VP test, indole test, the oxidase test, the citrate usage test, the starch hydrolysis test, the catalase test, and the gelatine hydrolysis test. Four different bacterial strains were identified based on their physical and morphological characteristics. The isolates identified were of *Bacillus cereus* (N1), *Pseudomonas aeruginosa (*N2), *Breuvendimonos terrae* (N3) and *Bacillus subtilis* (N4) species.

4. Discussion

Buckle et al., (1995), said that, in applied microbiology, when active feather keratin degraders are needed, the use of microbial keratinase is being investigated. Gram positive bacteria degrade keratin the most, while there are a few examples of gram - negative bacteria destroying feathers. Bacteria which produce the keratinase enzyme, were isolated and characterized as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Breuvendimonos Terrae* and *Bacillus Subtilis* respectively. A 16s r RNA sequencing analysis verified the identity. After three days, the isolated bacteria demonstrated efficient destruction. **Noval and Nickerson (1959)** discovered a number of enzymes that may breakdown keratin. Keratinases are the enzymes that do this. A lot of bacteria were recovered from a dump site of feathers in this experiment. These bacteria have keratinolytic activity, which allowed them to breakdown keratin wastes. Feathers provided the majority of their sulphur, energy, nitrogen, and carbon. Temperatures of 30, 35, 40, and 45°C were used to incubate the bacteria. **Tiwary E et al., (2013)** observe that keratin degradation has been found to be primarily restricted to Gram - positive bacteria, such as Bacillus, Streptomyces, & a few types of Gram - negative bacteria, in keeping with this research. It has previously been reported that keratinase - producing strains of *B. subtilis*, *B. cereus* and *B. licheniformis*, have been isolated. Keratin degeneration has long been associated with dermatomycosis; specific parasites, such as Aspergillus, Actinomyces, and the Streptomycin group from Actinomyces, were thought to be keratinase producers. It was primarily a meeting place for therapeutic mycologists. Nonetheless, the first study on segregation and presentation of the quill debasing bacteria *Bacillus licheniformis* PWD - 1 revealed its biotechnological and ecological value (**Williams S. et al., 1990).** The majority of their study focused on plume reuse and quill supper generation, and they identified KerA from *B. licheniformis* as a possible keratinase. KerA was fully characterised for a long period, involving its succession and articulation in several heterologous hosts (**Rani Gupta et al., 2013).** Efforts were undertaken to generate it, with KerA quality being improved by chromosomal reconciliation and the framing of asporogenic *B. licheniformis* strains (**G. Wang et al., 2014).** As a result, KerA received a large number of papers and licences. The role of disulfide reductase, cell redox, & substrate explicitness were all explored in attempts to understand the mechanism of keratin corruption (**Yamamura et al., 2002).** Regardless, keratinase research did not take off until the discovery of KerA's prion debasing capabilities in 2003, when the Mad Cow disease was making headlines. In 2003 - 04, Europe was ravaged by the Mad Cow plague. Meat was outlawed across the UK. Around the same time, the significance of keratinase in prion corruption was recognised by a publication from Shih's group in 2003, which demonstrated for the first time that keratinase from *B. licheniformis* PWD - 1 (KerA) may debase prion protein of scrapie - infected cerebrum tissue (**Langeveld et al., 2003).** Since then, there has been an increase in the number of publications on keratinases from various microbes that may effectively corrupt prion, & keratinase is increasingly being seen as a viable green solution for prion sterilisation. In this manner, the overall inquiry into keratinase may be divided into four sections. At the moment, keratinase research is at an all - time high, with papers being written on a wide range of keratinolytic bacteria in the hunt for a viable keratinase manufacturer. However, today's commercial agreements, such as Valkerase, Prionzyme, & Versazyme, are all based solely on KerA. Regardless, the only way to gain commercial size right now is to use keratinase. In order to investigate a large area of keratinase research, detailed surveys have been given that emphasise the good diversity, representation, and usage of keratinases. The first study, conducted by **Onifade et al. in 1998**, focused on keratinase characteristics, keratinolysis components, and biotechnological applications of keratinases for plume feast formation. After a lengthy hiatus, **Gupta R.** published a point - by - point audit in **2006**, which included origins, characteristics, and possible current applications of keratinases. **Brandelli et al., (2008)** evaluated the topic twice, the first time looking at qualities and creating applications, and the second time looking at structure, catalysis, and substrate specificity, generation, and applications. Despite these limitations, Suzuki et colleagues investigated keratinases from thermophilic small - scale living forms (**Suzuki et al., 2006).** Keratinase research is now moving at a breakneck speed, with new catalysts being discovered on a regular basis. **Kulkarni and Jadlav**

conducted a similar investigation in 2014 and were able to identify keratinolytic Breuvedimonosterrae species. Keratinolytic *Bacillus cereus* and *Pseudomonas aeruginosa* were identified in this experiment. *B. cereus* is a Gram - positive, rod - shaped bacterium which forms endospores and is facultatively anaerobic. The existence of the kerA gene might explain the existence of keratinase, a serine protease (**Lin et al., 1995).** Some serine proteases, such as subtilisin's, are identical in sequence and structure across Bacillus species. The same might be said for keratinase, which might open the door to a molecular method to screening microorganisms that degrade keratin. **Jeevana Lakshmi et al., (2013)** identified two native strains of *B. cereus* and *B. subtilis* in 2013. Furthermore, they have pinpointed the ideal circumstances for achieving maximum enzyme synthesis. This adds to the growing body of evidence indicating *B. cereus* is a keratinolytic bacteria. When *Bacillus sp*. is subjected to environmental challenges such as nutrition constraint, it has been found to display distinctive physiological features. When resources are scarce, they evolve physiological features such as the production of proteases, which enable them to use alternate carbon and nitrogen sources. For example, known keratinases are inducible and highly specific for substrates like hair, wool, and feathers (**Kaul and Sumbali, 1997).** These bacteria may have had their keratinase genes activated, allowing them to adapt to substrates like feathers. Isolation of these bacteria might be useful in increasing the nutritional quality of meals containing treated chicken feather wastes. Furthermore, this would open up possibilities for new methods of minimising feather waste while also limiting the formation of harmful microbe pools. According to **Cheng et al., 1995**, Bacillus Pwd - 1 has the maximum keratinase activity in 2 percent feather. The time necessary for Bacillus sp. to achieve maximal enzyme synthesis was assessed, and keratinase output peaked after 96 hours of incubation. Bacillus sp. culture in feather meal medium yielded the highest keratinolytic yield after 24 hours. The bacillus species in this investigation had the highest keratinase activity after 72 hours of development. After 48 hours of development, **Radha and Gunasekeran (2007)** found that the *Bacillus licheniformis* MKU generated the most keratinase. **Lin et al. (1999)** found that *Bacillus licheniformis* strains produced the most keratinase between 48 and 60 hours of development. After 48 hours of development, **Refai et al. (2005)** discovered that *B. pumilus F49* had the highest keratinase activity. However, **Ramnani and Gupta (2004)** found that *Bacillus licheniformis RGI* boosted keratinase activity after 72 hours of development. In raw feather medium, Microbacterium sp. kr10 exhibits maximal keratinase activity after 36 hours, which coincides with the termination of exponential phase (**Thys et al., 2004). Son et al. (2008)** found keratinolytic functions in the scale of 14.6e16.7 U mg1 for *Bacillus pumilus* grown - up in a medium supplemented with children's scalp hair, while **Lal et al. (1999)** found a highest keratinase activity of 4.89 kU ml1 for *Bacillus subtilis S1* grown in a medium supplemented with children's scalp hair. Likewise, utilizing feather as the inducer, **Joshi et al. (2007)** observed maximal keratinase activity of 50 and 30.5 U ml1 for *Bacillus sp. PW - 1* and *Bacillus licheniformis PWD1*. **Nagal and Jain (2010)** found that *B. cereus* cultured on feathers had a maximal keratinase activity of 39.1 U ml1. The maximal activity recorded for *B. cereus LAU 08*, on the other hand, were often greater than those reported for bacterial keratinases. **Suntornsuk (2003)** investigated the factors that influence plume debasement by *Bacillus sp. FK 46*. Same experts recently restricted strain FK - 46 from a soil test. The plume was found to be completely corrupted under the given conditions: 1 percent intact chicken quill as substrate, beginning medium pH of 9, 5 percent bacterial inoculum, 37 °C, and 250 rpm shaking rate. Methanol, glucose, Triton X - 100, and Tween 80 had no effect on the plume decomposition.

5. Conclusion

The keratinases are a category of proteolytic enzymes which are efficient of catalysing the hydrolysis and cleavage of the very stable & fibrous proteins: keratins. Keratinases have several commercial, scientific, and industrial applications, including in cosmetics, biomedicine, renewable energy production, and biological control. The goal of this work was to partly purify, identify, & characterise keratinolytic microorganisms & their enzymes for commercial use.

β - keratin, a fibrous & insoluble structural protein heavily cross connected by disulfide connections, makes up about 90 percent of the protein in feathers. The use of keratinolytic bacteria to degrade feathers is a cost - effective and environmentally favourable option. These keratinolytic organisms create keratinases, which might be used to decompose feather waste, and the digested products might be utilised to make fertilisers, animal feed, or natural gas. These keratinolytic isolates might be a good choice for degrading feather keratin and using it to make animal feed protein.

We identified four separate types of keratinolytic bacteria in this study. The experimental feather - degrading bacteria had a significant keratinolytic action when cultivated in broth comprising native feather, showing that they were obtained from chicken waste. The feathers were completely deteriorated during the harvest. The microorganisms might be useful in biotechnological keratin hydrolysis operations. Segregates were analysed with several biochemicals to complete the characterization of isolated strains. The isolates were identified to be *Pseudomonas aeruginosa, Bacillus cereus, Breuvendimonos terrae* and *Bacillus subtilis.* They were cultivated on feather broth to determine keratinase activity.

Due to the diversity of optimization tactics that may be used to achieve raised enzyme yields, & well - established fermentation technology, participating in a scale - up of keratinase production is a beneficial course of action. This category (which comprises over 90% of the literature & information on keratinases) represents a very important piece of the research and development puzzle, and so deserves to be acknowledged as an important catalyst for both discovery and innovation. It is true that the microbial diversity found via these conventional ways is far greater than what is known with modern technology. Since our current understanding of keratinases has been greatly enhanced by using genomic databases and metagenomic techniques, our current knowledge of these enzymes will be improved even more.

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