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Degradation of Keratinous Waste Products by Keratinolytic Bacteria Isolated from soil

Harison Masih and Sandeep Singh

Department of Microbiology and Fermentation Technology (MBFT), Jacob School of Biotechnology and Bioengineering (JSBB), Sam Higginbottom Institute of Agriculture Technology and Sciences (SHIATS), Naini, Allahabad, Utter Pradesh, India, 211007

Corresponding author: Harison Masih, harisonmasih555@gmail.com, 8005156879

Abstract:

Keratins are the most abundant protein in epithelial cells of vertebrates and represent the major constituents of skin and its appendages such as nail, hair, feather, and wool. World-wide poultry processing plants produce millions of tons of feather as a waste product annually, which consist of approximately 90% keratin feathers. These feathers constitute a sizable waste disposal problem. Several different approaches have been undertaken for disposing these feather wastes. In the present work soil samples were collected from barber shop and chicken shop waste dump of Allahabad. Among the bacterial isolates three strains (S-1, S-2 and S-3) showed evidence of keratin degradation. Strain S-1 and S-2 were identified as *B. subtilis* and strain S-3 was identified as *B. licheniformis*. The keratin degradation was evident by degradation of feathers, sheep wool and hairs used as substrate. Strain S-3 was found to be the best strain for the degradation of keratin waste.

Key words: keratin, *B. subtilis*, *B. licheniformis*, feather degrading bacterium, poultry wastes.

1. Introduction

Keratins are insoluble proteins from feathers, wool, hooves, scales, hair, nails (hard keratins) and stratum corneum (soft keratins). These proteins which belong to the scleroprotein groups are compounds that are extremely resistant to the action of physical, chemical and biological agents. Mechanical stability and high resistance to proteolytic degradation of keration are due to their disulfide bonds, hydrogen bonds, salt linkages and cross linkings **[1].**

The feathers which are hydrolysed by mechanical or chemical treatment can be converted to feed-stuffs, fertilizers, glues and foils. These are also used for the production of amino acids and peptides. Current commercial production of feather meal involutes, treatment at elevated temperature and high pressure. This process in addition to being energy intensive results in the loss of some essential amino acids **[2]**. Because of environmental considerations the use of keratinolytic enzymes in the production of amino acids and peptides is becoming attractive for biotechnological applications. Due to insoluble nature of keratin, it is resistance to enzymatic digestion by plants, animals and many known microbial proteases. Therefore the keratinase producing microorganisms have been described having the ability to degrade feather. They are general species of fungi, actinomyces and bacteria viz., *Doratomyces microsporus, Aspergillus sp, Alternaria radicina, Trichurus spiralis, Stachybotrys atra, Onygena sp, Absidia sp, Rhizomucor sp*. **[3]**, *Streptomyces pactum, S. albs, S. thermoviolaceus, S. fradiae* **[4]**, *S.thermonitrificans* **[5]**, *Flavobacterium pennavorans, Bacillus sp*. **[6]**, *Stenotrophomonas sp*. **[7]**, *Bacillus licheniformis* and *B. pumilus* **[8]** and *Vibrio sp*. **[9]**.

Keratinase producing microorganisms have the important industrial application in fermentation technology. Submerged fermentation of poultry waste by microorganism producing keratinase helps in the conversion of non-soluble keratin (feather) into soluble protein or polypeptide **[10]**.These protein byproduct may be used as animal and livestock feed, and as leather filling agents **[11]**. Keratinase has also emerging application in dehairing process in leather industry instead of sodium sulphides **[12]** and also used as a detergent to remove strains on cloth **[13]**. Valorization of keratin containing wastes like feathers from poultry farms and hair from leather industries may have the potential in development of non polluting processes. The scope of this work is to degrade the poultry feather wastes (insoluble protein) to soluble protein.

Enzymatic dehairing in tanneries has been envisaged as an alternative to sulfides **[14], [15], [16], [17]**. Tanneries are constantly concerned about the obnoxious odor and pollution caused by the extremely toxic sodium sulfide used in the dehairing process step **[18]** Deaths due to this toxic chemical process have even been reported. Worldwide, it is estimated that 315 million bovine leathers are produced per year. Considering a waste treatment cost of $0.30 per m2 of leather produced more than $1 million is spent per day to treat the waste from tanneries around the world. keratinase has the potential to replace sodium sulfide in the dehairing process.

Keratinous waste constitute a troublesome environmental contaminant that is produced in large quantities in commercial poultry processing plants and their utilization is of economic value as well as ecological significance. Feather waste make a serious problem as environmental pollutant and recently in outbreaks of H5N1 virus. Currently, feather waste is utilized on a limited basis as a source of nitrogen for plants or as a dietry protein suppliment for animal feedstuffs, prior to its use for that purpose, feathers were chamically treated to increase the digestibility and reduse the rigidity, this procedure has a disadvantage, in that certain heat sensitive amino acids are degraded. That is why the enzymetic biodegradation may be a better alternative to improve their nutritional value and offers cheap and mild reaction conditions for the production of valuable products.

The present work was directed towards the isolation of keratinolytic bacteria from soil and their utilization for degradation of various keratinous waste products.

2. Materials and Methods

2.1 Place of work

The present study entitled **"Degradation of Keratinous Waste Products by Keratinolytic Bacteria Isolated from soil"** was conducted in the Department of Microbiology and Fermentation Technology**,** Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-University), Allahabad (U.P.).

2.2 Isolation

For the isolation and identification of bacteria, soil samples were collected from different poultry soaps waste dumps. Then 1gm of each sample was dissolved in 9ml of sterile distilled water and this suspension was serially diluted up to 10^{-6}. A 0.1 ml of diluted suspension was spreaded over the solid nutrient agar plates and incubated for 24 hrs at 37 ±1°C the colonies grown were screened for the keratin degrading isolates.

2.3 Screening of Keratin degrading isolates

Skim milk agar was prepared and the above colonies were streaked on milk agar plates for testing the caseinolytic activity of the organism. Isolates were inoculated onto plates and incubated at 37°C for 24 h. Strains producing clearing zones in this medium were selected **[19]**.

2.4 Identification of isolates

The isolates were identified on the basis of cultural, morphological and biochemical characters as given in Bergey's Manual of Systematic Bacteriology **[20].**

2.5 Growth and maintenance of isolates

Bacterial isolates used in present study were grown on nutrient agar slants at 37°C for 24 hrs and maintained on nutrient agar slants at 5°C.

2.6 Preparation of Inoculum

A 100 ml nutrient broth solution was prepared and sterilized at 121°C for 20 min. The medium was inoculated under

aseptic conditions with bacteria. The broth culture was incubated for 14 hrs on a rotary shaker (150 rpm) at 30°C and was used for inoculating the production medium.

2.7 Degradation of different keratinous wastes by the isolated bacteria

For studying the biodegradation of different keratinous materials, the keratinous wastes (chicken feather, sheep wool, human hair) were fragmented into pieces with about 1 cm long and added to the fermentation media as a sole source of carbon and nitrogen. These sources were added separately to the fermentation media at 1% w/v. The percent of keratinous waste degradation was determined.

2.8 Determination of degree of degradation (DD)

The residual feather was washed, dried and scaled to calculate DD (fig.1) by using following equation-**[21]**

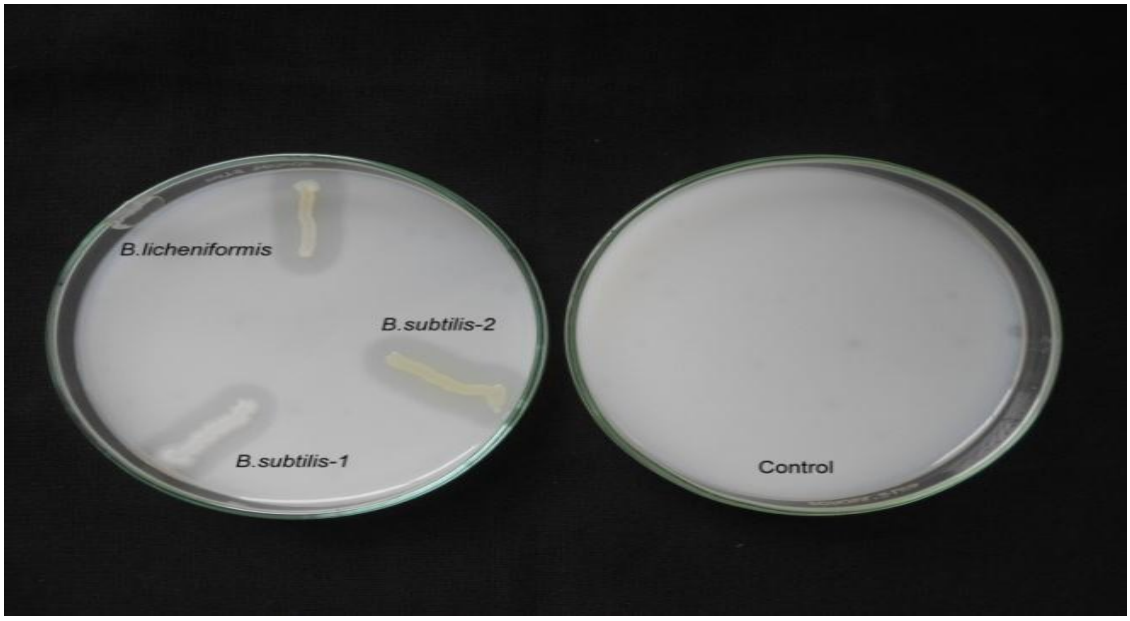
DD (%) = (TF-RF) ×100/TF

Where, TF is the total feather and RF is the residual feather

3. Results and discussion

3.1 Isolation

The purpose of this study was to isolate bacterial strains which degrade keratinous waste products. Soil samples were collected from barber shop and poultry soaps waste dumps. Three isolated strains were able to form clear zones on skimmed milk agar plates (photograph-1) since casein was hydrolyzed by the extracellular proteolytic enzyme secreted by the isolated strains. All the three strains grew well on feather meal agar plates.



Photograph 1. Screening of keratin degrading isolates on skimmed milk agar

3.2 Identification of isolates

In the present study isolated strain S-1 and S-2 were identified as *Bacillus subtilis* and S-3 was identified as *Bacillus licheniformis* on the basis of cultural, morphological and biochemical characters (Table.1).

Previous studies conducted by **[22], [23]** for the isolation of keratinolytic organism from soil and other natural sources, reports *Bacillus* sp. as a potential keratinolytic organism and its possible use in field studies for biodegradation of feather.

3.3 Degradation of different keratinous wastes by the isolated bacteria

Biodegradation of three different keratinous materials (chicken feather, sheep wool, human hair) were studied (Photograph-2). When feathers were used as keratinous waste, strain-3 was showing the best result followed by strain-1 and strain-2 respectively (Table.2, Figure.1). Similarly when human hairs were used as keratinous waste, strain-3 was showing the best results followed by strain-1 and strain-2 respectively (Table.3,

Figure.1). At the same time when seep wool was used as keratinous waste, strain-3 was showing the best result followed by strain-1 and strain-2 respectively (Table.4, Figure.1). According to the results of biodegradation of different substrates, strain-3 (*B.licheniformis*) was showing the promising results.

Similar results were observed by **[24]** while studying feather and hair degradation by some potential bacterial isolates. Similarly **[25], [26], [27]** reported the degradation of feather powder, guinea pig hair, human hair and nails, cow horn and hooves by the species of *Bacillus* for the production of keratinolytic enzyme.

Table.1 Morphological and biochemical characterization of isolates for identification

Details of experiment	Observations		
	S-1	S-2	S-3
Shape of bacteria	Rods	Rods	Rods
Endospore formation	$+^{ve}$	$+^{ve}$	$+^{ve}$
Motility	$+^{ve}$	$+^{ve}$	$+^{ve}$
Gram character	$+^{ve}$	$+^{ve}$	$+^{ve}$
Colony characteristics			
Growth	Rapid	Rapid	Rapid
Shape	Circular flat	Circular flat	round
Margin	undulate	undulate	undulate
Color	Cream white	Cream white	white
Opacity	opaque	Opaque	opaque
Biochemical characteristics			
Indole production	$-^{ve}$	$-^{ve}$	$-^{ve}$
Methyl red reaction	$-^{ve}$	$-^{ve}$	$+^{ve}$
Voges-proskaure reaction	$-^{ve}$	$+^{ve}$	$+^{ve}$
Citrate utilization	$-^{ve}$	$-^{ve}$	$+^{ve}$
Gelatinase	$+^{ve}$	$+^{ve}$	$+^{ve}$
Nitrate reduction	$+^{ve}$	$+^{ve}$	$+^{ve}$
Starch hydrolysis	$+^{ve}$	$+^{ve}$	$+^{ve}$
Caesinase	$+^{ve}$	$+^{ve}$	$+^{ve}$
Catalase	$+^{ve}$	$+^{ve}$	$+^{ve}$
H_2S production	$-^{ve}$	$-^{ve}$	$-^{ve}$
Arginine	$-^{ve}$	$-^{ve}$	$-^{ve}$
Esculin hydrolysis	$+^{ve}$	$-^{ve}$	$+^{ve}$
Urease	$-^{ve}$	$-^{ve}$	$-^{ve}$
Carbohydrate fermentation			
Glucose	A^+/G^-	A^+/G^-	A^+/G^+
Lactose	A^-/G^-	A^-/G^-	A^+/G^-
Mannitol	A^+/G^-	A^+/G^-	A^+/G^+
Fructose	A^+/G^+	A^+/G^+	A^+/G^+
Sucrose	A^+/G^-	A^+/G^-	A^+/G^+
Maltose	A^+/G^+	A^-/G^-	A^+/G^+
Sorbitol	A^+/G^+	A^+/G^+	A^+/G^+
Xylose	A^+/G^-	A^+/G^-	A^+/G^+
Arabinose	A^+/G^-	A^+/G^-	A^-/G^-

Table2. Bacterial strains showing feather degradation

BACTERIA	INITIAL WEIGHT [FEATHERS]	FINAL WEIGHT [AFTER 4 DAYS]	DEGRADATION (%)
STRAIN- 1	1gm	0.65gm	35
STRAIN- 2	1gm	0.70gm	30
STRAIN- 3	1gm	0.30gm	70

Table 3.Bacterial strains showing hair degradation

BACTERIA	INITIAL WEIGHT [HAIRS]	FINAL WEIGHT [AFTER 4 DAYS]	DEGRADATION (%)
STRAIN- 1	1gm	0.76gm	24
STRAIN- 2	1gm	0.83gm	17
STRAIN- 3	1gm	0.50gm	50

Table 4.Bacterial strains showing sheep wool degradation

BACTERIA	INITIAL WEIGHT [SHEEP WOOL]	FINAL WEIGHT [AFTER 4 DAYS]	DEGRADATION (%)
STRAIN- 1	1gm	0.72gm	28
STRAIN- 2	1gm	0.89gm	11
STRAIN- 3	1gm	0.47gm	53

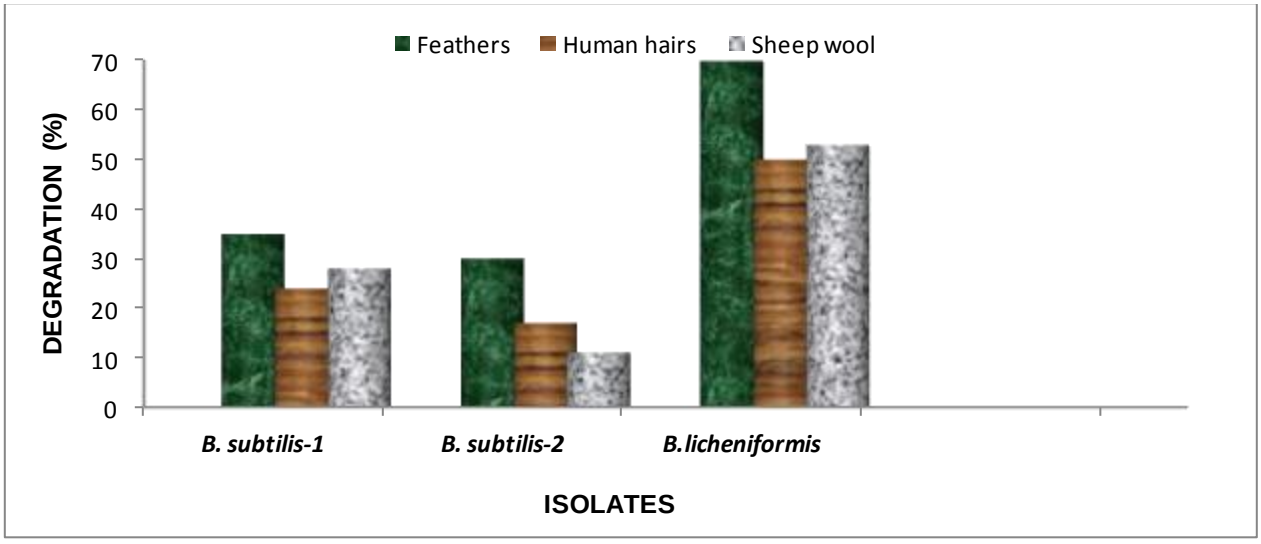
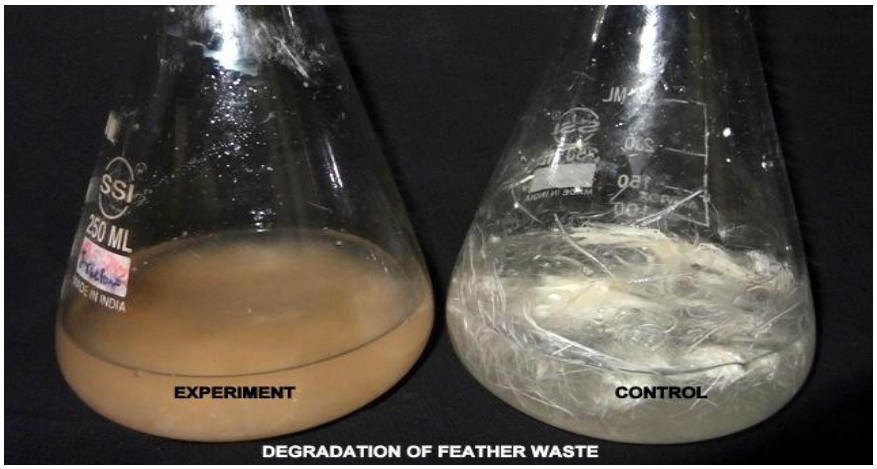
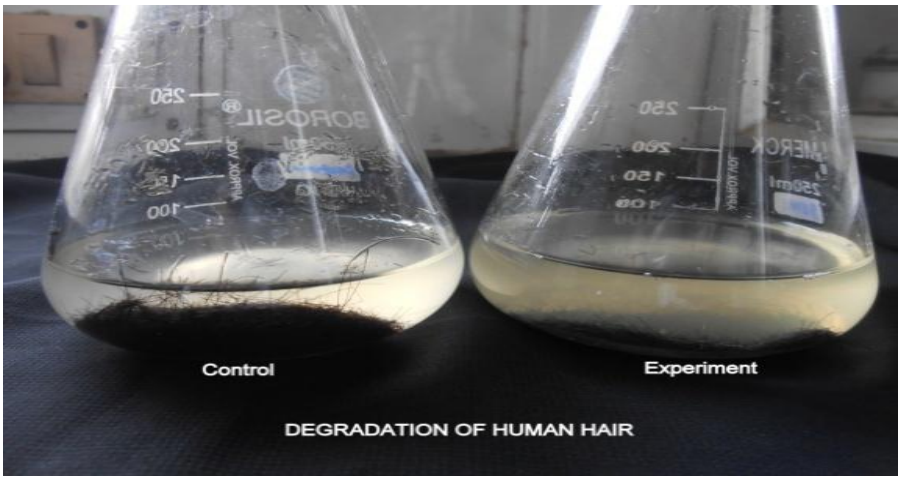


Fig.1 Degree of degradation of keratinous waste by bacterial isolates

(a)



(b)



(c)

Photograph-2. (a) Degradation of sheep wool by *B. licheniformis*
(b) Degradation of feather waste by *B. licheniformis*
(c) Degradation of human hair by B. licheniformis

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Author Profile

Harison Masih received his PhD. Degree from……. And currently working in Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad U.P., this project was funded by the Sam Higginbottom Institute of Agriculture Technology and Sciences, Allahabad U.P.