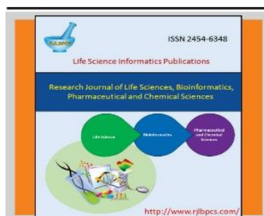

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MICROBIAL DEGRADATION OF POULTRY FEATHER BIOMASS BY KLEBSIELLA SP. BTSUK ISOLATED FROM POULTRY WASTE DISPOSAL SITE

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ABSTRACT: A novel feather degrading microorganism was isolated from poultry waste disposal site, Kolhapur, India degraded native poultry feather by keratinolytic activity. Bacterium was identified as *Klebsiella* sp. BTSUK using 16S rRNA gene sequence analysis. *Klebsiella* sp. BTSUK showed prompt hydrolysis of native feathers within 60 h and produced the highest level of keratinase activity (77.9 U ml^{-1}). Keratinous materials like silk, human hair, wool and chicken feathers were tested for keratin degrading ability of the bacterium. However, *Klebsiella* sp. BTSUK showed more specificity towards chicken feathers degradation (78.65%) with maximum solubilized protein concentration at 60 h (3.33 mg ml^{-1}) and amino acid content at 72 h (3.82 mg ml^{-1}). This keratinolytic ability of *Klebsiella* sp. BTSUK would be beneficial to utilize in environmental-friendly processes such as bioconversion of feathers biomass, representing an alternative way of waste management that could lead to the production of value-added products such as amino acids and protein hydrolysates as bioenhancer in agriculture; keratinase enzyme for ecofriendly leather processing.

KEYWORDS: Poultry feather, Keratin, *Klebsiella* sp. BTSUK, Amino acids, Protein

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1.INTRODUCTION

The growing global population is associated with increasing demand for food, but beside there was a need to reduce the gradual deterioration of environment. Enormous numbers of waste are generated through various food industries among which meat industry produces a waste biomass consisting noticeable quantities of organic residues such as feather, bones, blood etc. Chicken feather is the major by-product generated in millions of tones from the commercial poultry processing which is accumulating at higher rate (Zhao et al. 2012).Common methods for disposing these wastes are burning, incineration, which has adverse effect on environment. The disposal of chicken carcasses presents significant environmental, biological, and financial problems for the poultry industry. This waste contains a large amount of the useful proteins such as keratin and collagen, which contributes about 8-10% to the chickens live body weight, while dried feather contains 85–99% proteins (Papadopoulos, 1985; Agrahari and Wadhwa, 2010). Bioconversion of keratinous materials has been proposed as a waste treatment alternative to the disposal of feather keratin, to produce feather meal through thermal processing, resulting in a low nutritional value product (Wang and Parsons, 1997).Bacterial keratinases are of particular interest because of their action on insoluble keratin substrates, and generally on a broad range of protein substrates (Lin et al. 1999). The chain is tightly packed in the α-helix (α-keratin) or β-sheet (β-keratin) into a supercoiled polypeptide chain (Parry and North, 1998), resulting in mechanical stability and resistance to common proteolytic enzymes such as pepsin, trypsin, and papain. In addition, cross-linking of protein chains by cysteine bridges confers high mechanical stability and resistance to proteolytic degradation of keratins. Nevertheless, feathers do not accumulate in nature, since structural keratin can be degraded by special microbes (Onifade et al. 1998). Keratinases are the enzymes produced by microorganisms to hydrolyze the keratin waste and utilize it as nitrogen, carbon and energy source for growth and development. Keratinase enzyme has application in ecofriendly leather processing for dehairing of leather (Gurav and Jadhav, 2012). Similarly, feather hydrolysates produced by bacterial keratinases have been used as additives for animal feed (Williams et al. 1991). In addition, keratin hydrolysates have potential use as organic fertilizers, production of edible films and rare amino acids like leucine, valine, methionine, glycine, cysteine, serine, phenylalanine, tryptophan, lysine, and tyrosine (Gurav and Jadhav, 2012). Feather hydrolysate rich in protein and amino acids was used as a biofertilizer in the banana cultivation which enhanced the vegetative growth, nutrient uptake in plants, abiotic and

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biotic stresses tolerance resulting in increased crop yield, and also increased the fruit nutritional quality (Gurav and Jadhav, 2013). In the present study, a feather degrading bacterial strain was isolated from poultry waste disposal site, Kolhapur, India. The *Klebsiella* sp. BTSUK was explored to hydrolyze the native poultry feather. Analysis of enzyme activity and products of feather degradation was executed.

2.MATERIALS AND METHODS

Chemicals and keratinous substrates

Standard keratin, bovine serum albumin, standard amino acids, di-sodium hydrogen orthophosphate (Na_2HPO_4), potassium di-hydrogen orthophosphate (KH_2PO_4), ammonium chloride (NH_4Cl), sodium chloride (NaCl), magnesium sulphate ($MgSO_4$), tri-chloro acetic acid (TCA), folin-phenol reagent were purchased from Himedia Pvt. Ltd., Mumbai, India. All the chemicals used were of analytical grade and highest purity. Chickens were first slaughter and the feathers were scalded from their body in an automated machine at chicken slaughtering center. This obtained feather biomass was free of body parts, washed under tap water in the mesh tray. Chicken feathers were then dried under sun light and stored for the further experiments. Other keratinous substrates such as human hair (from barber shop), sheep wool and silk cocoons were collected from local market, Kolhapur, India.

Isolation of keratin utilizing microorganism

The strains BTSUK was first screened for proteolytic potential of milk agar plate followed by cultivating then on keratin agar plates containing basal salt medium (BSM) (g l^{-1}) KH_2PO_4 (4), Na_2HPO_4 (6), NH_4Cl (4), NaCl (5) and $MgSO_4$ (0.1) and keratin powder (0.1%) as a sole source of carbon, nitrogen and energy and cultures were maintained on the same medium (Gurav and Jadhav, 2012).

Molecular identification of bacteria

The identification of the strain was performed by 16S rRNA gene sequence analysis at Chromous Biotech Pvt. Ltd., Bangalore, India. The obtained nucleotide sequence was submitted to GenBank under accession number JX477370. This bacterium was aligned with the non-redundant database present in NCBI using BLASTn program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the homologous sequences of species obtained were used for phylogenic analysis. The phylogenetic tree was constructed with $MEGA_4$ software (AZ, USA). The evolutionary history was inferred using the

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neighbor-joining method (Saitou et al. 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein et al. 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+noncoding. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option).

Feather degradation

The feather degradation was carried out in sterilized liquid BSM containing native chicken feathers (1% w/v) inoculated with *Klebsiella* sp BTSUK and incubated on rotary (140 rpm) at 37 °C. Aliquots were withdrawn aseptically from broth at regular time interval to investigate keratinase activity (Cai et al. 2008).

Keratinase enzyme, soluble protein and amino acid analysis

The reaction mixture consisted of 1.0 ml crude extracellular enzyme diluted with Tris-HCl buffer (pH 8.5) and 1.0 ml of 0.1% (w/v) standard keratin (dissolved in buffer) as a substrate. This mixture was incubated at 37 °C for 10 min and the reaction was stopped by adding 2.0 ml (0.4 mol l^{-1}) trichloroacetic acid (TCA) and the supernatant after centrifugation was used to determine the enzyme activity at 280 nm (Gurav and Jadhav, 2012). The soluble protein and amino acid quantification was done using the supernatant from feather degradation medium by Lowry et al. (1951) and Moore et al. (1957) methods.

Keratinase activity and percentage degradation on different keratin substrates

The *Klebsiella* sp. BTSUK was tested to degrade keratin substrates like chicken feathers, human hair, wool and silk. The BSM was supplemented with different keratinous substrates (1.0% w/v) and incubated at 37°C on orbital shaker (140 rpm) and analyzed for keratinases activity.

The percentage degradation of feathers, hair, silk and wool (1.0 % w/v) was determined by considering the dry weight of substrate remaining after degradation. The results were expressed as percentage of the initial weight (considered as 100%) and calculated by comparison between the dry weight of residual substrate before and after hydrolysis (Cortezi et al, 2008).

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3. RESULTS AND DISCUSSION

Isolation And Phylogenetic Analysis Of Bacterial Isolate

The isolated bacterium exhibited strong proteotytic activity with a zone diameter 3 mm on milk agar plate. Growth was also observed my keratin agar plates demonstrated the keratinolytic ability of the bacterium. The identification of this bacterial strain was done by 16S rRNA gene sequence analysis. The obtained nucleotide sequence was submitted to GenBank under accession number JX477370 and strain was named as *Klebsiella* sp. BTSUK. Phylogenetic position of *Klebsiella* sp. BTSUK in relation to the earlier data in GenBank database is as illustrated in figure 1. The branching pattern was generated by the neighbor-joining method. The number of each branch indicates the bootstrap values while the digits adjacent to nodes are the statistical frequencies of the indicated species.

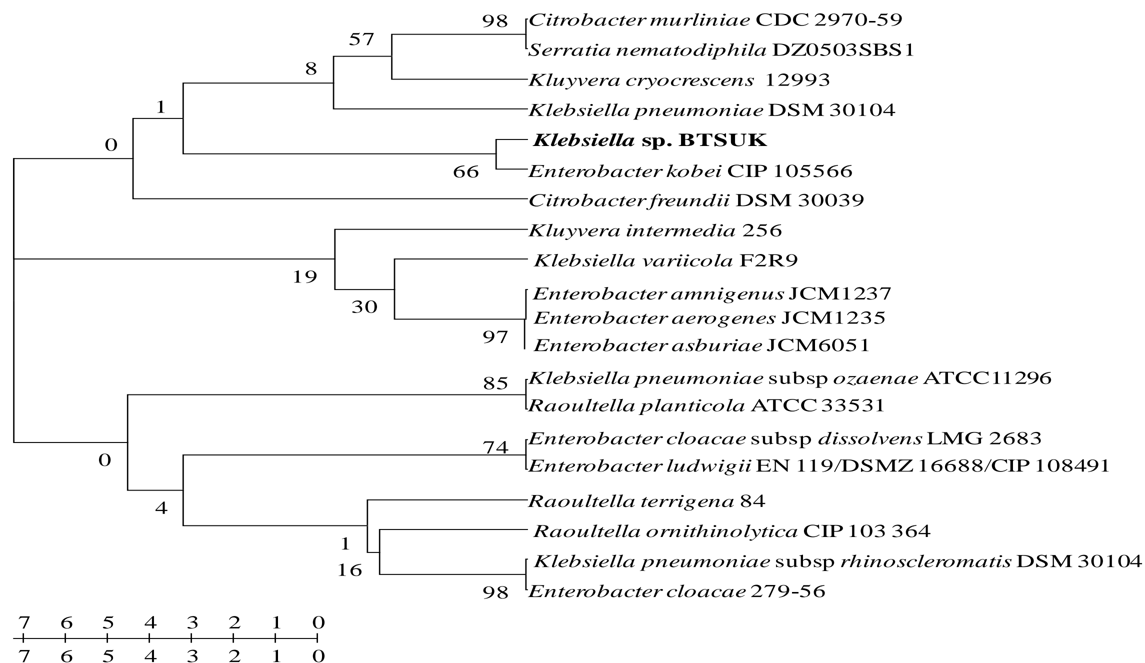

Figure 1: Phylogenetic position of sp. BTSUK within the genus *Klebsiella* and allied bacteria. The branching pattern was generated by neighbor-joining method. The number of each branch indicates the bootstrap values.

Biodegradation of feathers by *Klebsiella* sp. BTSUK

The *Klebsiella* sp. BTSUK degraded native chicken feathers and utilized it as a source of carbon, nitrogen, sulphur and energy for its growth (Figure 2). The keratinase activity was assessed after 12 h was 17.6 U ml^{-1} and reached its peak level at 60 h which was 77.9 U ml^{-1} and declined with further incubation (Figure 3). Soluble protein concentration in the medium increased with time as feather

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degradation proceeded and attended highest concentration at 60 h i.e. 3.33±0.001 mg ml^{-1}. Similarly, free amino acids were evaluated which was parallel to the soluble protein content; the increase in the amino acid content was observed till 72 h was 3.82±0.003 mg ml^{-1} this may be due to peptides continued to degrade and were further transformed into amino acids (Figure 4). Similarly, rise in the pH of the feather degradation medium was observed from 7.2 to 8.4. The alkalization of the culture medium was an important factor connected with the process of deamination of simpler proteins which release the ammonia in the surrounding medium (Kunert, 1976).

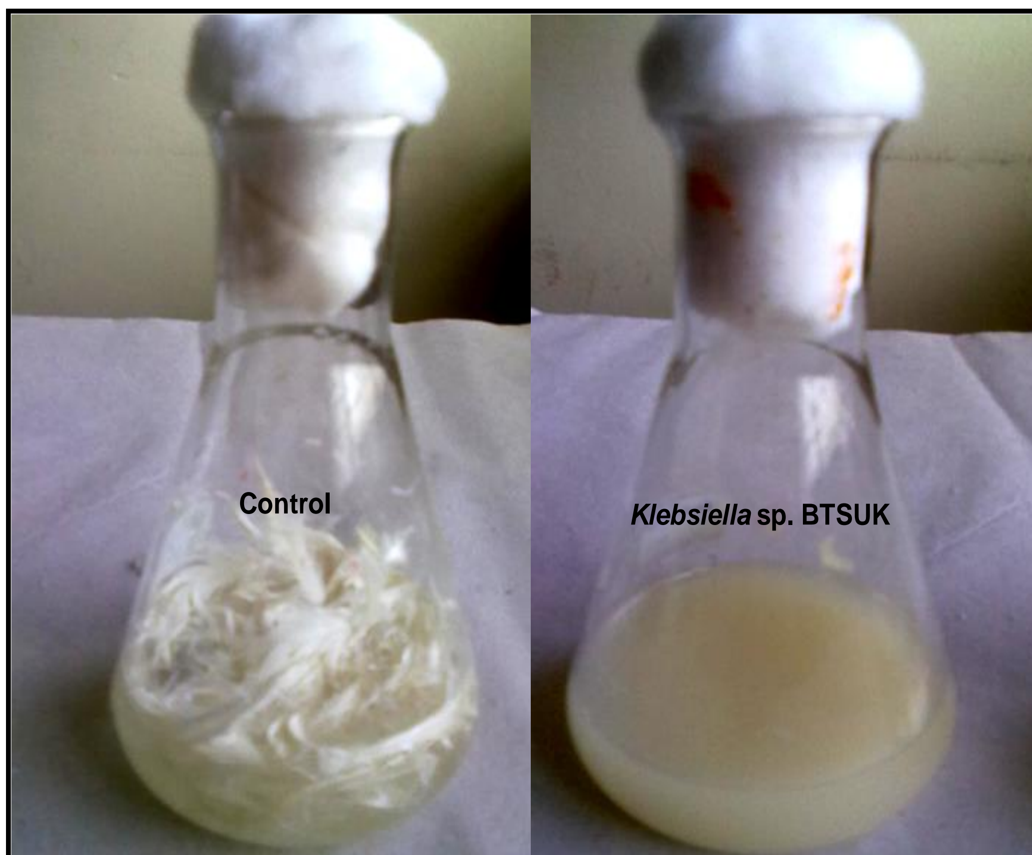

Figure 2: Enzymatic dehairing of chicken feather using *Klebsiella* sp. BTSUK; 'Control' flask consists of feather incubated without bacterium and '*Klebsiella* sp. BTSUK' flask inoculated with bacterium showed complete degradation of native feathers within 60 h.

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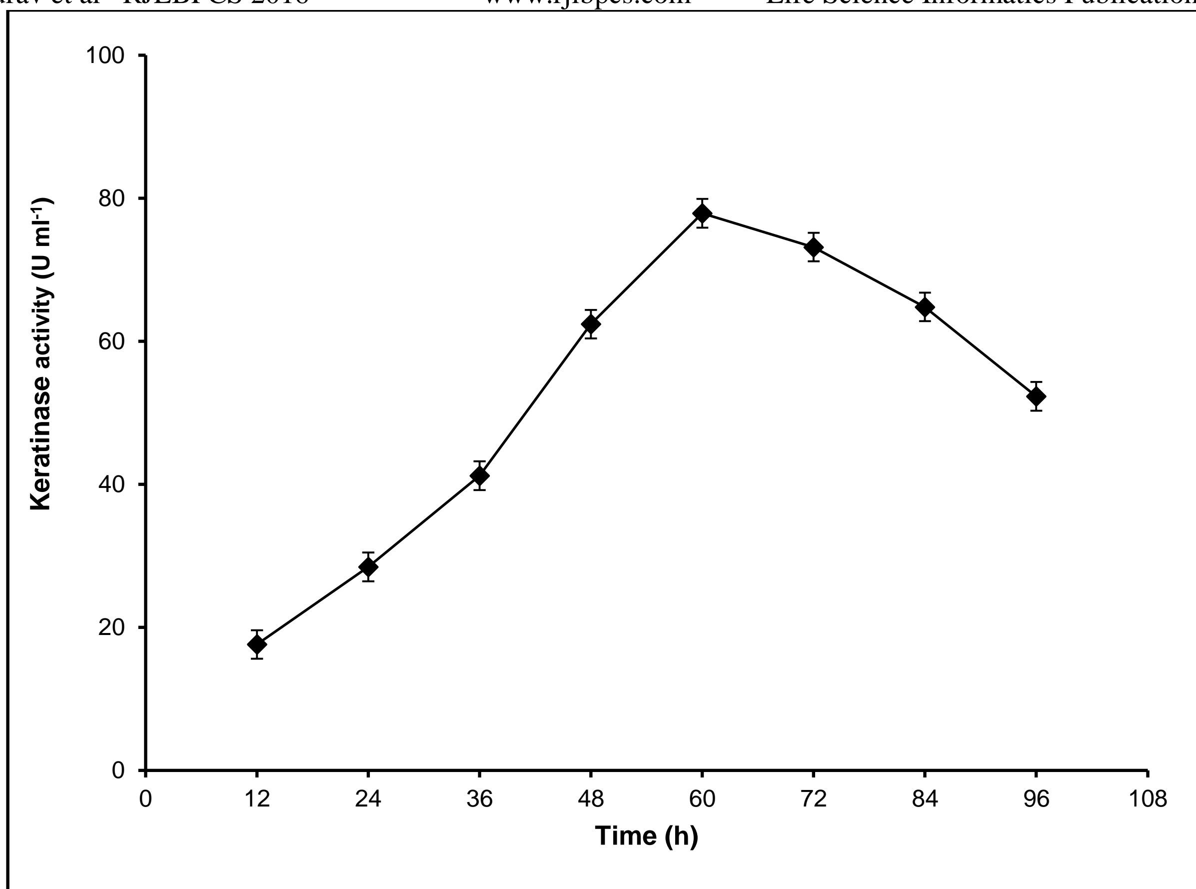

Figure 3: Keratinase activity of *Klebsiella* sp. BTSUK at different time intervals.

This study demonstrated the role of *Klebsiella* sp. BTSUK in degradation of the feather has been reported for the first time. This bacterium secreted the keratinase enzyme and hydrolyzed the native chicken feathers within 60 h without any pretreatment by chemical or mechanical means which helps to reduce the cost of waste processing and production of value added products. The microbial processed keratinous wastes has represented an attractive alternative in the bioconversion waste into animal feed having same nutritional quality as soybean meal currently used as animal feed (Williams et al. 1991). Similarly, the keratin waste can serve as raw material in the production of biocompatible proteinous materials which are environmentally friendly and can be applied in textile, building materials, films, microcapsules, sponges, bioplastics, gels, edible materials, coatings or fibres, compostable packaging and in the production of rare amino acids like serine, cysteine, proline etc. (Tanabe et al. 2004).

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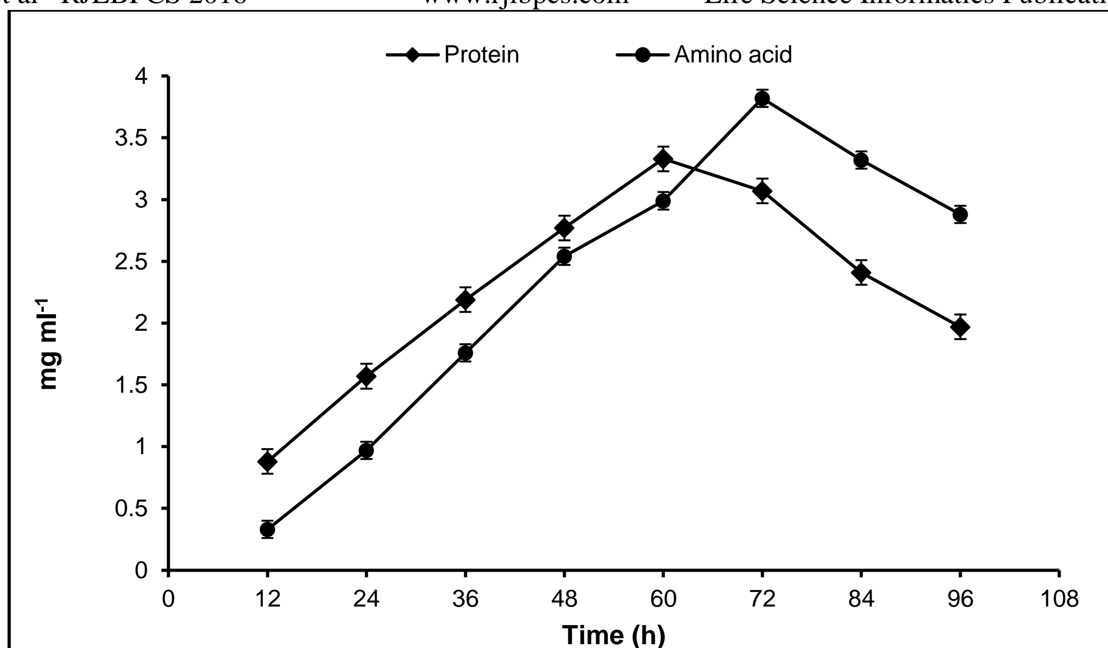

Figure 4: Concentration of soluble protein and free amino acid in feather degradation medium at different time.

Hydrolysis of different keratins

The *Klebsiella* sp. BTSUK was tested for its capability to degrade various keratinous substrates like chicken feather, human hair, sheep wool and silk with respect to their percent degradation and corresponding keratinase activity. This bacterium was most attracted by native chicken feathers and degraded 78.65%, followed by wool 42.14%, hair 31.12% and silk 19.50% (Table 1). Also the keratinase enzyme activity on the different keratin substrate was evaluated which was higher during feather hydrolysis as shown in table 1. Previously, Akhatar and Edwards, (1997) found that cystine content in feather keratin was lower than the wool and hair keratin. This was helpful to hydrolyse the feather waste most efficiently. The enzymatic hydrolysis of chicken feathers under mild reaction conditions will be more eco-friendly and cost effective option for the waste treatment.

Several keratinases have been previously isolated from a diverse bacteria, but the mostly studied genus is *Bacillus* and Actinomycetes, which were isolated from various sources like bird feathers, feather waste processed by fermentation, composting etc. (Kim et al. 2001).

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	Keratinase activity ($U\ ml^{-1}$)	Percentage degradation (%)
Feather	76.0 ± 0.212	78.65 ± 0.078
Wool	34.8 ± 0.131	42.14 ± 0.091
Hair	26.5 ± 0.070	31.12 ± 0.043
Silk	16.3 ± 0.092	19.50 ± 0.010

Table 1: Hydrolysis of different keratins and keratinase activity.

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