ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 9 (5): 482-489, 2010
ISSN 1682-8356

Degradation of Chicken Feather a Poultry Waste Product by Keratinolytic Bacteria Isolated from Dumping Site at Ghazipur Poultry Processing Plant

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Abstract: Feathers are byproduct waste of poultry processing plant and produced in large amount. A small percentage of feather waste is steamed, chemically treated, ground, to form feather meal a dietary protein supplement for animals. Alternatively, keratin can be biodegraded by some Keratinolytic bacteria and in this study Keratinase producing bacteria and their Keratinolytic enzyme production was investigated. Soil sample was collected from Ghazipur poultry waste site, Ghaziabad, India, a feather dumping site. Soil sample were inoculated in three enrichment media and colonies producing clear zone in feather meal agar were selected and identified as *B. megaterium* SN1, *B. thuringenesis* SN2, *B. Pumilis* SN3 were able to degrade chicken and pigeon feathers. They produced extracellularly Keratinolytic enzymes in enrichment media with 10% Feather meal powder. We report that Keratinase and Protease activity were detected in the culture supernatant and optimal medium for extracellular production of Keratinase and Protease is feather meal media 2 at pH (7.5) and temperature (30°C). There was complete degradation of feathers in 120 h of incubation and 0-80% Ammonium sulphate fraction showed 1.5 fold purification for Keratinase and 1.3 fold purification for Protease over the crude enzyme preparation. The keratinous waste can be biologically degraded by enzymes or the microbe itself to form useful products.

Key words: Bacillus, feather, keratinolytic enzymes

INTRODUCTION

Worldwide 24 billion chickens are killed annually and around 8.5 billion tonnes of poultry feather are produced. According to a recent report in leading news paper India's contribution alone is 350 million tonnes. The poultry feathers are dumped, used for land filling, incinerated or buried, which involves problems in storage, handling, emissions control and ash disposal. Discarded feather also causes various human ailments including chlorosis, mycoplasmosis and fowl cholera (Williams *et al*., 1991).

Feather is pure keratin protein and is insoluble and hard to degrade due to highly rigid structure rendered by extensive disulphide bond and cross-linkages. The keratin chain is insoluble, high stable structure tightly packed in the α-helix (α-Keratin) and β-sheets (β-keratin) into super coiled polypeptide chain (Parry and North, 1998). 90% of the feather contain β-keratin by mass (Onifade *et al*., 1998) and β-keratin are extensively cross linked. Cross-linking of protein chains by cysteine bridges confers high mechanical stability and resistance to proteolytic degradation by pepsin, trypsin and papain. The disulphide bonds of β-keratin can be reduced by the enzyme disulphide reductase (Yamamura *et al*., 2002) followed by proteolyitc keratinases (Gupta and Ramnani, 2006). Feather can be utilized so that it can be used as animal feed, this can prevent accumulation of feather in the environment and decrease the development of pathogenic strains. Biotechnological processing of feathers for the production of feather meal, instead of chemical processing is preferred as it preserves the essential amino acids (Methionine, Lysine, Histidine) (Riffel *et al*., 2003).

Innovative solution for waste disposal along with biotechnological alternative for recycling of such wastes is of utmost importance. Structural keratin can be degraded by some proteolytic micro-organisms as reported by (Onifade *et al*., 1998). Keratinase are specific protease that degrade keratin specifically. It is produced by Saprophytic and Dermatophytic Fungi and some *Bacillus* species. Feather degrading bacteria are physiologically diverse and approximately 99% of Bacterial species are unculturable because of their ability to enter non culturable state or because no culture methods have been established (Amann *et al*., 1995). A number of keratinolytic microorganisms have been reported, including some species of fungi such as *Microsporum* (Essien *et al*., 2009), *Trichophyton* (Anbu *et al*., 2008) and from the bacteria *Bacillus* (Cai and Zheng, 2009; Macedo *et al*., 2005; Pillai and Archana, 2008) and *Streptomyces* (Syed *et al*., 2009; Szabo *et al*., 2000; Tatineni *et al*., 2008) and actinomycetes (Bockle *et al*., 1995; Young and Smith, 1975). Increase in keratinolytic activity is also found to be associated with

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thermophilic organisms, which require high energy inputs to achieve maximum growth and the decomposition of keratin wastes (Friedrich and Antranikian, 2002).

Till date most of purified keratinizes known cannot completely solubilize native keratin (Ignatova *et al*., 1999; Ramnani *et al*., 2005), their exact nature and uniqueness for keratinolysis is still not clear There is always a requirement of isolation of enzymes from new sources to meet the industrial and environmental demand.

Keratinolytic enzymes have found important utilities in biotechnological processes involving keratin-containing wastes from poultry and leather industries, through the development of non-polluting processes. After hydrolysis, the feathers can be converted to feedstuffs, fertilizers, glues, films and as the source of rare amino acids, such as serine, cysteine and proline (Gupta and Ramnani, 2006; Cai and Zheng, 2008; Cao *et al*., 2009). In this study we report the isolation of three mesophilic bacteria that produce Keratinolytic enzymes. which can efficiently degrade chicken and pigeon feather within 120 hrs of incubation. Earlier studies from our lab involving screening of micro-organism from same soil sample of dumping site of Gazipur poultry processing plant, we have reported isolation of *Pseudomonas thermaerum* GW1, GenBank accession GU95151, this bacteria showed proteolytic activity but not keratinolytic activity (Gaur *et al*., 2010).

MATERIALS AND METHODS

Isolation and screening of keratinase producing bacteria: Soil was collected from a regular feather dumping site of Ghazipur poultry processing plant, Ghaziabad, India in sterilized sampling bags. The samples were brought in winter to the laboratory and processed for analysis on the same day. Soil samples were suspended in Peptone broth and kept for growth at 30°C for 3 days. This suspension was reinoculated in three media, Horikoshi media, Feather meal media 1 and Feather meal media 2 at 30°C at 160 rpm for 7 days. They were used for keratinase production. They contained the following constituent:

I Horikoshi media (g/l): Soluble starch, 5; Peptone, 5; Glucose, 5; K_2HPO_4, 1; $MgSO_4$ $7H_2O$, 0.2; Na_2CO_3, 1; Yeast extract, 5 and Feathers, 10; pH 7.5.

II The Feather meal media 1 (g/l): NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4 and Feather, 10; pH 7.5.

III The Feather meal media 2 (g/l): NH_4Cl, 0.5; NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl_2.6H_2O$, 0.1; Yeast extract, 0.1 and Feather, 10; pH 7.5.

The flask was incubated at temperature of 30°C on a rotary shaker at 160 rpm for 7 days. Feather degradation in culture broth was confirmed visually.

The culture broth in which feather degradation was confirmed was screened for keratinolytic activity. Feather meal agar that composed of (g/l): NH_4Cl, 0.5; NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl_2.6H_2O$, 0.1; Yeast extract, 0.1 and Feather meal powder, 10, agar powder, 20 and pH was maintained at 7.5 at 30°C for 72 h, Strain which exhibited the largest clearing zones were selected, identified and grown in cultivation media for enzyme production.

Feather meal powder preparation: Poultry feathers was washed extensively, boiled at 30-40 psi for 2-3h. Dried in hot air oven for 4 h at 50°C. The dried feathers were pulverized and the powder was used as feather meal.

Morphological studies of isolated bacterial strains: Bacterial identification was conducted on morphological, physiological and biochemical tests. Results were compared with *Bergey's Manual of Determinative Bacteriology*, 8th edition (Buchanan and Gibbons, 1974). Genus Bacillus: Agriculture Handbook No. 427 (Gordon *et al*., 1973). The strain were also identified by chromogenic method on the bacillus differential agar from Himedia, India, M1651 recommended for rapid identification of *Bacillus* species from a mixed culture. The medium contains peptic digest of animal tissues and meat extract, which provide nitrogenous compounds. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by the pH indicator phenol red. Mannitol fermenting organisms like *B. megateruim* yield yellow colored colonies, *B. thuringiensis* will grow as blue colonies and *B. pumilis* will also grow as green colonies on this medium. Results are summarized in Table 1. Growth determination of bacteria was done taking absorbance at 600 nm of bacterial growth media (Fig. 4) at regular intervals.

Growth condition for Protease and Keratinase production: Seed culture of the three isolated strains were prepared in 500 ml Erlenmeyer conical flask containing 100 ml of the culture media that was maintained at 30°C at 160 rpm and washed feather 10% in cultivation media. After five days of incubation, the crude culture broth was centrifuged (10,000g, 4°C, 30 min) and cell free supernatant was subjected to 0-80% ammonium sulphate precipitation. After chilling at 4°C for 1 h, the resulting precipitate was collected by centrifugation (10,000 g, 4°C, 30 min) and dissolved in a minimal volume of Tris-Cl buffer 10 mM (pH 8.0) and dialyzed overnight against 4 liters Tris-Cl buffer 10 mm (pH 8.0). The dialysed protein fraction was checked for protease and keratinase activity by the modified method of Tsuchida *et al*. (1986) and Cheng *et al*. (1995) respectively. Standard strain of *Bacillus. licheniformis* (MTCC 1483) was also studied for comparative purposes.

Table 1a: Results of morphological, physiological, cultural, biochemical characteristic of three isolated bacterial strain SN1, SN2, SN3 were coducted. Collectively these characteristics indicated that the isolates were of genus *Bacillus*

	Observations		
Details of experiment	*B. megaterium* SN1	*B. thuringenesis* SN2	*B. Pumilis* SN3
Shape of Bacteria	Rod	Short rod	Rod
Endospore formation	+	+	+
Motility	Motile	Highly motile	Motile
Gram character	+	+	+
Anaerobic growth	–	–	–
Colony characteristics			
Growth	Rapid	Rapid	Rapid
Shape	Circular	Irregular	Circular
Surface	Smooth shiny	Smooth	Smooth shiny
Margin	Entire	Entire	Entire
Color	Cream	White	Cream
Elevation	Convex	Flat	Convex
Consistency	Buttery	Viscous	Buttery
Opacity	Opaque	Opaque	Opaque
Biochemical characteristics			
Glucose	-/-	A/-	-/-
Lactose	-/-	A/-	-/-
Mannitol	-/-	A/-	-/-
Indole production	–	–	–
Methyl red reaction	–	+	+
Voges-proskaure reaction	–	–	–
Citrate utilization	–	–	–
Catalase	+	+	+
Gelatinase	–	+	+
Caesinase	+	+	+
Amylase	–	–	–
Cellulase	–	–	–
Deaminase	–	–	–

Symbol: +: Positive; -: Negative; A/- :Acid/No gas; -/- :No acid/No gas

Table 1b: Table depicts that growth on Triple sugar iron (TSI) agar. *B. megaterium* SN1, *B. Pumilis* SN3 showed alkaline reaction on slant and acidic reaction on butt. *B. thuringenesis* SN2 showed acidic reaction both on slant and butt

Bacterial Isolates	Color and reaction of slant	Color and reaction of butt	Gas production	Hydrogen sulphide production
B. megaterium SN1	Red, Alkaline	Yellow, Acidic	–	–
B. thuringenesis SN2	Yellow, Acidic	Yellow, Acidic	–	–
B. Pumilis SN3	Red, Alkaline	Yellow, Acidic	–	–

Protein concentration: Protein concentration was determined by the method of Bradford (1976) with bovine serum albumin as a standard.

Hydrolysis of protein substrates: Protease activity with various protein substrates including keratin, casein, gelatin and bovine serum albumin (2 mg/ml) was assayed by mixing 100 µl of the enzyme and 900 µl of assay buffer containing the protein substrates. After incubation at 50°C for 20 min, Reaction was terminated by the addition of an equal volume of 10% chilled trichloroacetic acid then the reaction mixture was allowed to stand in ice for 15 min to precipitate the insoluble proteins. The undigested proteins were removed by filtration or centrifugation at 10,000 rpm for 5 min and amino acid released was assayed. The supernatant was separated by centrifugation at 10,000 rpm for 10 min at 4°C; the acid soluble product in the supernatant was neutralized with 5 ml of 0.5 M Na_2CO_3 solution. The color developed after adding 0.5 ml of 3 fold diluted Folin-Ciocalteau reagent was measured at 660 nm. All assays were done in triplicate.

Determination of keratinase activity: The keratinase activity was assayed by the modified method of Cheng *et al.* (1995) by using keratin as a substrate. The reaction mixture contained 200 µl of enzyme preparation and 800 µl of 20 µg/ml keratin in 10 mm Tris buffer, pH 8. The reaction mixture was incubated at 45°C for 20 min and the reaction was terminated by additing 1 ml of 10% chilled trichloroacetic acid. The mixture was centrifuged at 10,000 g for 5 min and the absorbance of the supernatant fluid was determined at 440 nm. All assays were done in triplicate. One Unit (U) of enzyme activity was the amount of enzyme that caused a change of absorbance of 0.01 at 440 nm in 20 min at 45°C.

Determination of protease activity: Protease activity was assayed by a modified method of Tsuchida *et al.* (1986) by using casein as substrate. 100 µl of enzyme

solution was added to 900 µl of substrate solution (2 mg/ml casein in 10 mM Tris-Cl buffer, pH 8.0). The mixture was incubated at 50°C for 20 min. Reaction was terminated by the addition of an equal volume of 10% chilled trichloroacetic acid then the reaction mixture was allowed to stand in ice for 15 min to precipitate the insoluble proteins. The supernatant was separated by centrifugation at 10,000 rpm for 10 min at 4°C, the acid soluble product in the supernatant was neutralized with 5 ml of 0.5 M Na_2CO_3 solution. The color developed after adding 0.5 ml of 3 fold diluted Folin-Ciocalteau reagent was measured at 660 nm. All assays were done in triplicate. One protease unit is defined as the amount of enzyme that releases 1 µmol of tyrosine per ml per minute under the above assay conditions. The specific activity is expressed in the units of enzyme activity per milligram of protein.

RESULTS

Isolation, characterization of keratinolytic strains: It was found that the enriched feather degrading culture contained micro-organism exhibited keratinolytic activity The feathers were fully solubilized within 120 h of incubation with the microbes from selected soil (Fig. 1). Three bacterial strains that visually degraded feather were isolated allowed to grow on medium containing feather meal powder as sole carbon and nitrogen source. The strain SN1, SN2, SN3 were selected as they produced clear zones on incubation at 30°C for 72 h suggesting the presence of keratinolytic activity (Fig. 2). The identification of the keratinolytic bacteria was based on cell morphology, colony morphology, and several biochemical tests (Table 1a). Isolates SN1, SN2, SN3 were determined to be Gram-positive, sporulating, motile bacilli. The isolate SN1, SN2 formed yellow colored colonies and SN3 showed white colored colony on feather meal agar plate. These results suggested that these three strains belong to genus *Bacillus*. On the basis of morphological characteristic and cultural characteristic on Hicrome *Bacillus agar* and was identified (Table 1b). They (SN1, SN2 and SN3) were further identified to be as sample *B. megaterium*, *B. thuringenesis*, *B. Pumilis* respectively (Fig. 3). These strains degraded the chicken feathers and pigeon feathers (figure not shown) completely. Their cultural characteristic in the differential media is given in Table 2 with *B. megaterium* SN1 showing yellowish green, irregular colonies, *B. thuringenesis* SN2 showed blue circular colonies, *B. Pumilis* SN3 showed green, flat, circular shiny colonies.

Factors affecting growth and enzyme production: Bacterial growth and enzyme production of keratinase and protease by the microbes of soil was monitored during growth in Horikoshi media, Feather meal

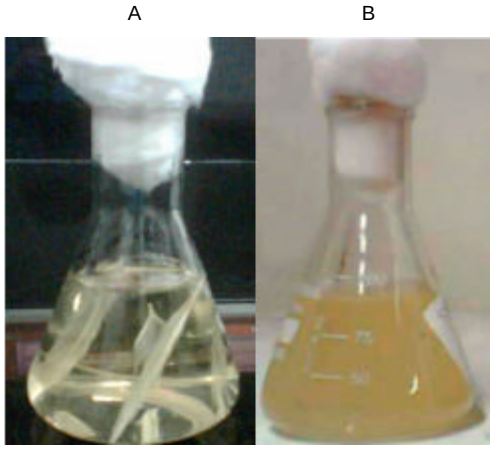

Fig. 1: Degradation of chicken feathers by the bacterial strain isolated from soil of Ghazipur poultry dumping site, India, in submerged cultivation at 30°C. (A) Feather control without the bacterial strain, (B) feather after 120 hrs of incubation with the bacterial strain showed complete degradation

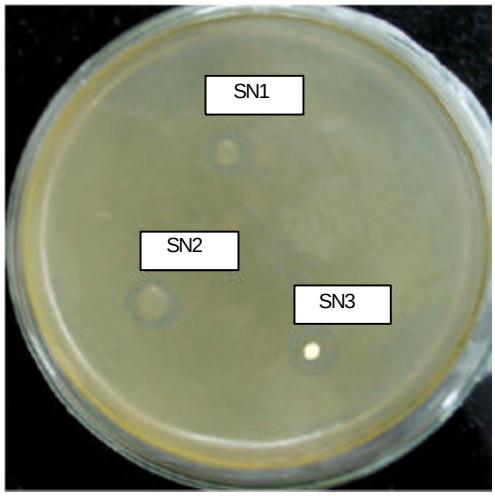

Fig. 2: Production of clear zones in feather meal powder (10g/l) agar plates by keratinolytic bacteria. Three strains were identified *Bacillus* SN1, *Bacillus* SN2 and *Bacillus* SN3 that produced clear zone. Microbial culture was spreaded on feather meal agar plate and incubated at 30°C for 72 h

media 1. Growth determination of three bacteria was done taking absorbance at 600 nm of bacterial growth media at regular intervals from 0-144 h (Fig. 4). Log phase of growth is from 24 h till 48 h for all the strains and in stationary phase till 120 h. Hydrolysis of protein substrates (Keratin, Casein, Gelatin, Bovine Serum

Table 2: Cultural characteristics on Hicrome *Bacillus agar*. Morphological characteristic and cultural characteristic of the three isolates on Hicrome *Bacillus agar* Himedia M1651. Strains SN1, SN2, SN3 were classified as *B. megaterium*, *B. thuringenesis*, *B. Pumilis* respectively

Colony characteristics	*B. megaterium* SN1	*B. thuringenesis* SN2	*B. Pumilis* SN3
Growth	Luxuriant	Luxuriant	Luxuriant
Shape	Irregular, large	Circular, Small	Circular, Small
Surface	Smooth	Smooth	Smooth shiny
Margin	Irregular	Entire	Entire
Color	Yellowish green	Blue	Green
Elevation	Convex	Flat	Flat
Opacity	Opaque	Opaque	Opaque

Fig. 3: Growth of bacterial strain SN1, SN2, SN3 on chromogenic differentiation agar Hicrome bacillus agar from Himedia M1651. Yellowish green colonies represent *B. megaterium* SN1; Blue colonies represent *B. thuringenesis* SN2; Green colored colonies represent *B. Pumilis* SN3

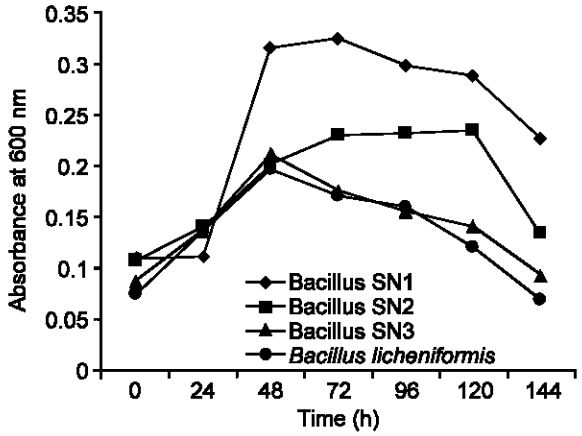

Fig. 4: Growth determination of bacterial strains *B. megaterium* SN1, *B. thuringenesis* SN2, *B. Pumilis* SN3, *Bacillus licheniformis* 1483 (Standard strain collected from MTCC was used for comparative study) were done taking absorbance at 600nm of bacterial growth media. It is observed that Log phase is from 24 h till 48 h and stationary phase is till 120 h for all the 3 strains

Albumin 2 mg/ml) by cell free supernatant collected after 120 h of bacterial growth {strains identified were *B. megaterium* SN1, *B. thuringenesis* SN2, *B. Pumilis* SN3, *Bacillus licheniformis* 1483 (Standard strain collected

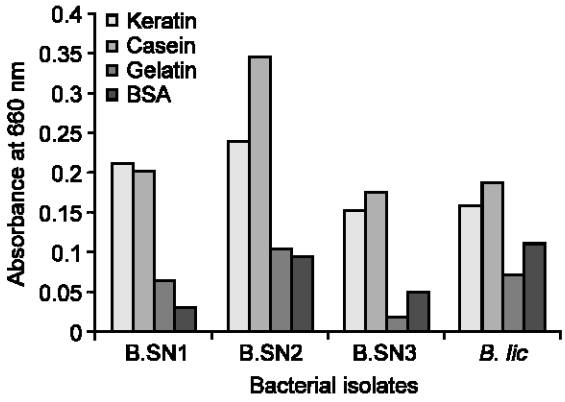

Fig. 5: Hydrolysis of protein substrates (Keratin, Casein, Gelatin, Bovine Serum Albumin 2mg/ml) by cell free supernatant collected after 120 h of bacterial growth {strains identified were *B. megaterium* SN1, *B. thuringenesis* SN2, *B. Pumilis* SN3, *Bacillus licheniformis* 1483 (Standard strain collected from MTCC was used for comparative study)}. All strains preferred Keratin and casein as preferred substrate Gelatin was least preferred source as substrate. Maximum utilization of keratin and casein was by strain SN2 as seen by the absorbance

from MTCC was used for comparative study)}. All strains preferred Keratin and casein as substrate Gelatin was least preferred source as substrate. Maximum utilization of keratin and casein was by strain SN2 as seen by the absorbance (Fig. 5).

The maximum protease activity was seen at 120 h (5th day) of incubation in feather meal media 2 (Fig. 6) and the maximum keratinase activity was seen on the 144 h (6th day) of incubation in feather meal media 2 (Fig. 7). Relatively the bacterial culture could produce 2.4 times more protease in feather meal media 2 and 2.53 times more keratinase in feather meal media 2 than in peptone broth (Table 3 and 4). Comparison was made standard strain of *Bacillus. licheniformis* (MTCC 1483).

DISCUSSION

Animal feed typically includes a carbohydrate source and a protein source. Common protein sources used in

Table 3: Purification table that compares the yield, Specific Activity of protease from micro-organisms grown in Peptone Broth and the enriched media (Hori koshi, Feather meal media 1 and Feather meal media 2) Enzyme assay was determined as mentioned in Material and Methods. The Standard strain *B licheniformis* (Crude and 0-80% Ammonium sulphate) was kept as control

Purification step	Protein (mg)	Total activity (U^a)	Specific activity (U/mg)	Yield (%)	Purification (Fold)
Peptone broth	138.75	1095250	7893.69	100	1
Horikoshi media (0-80% A.S.)	217.5	1194075	5503	1.090	0.697
Feather meal media 1 (0-80% A.S.)	63	51770	1297	0.0746	0.1644
Feather meal media 2 (0-80% A.S.)	279.5	2850750	10199	2.6028	1.292
Crude (*B.lic*)	137.95	782177.5	5670.05	100	1
B.lic (0-80% A.S.)	176.25	509362	2890.81	0.6512	0.5098

Table 4: Purification table that compares the yield, Specific Activity of keratinase from micro-organisms grown in Peptone Broth and the enriched media ((Hori koshi, Feather meal media1 and Feather meal media 2) The Standard strain *B licheniformis* (Crude and 0-80% Ammonium sulphate) was kept as control Enzyme assay was determined as mentioned in Material and Methods

Purification step	Protein (mg)	Total activity (U^a)	Specific activity (U/mg)	Yield (%)	Purification (Fold)
Peptone broth	138.75	11,500	82.88	100	1
Horikoshi media (0-80% A.S.)	217.5	23,900	110.138	207.82	1.328
Feather meal media 1 (0-80% A.S.)	63	4,650	73.80	40.80	0.890
Feather meal media 2 (0-80% A.S.)	279.5	33,900	121.28	294.78	1.463
Crude (*B.lic*)	137.95	9,050	65.61	100	1
B.lic (0-80% A.S.)	176.25	18,500	104.96	204.41	1.596

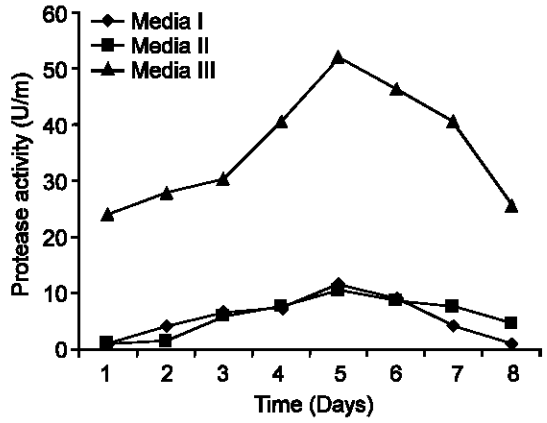

Fig. 6: Protease activity produced by feather degrading bacterial strains of feather dumping site, activity was measured by a modified method of Tsuchida *et al.* (1986) by using casein as substrate. One protease unit is defined as the amount of enzyme that releases 1 µmol of tyrosine per ml per minute under the above assay conditions. The specific activity is expressed in the units of enzyme activity per milligram of protein. In different culture media Horikoshi media, Feather meal media 1, Feather meal media 2. Maximum protease units 54 U/ml were seen in feather meal media 2 (Media III in graph)

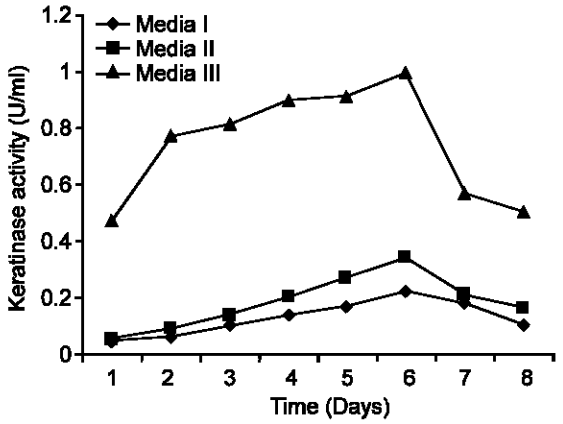

Fig. 7: Keratinase activity produced by feather degrading bacterial strains dumping site, activity was measured by a modified method of Cheng *et al.* (1995) by using keratin as a substrate. One unit (U) of enzyme activity was the amount of enzyme that caused a change of absorbance of 0.01 at 440 nm in 20 min at 45°C. In different culture media Horikoshi media, Feather meal media 1, Feather meal media 2. Maximum activity 1.2 U/ml was produced in feather meal media 2 (Media III in graph)

animal feed include soy meal; fish meal; blood meal; meat or poultry by-products and meat and poultry meal. These protein sources are generally expensive Feather waste too is high in protein and very inexpensive, but cannot be used directly in animal feed, as it is difficult for animals to digest. Typical treatments to f orm feather meal are expensive. These treatments also tend to destroy some amino acids, which are heat-sensitive amino acids. This lowers the quality of the protein in the feed. Due to these problems, feather meal is not extensively used in feed, despite the expense of other sources of dietary protein. It is reported that keratinolytic bacteria can degrade feathers.

We attempted to isolate feather degrading bacteria from soil samples collected from regular dumping site for poultry waste. From these soil samples, one soil sample showed ability to degrade chicken feathers. Feather was completely degraded in Feather meal media 2 at 30°C within 5 days of incubation. This media showed the presence of keratinase and protease activity into the cell culture supernatant. Other samples were not degraded feather showed no enzyme activity and they were discarded. Micro-organisms of the selected soil sample was further maintained in enrichment medias Horikoshi media, Feather meal media 1, Feather meal media 2. Growth of bacteria, keratinase and protease production was monitored at regular intervals. Our results depict that optimal medium for protease and keratinase enzyme production is feather meal media 2. The Keratinolytic activity of crude enzyme was confirmed because the activity of enzyme on he feather was visibly degraded with the formation of soluble feather product and reduction in weight (Moreira *et al*., 2007). It is also known that most microbial Keratinase are inducible and it is substrate specific (Cheng *et al*., 1995) and various keratinous material chicken feather, feather meal, wool, bovine hair, humans foot skin are used as inducer for Keratinase. (Kumar *et al*., 2008; DeToni *et al*., 2002; Ignatova *et al*., 1999) keratinase has been purified using various strategies and molecular weight determined (Fuhong *et al*., 2010). Lower level of degradation of buffalo horn, goat hair, duck feathers is reported by Singh (1997). It is also reported that Keratinolytic activity of micro-organism is associated with the production of serine protease, metalloprotease (Gupta and Ramnani, 2006) with the exception of yeast which produce aspartic proteases (Monod *et al*., 2002).

Ammonium sulphate fraction of the crude cell free supernatant which was seeded with the soil inoculum showed 1.5 fold purification for Keratinase and 1.3 fold purification. for Proteases Bacteria were isolated from a poultry processing plant, that showed keratinolytic activity and ability to degrade chicken and pigeon feathers. Preliminary identification tests indicate that bacterial strains B. *megaterium* SN1, *B.thuringenesis* SN2, *B. Pumilis* SN3.

Conclusion: Through the strategy of isolation of Keratinolytic microorganisms utilized in this work, bacteria presenting high Keratinolytic activity were selected. Considering that feather protein has been showed to be an excellent source of metabolizable protein (Klemersrud *et al*., 1998) and that microbial Keratinases enhance the digestibility of feather keratin (Lee *et al*., 1991), these Keratinolytic strains could be used to produce animal feed protein. In addition, the selected isolates were able to grow and display Keratinolytic activity in keratin wastes (feathers). Utilization of these potential keratin degraders will definitely find biotechnological use in various industrial processes involving keratin hydrolysis. It would also solve the waste disposal problem of poultry waste and with limited resources recycling of Keratinacious waste would be beneficial financially and environmentally.

ACKNOWLEDGEMENT

We are thankful to Jaypee Institute of Information Technology University, Noida, India for providing the infrastructure facilities for this study.

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