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Short Communication

A study of the degradation of feather and hair wastes in an eco-friendly way so as to make the waste dumping soils fertile

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The current study was aimed at the degradation of feather and hair wastes in an eco-friendly way, which should further be helpful to make the waste dumping soils fertile. Degradation of feathers and hair was assessed by a highly potent Keratinophilic fungus namely *Chrysosporium tropicum*. The 60 day experiment was set up with sterile defatted feather and hair as substrates in a mineral medium along with the inoculum of the organism. The culture filtrate was analyzed every 10 days, for the release of catabolic products such as protein and keratinase, along with the concomitant increase in pH. Maximum degradation was found on the 40th day sample, where the protein released was 6.9mg/ml and the Keratinolytic activity was 8.56 KU/ml. The increase in pH (from neutral), towards alkalinity up to 40days (9.0) of incubation and decline thereafter, indicates that the release of soluble protein into the medium is maximum for 40 days. Among the two substrates used, Chrysosporium tropicum had more effect on hair than that of feather.

Key words: Feather waste, hair waste, Keratinophilic fungi, Chrysosporium tropicum.

INTRODUCTION

Environmental pollution and degradation of ecosystem have assumed significance owing to an increase in the accumulation of wastes from industries, agriculture and poultry. In India, poultry feather, animal hair and other keratin sources do not find suitable applications. Surveys conducted at different feather dumping soils in various places (Ali-shtayeh et al., 2001; Hubalek et al., Krysztof, Ali-shtayeh et al., Vidal et al., 2000; Moallaei et al., 2006; Vidyasagar et al., 2003) all over the world (Aleer Rose, 1980; Ali-shtayeh, 1989; Dominik et al., 1964; Filipello marchisio et al., 1991; Merkantini et al., 1983; Abdel-hafez et al., 1987) including at various places in India (Deshmukh, 1998; Anbu et al., Deshmukh, 2004; Ramesh et al., 2002) and in-and-around Visakhapatnam, indicated that several tons of poultry feather go as waste every day.

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On the other hand, as Andhra Pradesh is the home of many religious pilgrim centers and people believe that hair is a symbolic offering to the gods, representing a real sacrifice of beauty and in return, are given blessings in proportion to their sacrifice, hair is becoming a major keratin material being dumped around temples and pilgrimages which causes the occurrence of too many Keratinophilic fungal population in those soils.

In general Keratinophilic fungi are a group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight (Harish, 2000). Poultry feathers and human hair contain the major component of keratin. Keratins are scleroproteins composed of long polypeptide chains, which are insoluble in dilute acids, alkali solutions and also resistant to the action of pepsin, trypsin and other non-substrate specific proteases because of their high content of cystine. The higher the percentage of sulfur, the higher is the stability of keratin towards solubilization (Malcom, 2000).

The disruption of insoluble keratin is generally believed to be an enzymatic process (Deshmukh, 2004).

Keratinases are the key enzymes elaborated by Keratinophilic fungi for the degradation of keratin (Tawfik et al., 2001; Vidal et al., 2000). The enzyme is inducible and extra-cellular in nature.

Therefore, an attempt has been made to suggest the possibility of degradation of waste feather and hair by enzyme production in association with saprophytes with higher Keratinolytic potential.

MATERIAL AND METHODS

C Tropicum, a Keratinolytic saprophyte isolated and identified (in comparison with that of strain procured from IMTECH, Chandigarh – Code: MTCC2821) from soil was maintained on Potato Dextrose Agar medium (PDA) (Scrubbed and Diced Potaoes-200.0g; Dextrose-20.0g; Agar-15.0g; Distilled Water- 1000ml; pH-5.6).

Mineral medium was used for assay for keratin degradation (Di-Potassium hydrogen O-phosphate-1.500g; Magnesium sulfate-0.050g; Calcium chloride-0.025g; Ferrous sulfate-0.015g; Zinc sulfate-0.005g; Distilled water-1000ml; Sterile defatted feather or hair -10g; pH-7.5). Feathers or hair were defatted by soaking them in Methanol-Chloroform mixture (1:1) for 24 hours, washed with water and air dried.

Biochemical changes during hydrolysis of hair and feathers

50 ml of the prepared mineral medium with hair and feather was distributed in 250 ml Erlenmayer's flasks and were sterilized at 120^{O}C for 20 minutes. The liquid media were inoculated with 1 ml of spore suspension from the test fungi and incubated at 28±1^{O}C in static condition for a period of 60 days. During the period of incubation, samples were drawn at regular intervals of 10 days and analyzed to study the progress of feather degradation.

The culture filtrate was centrifuged at 4000 rpm fro 5 minute. and the collected supernatant was used to assess the:

1. Changes in pH (Systronics pH Meter)
2. Estimation of protein (Bradford, 1976)
3. Determination of Keratinolytic activity
4. Changes in pH

Changes in pH:nA clear cut evidence to assess the progress of poultry feather degradation was obtained by observing the changes in hydrogen ion concentration of the mineral media. The changes towards alkalinity of culture filtrate were measured by using a pH meter (Systronics pH meter) with glass electrode.

Estimation of protein (Bradford, 1976): To 1 ml of sample, 2 ml of Coomassie Brilliant Blue solution was added and the absorbance was taken at 595 nm after 2 to 30 min. of incubation. Amount of protein released in the culture filtrate was calculated with the help of standard prepared by using Bovine Serum Albumin (BSA) as the substrate.

Coomassie Brilliant Blue Solution: In a volumetric flask, 100mg Coomassie Brilliant Blue G-250 was dissolved in 50ml of 95% ethanol. Later 100ml of 85% phopshoric Acid was added and diluted up to 500 ml with distilled water. This solution was filtered through Whatmann No.1 filter paper and stored in cold room at 4^{O}C.

Determination of Keratinolytic Activity: Keratinolytic activity was assayed by a modified method, using Hair/Feather as substrate. 20 mg of Hair/feather from different temples, pilgrim and tonsure centers were cut into 1-3 mm long bits and were suspended in 3.5ml of Tris-HCl buffer (0.1M, pH7.8) to which 0.2 ml of culture filtrate was added. The mixture was kept in a water bath at 37^{O}C for 1 h after incubation, the assay mixture was dipped in ice cold water for 10 min. and the remaining feather was filtered out. The optical density of clear mixture was measured by UV-Visible-Spectrophotometer at 280nm against corresponding blank that was prepared in the same way, instead of enzyme solution, buffer was added.

Enzyme Unit: One unit of Keratinolytic activity (KU) was the amount of enzyme that could liberate products having an absorbance of 0.1 under the assay condition (1KU=0.100 corrected absorbance) (Kavitha et al., 2000).

RESULTS AND DISCUSSION

The amount of catabolic degradative products such as protein and keratinase production along with the simultaneous increase in pH was an indication of feather degradation.

Changes in pH: The changes in pH towards alkalinity were assessed during hydrolysis. In all setups with *C Tropicum*, there was a gradual increase in pH of the medium up to 40 days of incubation followed by decline thereafter (Figure 1).

The medium, set up for feather hydrolysis showed the pH: 7.9, 8.6, 8.8, 9.0, 8.5 and 7.9 while the setup for hair degradation showed the pH: 8.0, 8.8, 8.9, 9.2, 8.7 and 8.2 for 10th, 20th, 30th, 40th, 50th and 60th days respectively.

The set up with hair as substrate has shown maximum change in pH (7.5 to 9.2) than that of the feather (7.4 to 9.0).

The tendency towards alkalization of the medium may be due to the hydrolysis of feather and hair and the decline in pH at the end of the experiment may have been caused by the accumulation of degraded products especially acidic sulfur compounds in the media. This change in pH was an indicator for as well as pre-conditioner for Keratinolysis (Kavitha et al., 2000).

Estimation of protein: As shown in the figure 2, the maximum amount of protein was released by *C Tropicum*, in 40th day sample (6.9mg/ml) and is same in both the cases where the feather and hair are used as substrates.

A remarkable amount of protein was released by the keratinolytic activity of the *C Tropicum*, from all the samples: 4.9mg/ml, 5.4mg/ml, 6.1mg/ml, 6.9mg/ml, 4.8mg/ml, and 4.2mg/ml for 10th, 20th, 30th, 40th, 50th and 60th days respectively, when feather was used as substrate.

In accordance with the report of Kavitha et al., (2000), waste feathers and hair are valuable organic sources because of their high content protein.

Determination of keratinolytic activity: Among all the setup samples, maximum keratinase production registered

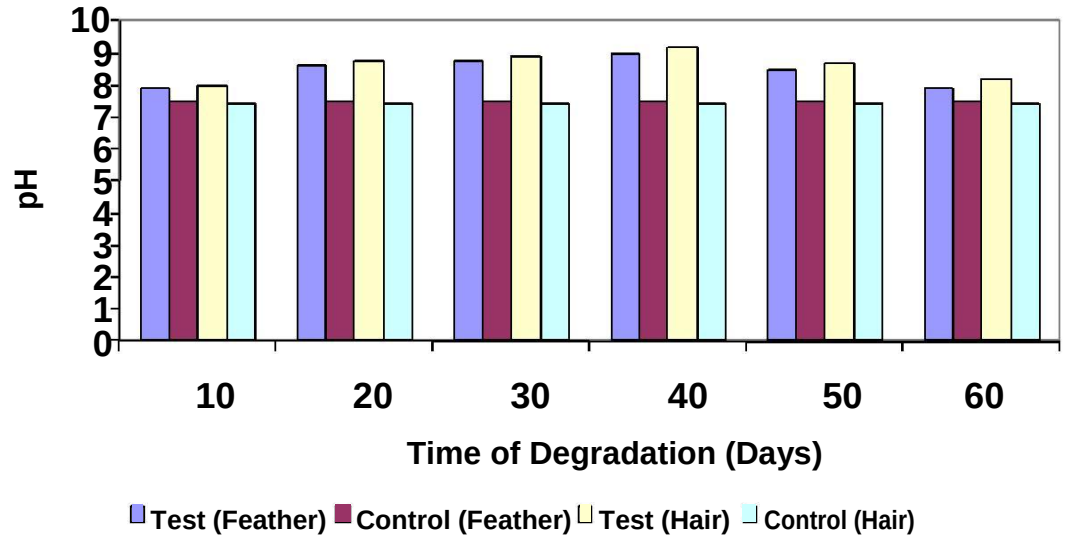

Figure 1. Changes in pH of mineral medium after degradation by *Chrysosporium tropicum*.

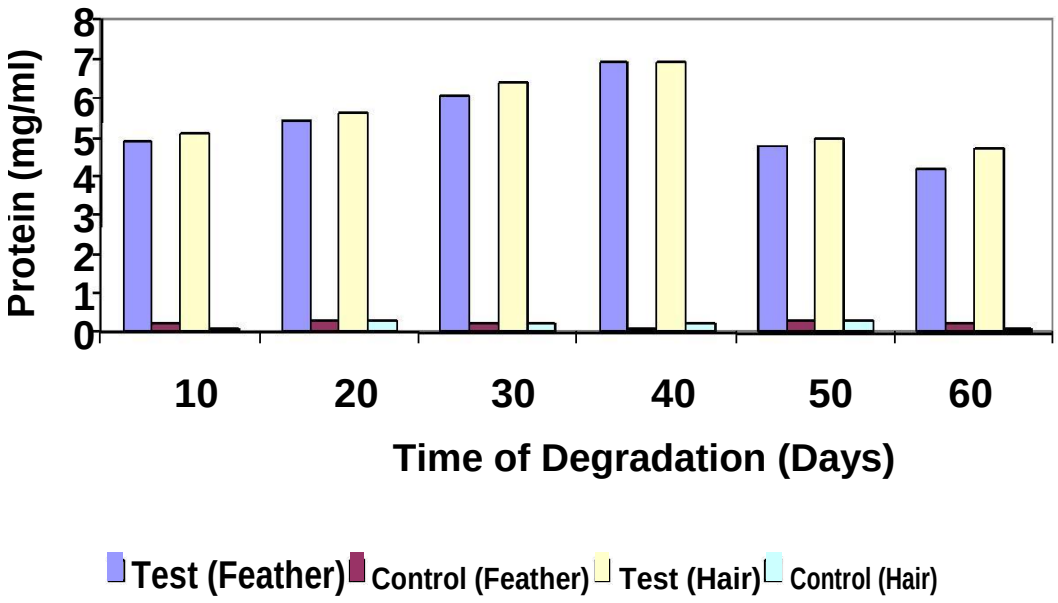

Figure 2. Estimation of protein after degradation by *Chrysosporium tropicum* (mg/ml)

was 8.56 KU/ml on the 40th day when hair was used as substrate.

It is evident from the results (Figure 3) that gradual increase in the keratinolytic activity of the *C Tropicum* up to 40 days and decline thereafter, on both the feather and hair as substrates, occurred. The keratinolytic activity values when feather was used as a substrate are the following: 5.12 KU/ml, 7.02 KU/ml, 7.93KU/ml, 8.35KU/ml, 6.52KU/ml and 6.18KU/ml when feather was used as substrate, the keratinolytic activity values are: 6.77 KU/ml, 7.37 KU/ml, 8.14KU/ml, 8.56KU/ml, 6.50KU/ml and 5.92KU/ml when hair was used as substrate, for 10th, 20th, 30th, 40th, 50th and 60th days respectively.

The results of the present study are in accordance with the work of Bohme et al., (1969) that, deamination was

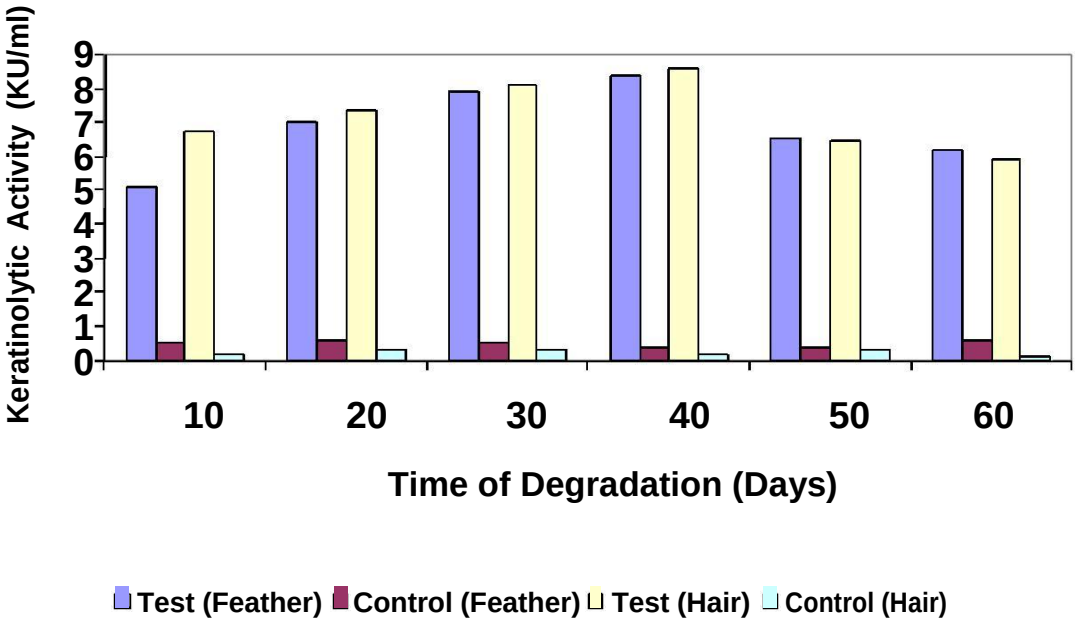

Figure 3. Determination of Keratinolytic activity by *Chrysosporium tropicum* (KU/ml)

the key reaction of keratinolysis. In adding support to the view of present study, Ramesh and Hilda (1998) reported that the use of fungal keratinase was an industrial tool in the conversion of waste hairs into resource.

The decline in keratinase activity on the 50th day may possibly be due to the end product inhibition caused by different catabolic degradative products of organic sulfur which is originally combined in feather and hair or due to the loss of accessory protein required for keratinase to act on feather or hair effectively. Such a conclusion was in accordance with the findings of Ramesh and Hilda (1998) and Kavitha et al., (2000). Hence, it is evident from the present investigation that the fungal strain of *C Tropicum* was a potent keratinolytic fungus both on feather and hair as substrate.

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