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Full Length Research Paper

Degradation of feather and hair by *Chrysosporium tropicum*: A potent keratinophilic fungus

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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 fungi 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 at every 10 days interval, for the release of catabolic products such as protein and keratinase, along with the concomitant increase in pH. Maximum degradation was found at the 40th day sample, where the protein released was 6.9 mg/ml and the keratinolytic activity was 8.56 KU/ml. There was increase in pH (from neutral) towards alkalinity up to 40 days (9.0) of incubation and decline thereafter, indicating the maximum release of soluble protein into the medium. Among the two substrates used, *C. 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 2000, Krysztof et al., 2000, Ali-shtayeh 1989, Vidal et al., 2000; Moallaei, 2006; Vidyasagar, 2003) all over the world (Aleer Rose, 1980; Ali-shtayeh, 1989; Dominik and Majchrowicz, 1964; Filipello marchisio et al., 1991; Merkantini et al., 1983; Abdel-hafez and El-sharouny, 1987) including India (Deshmukh, 1999; Anbu et al., 2004, Deshmukh, 2004) and in-and-around Visakhapatnam, indicated that several tons of poultry feather go as waste every day.

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 colonizes 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 and Michael, 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;

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Vidal et al., 2000). The enzyme is inducible and extracellular in nature.

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

MATERIALS AND METHODS

Chrysosporium 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 (PDA) medium.

Mineral medium was used for assay for keratin degradation (di-potassium hydrogen O-phosphate-1.500 g; magnesium sulfate-0.050 g; calcium chloride-0.025 g; ferrous sulfate-0.015 g; zinc sulfate-0.005 g; distilled water-1000 ml; sterile defatted feather or hair-10 g; pH-7.5). Feathers or hair were defatted by soaking them in methanol-chloroform mixture (1:1) for 24 h, 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°C for 20 min. The liquid media were inoculated with 1 ml of spore suspension from the test fungi and incubated at 28 ± 1°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 for 5 min and the collected supernatant was used to assess the changes in pH (Systronics pH Meter), estimation of protein (Bradford's method) and determination of keratinolytic activity (Kavitha et al., 2000).

Changes in pH

A 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 culture filtrate was measured by using a pH meter (Systronics pH meter) with glass electrode.

Estimation of protein

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. Standard solution was prepared by using bovine serum albumin (BSA) as the substrate (Bradford, 1976).

Determination of keratinolytic activity

Keratinolytic activity was assayed by a modified method, using hair/feather as substrate. 20 mg of hair/feather from different temple, pilgrim and tonsure centers were cut into 1 to 3 mm long bits and were suspended in 3.5 ml of Tris-HCl buffer (0.1 M, pH7.8) to which 0.2 ml of culture filtrate was added. The mixture was kept in a water bath at 37°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 at 280 nm against corresponding blank prepared in the same way except that 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 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 set ups 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 has shown the pH to be 7.9, 8.6, 8.8, 9.0, 8.5 and 7.9 while the set up for hair degradation was shown to be 8.0, 8.8, 8.9, 9.2, 8.7 and 8.2 for 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th} and 60^{th} days, respectively. The set up with hair as substrate showed 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 as well as pre-conditioner for keratinolysis (Kavitha et al., 2000).

Estimation of protein

As shown in Figure 2, maximum amount of protein was released by *C. tropicum*, at the 40^{th} day of incubation (6.9 mg/ml) for both the feather and hair substrates. A remarkable amount of protein was released by the keratinolytic activity of the *C. tropicum*, from all the samples; 4.9, 5.4, 6.1, 6.9, 4.8 and 4.2 mg/ml, when feather was used as substrate and 5.1, 5.6, 6.4, 6.9, 5 and 4.7 mg/ml when hair was used as substrate for 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th} and 60^{th} days, respectively. In accordance with the report of Kavitha et al. (2000), waste feathers and hair are the valuable organic sources because of their high protein content.

Determination of keratinolytic activity

Among all the setup samples, maximum keratinase

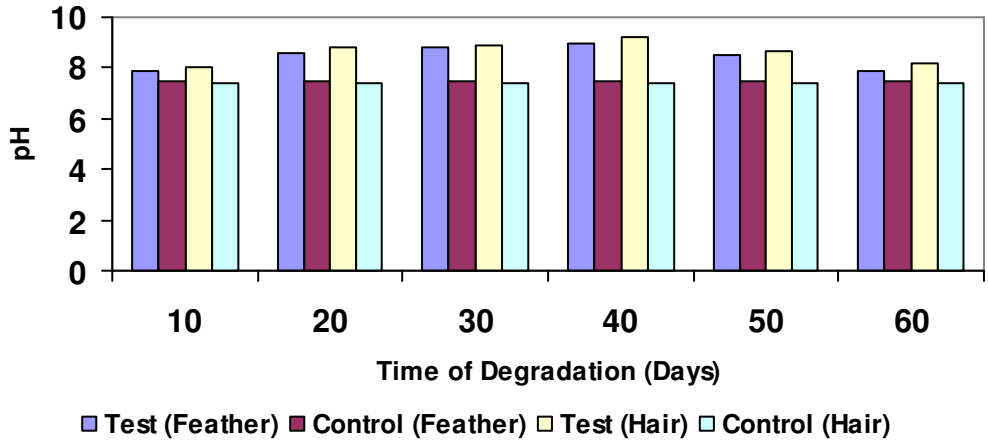

Figure 1. Changes in pH of mineral medium after degradation by *C. tropicum*.

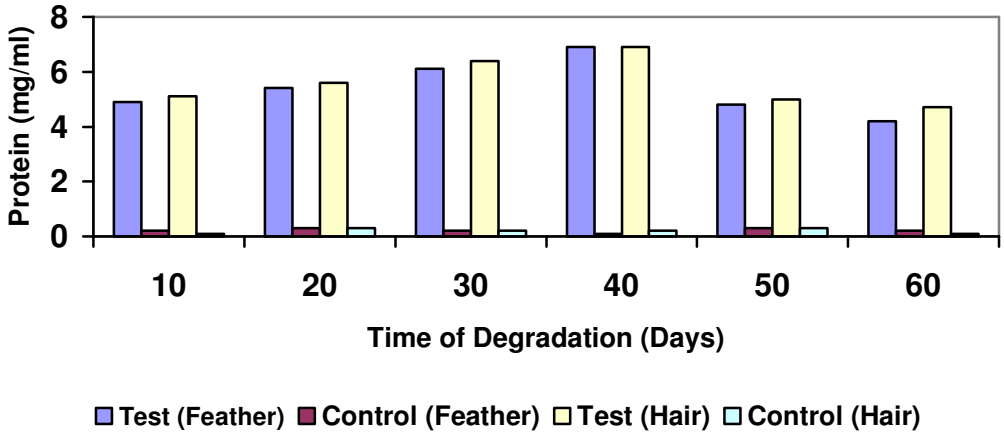

Figure 2. Estimation of protein after degradation by *C. tropicum* (mg/ml)

production registered was 8.56 KU/ml at the 40th day when hair was used as substrate. It is evident from the results (Figure 3) that there was gradual increase in the keratinolytic activity of the *C. tropicum* up to 40 days but decline there after, on both the feather and hair as substrates. The values are 5.12, 7.02, 7.93, 8.35, 6.52 and 6.18 KU/ml when feather was used as substrate, while 6.77, 7.37, 8.14, 8.56, 6.50 and 5.92 KU/ml when hair was used as substrate, for 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th} and 60^{th} days respectively.

The results of the present study are in accordance with the work of Hannelore and Ziegler, (1969) that showed that deamination was the key reaction of keratinolysis. In addition, Ramesh and Hilda (1998) reported that the use of fungal keratinase was an industrial tool in the conversion of waste hairs into resource. Gradual degradation was visible in subsequent microphotographs (Figures 4, 5 and 6) where the organism was observed to be penetrating through the hair strand. However, the decline in keratinase activity on the 50^{th} 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

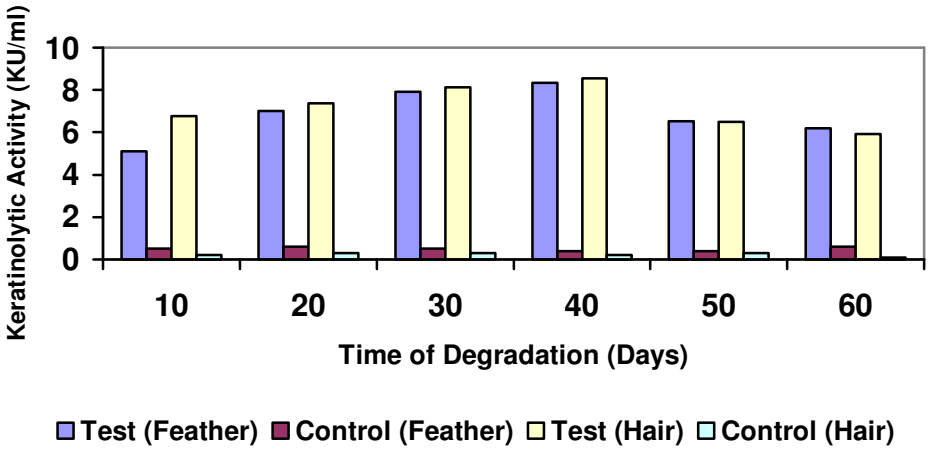

Figure 3. Determination of keratinolytic activity by *C. tropicum* (KU/ml)

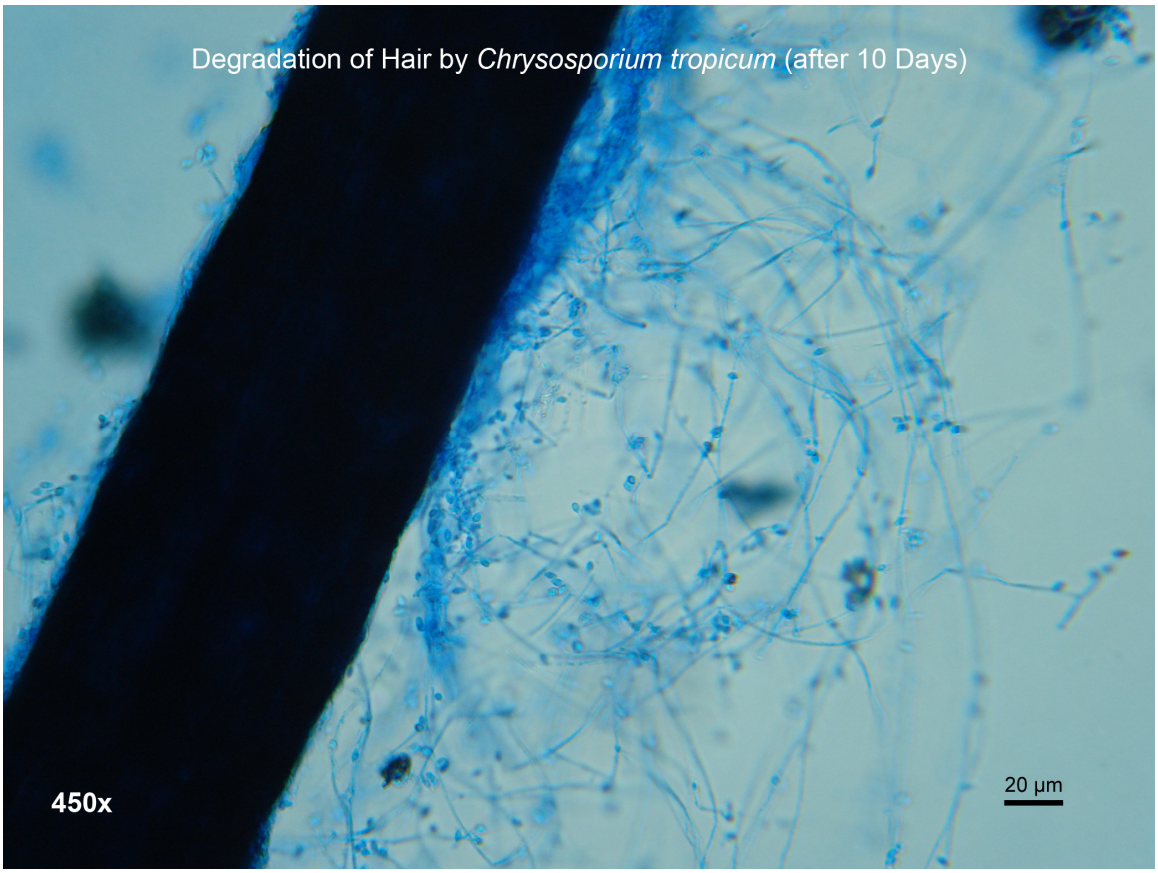

Figure 4. Microphotograph of *C. tropicum* on hair strand after 10 days of degradation.

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.

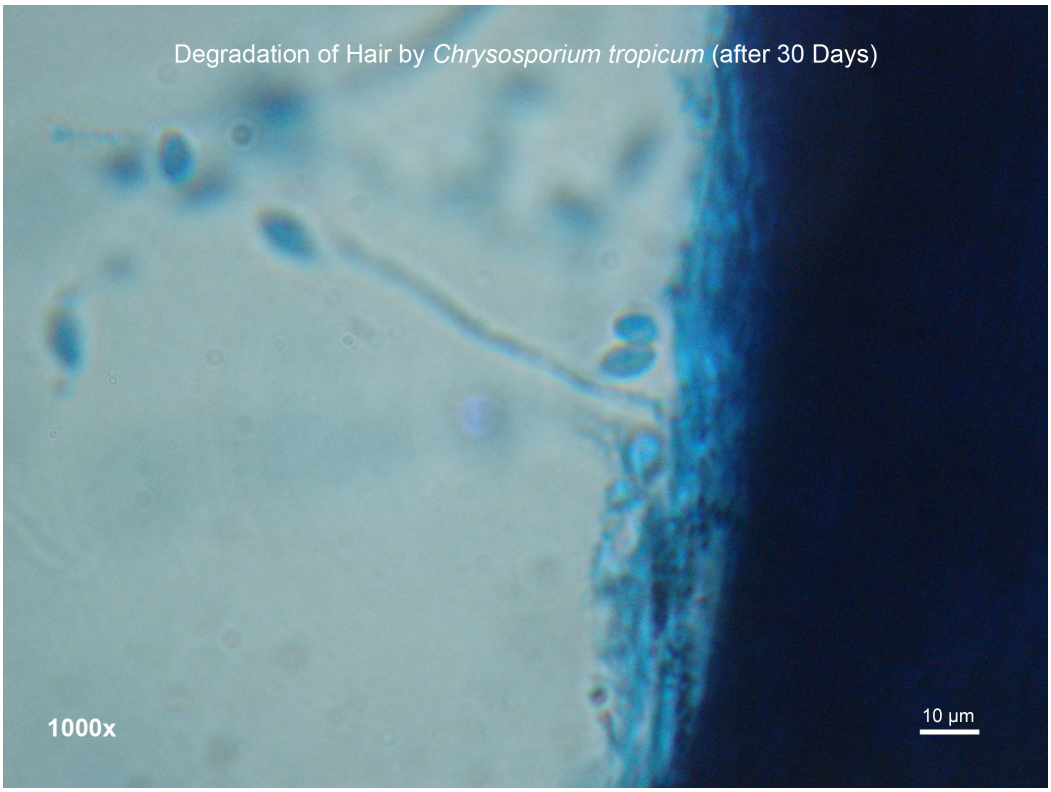

Figure 5. Microphotograph of *C. tropicum* on hair strand after 30 days of degradation.

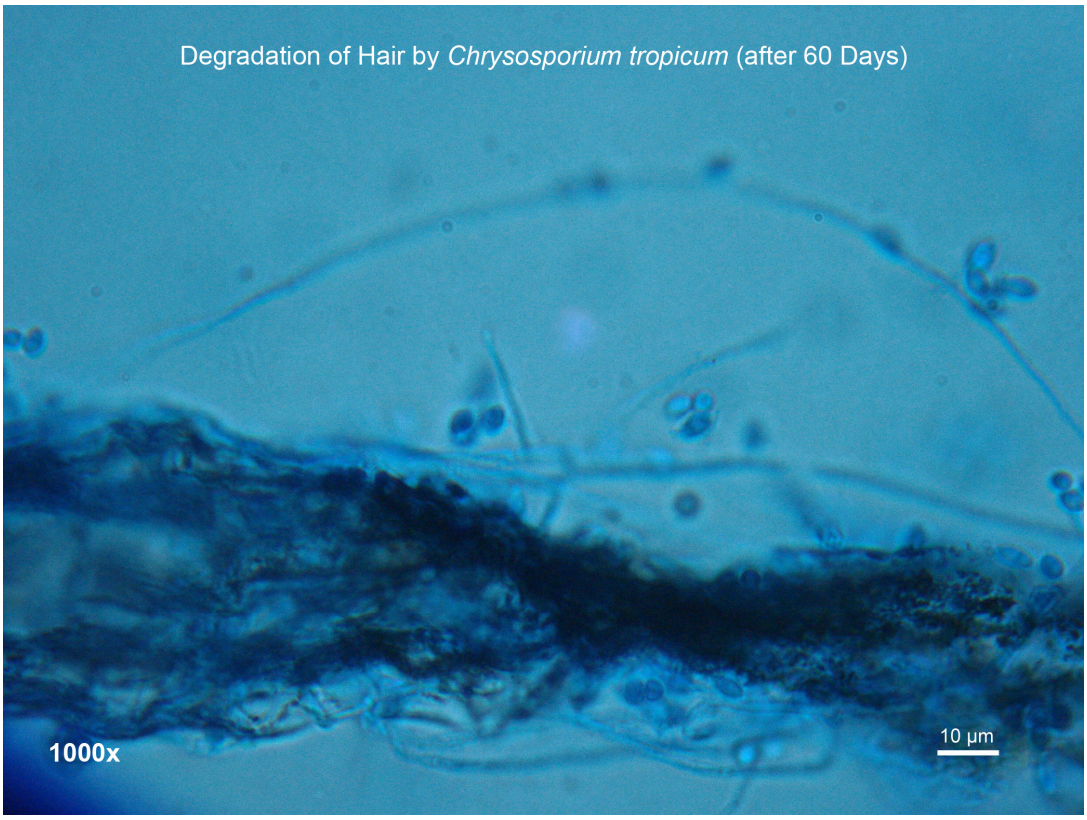

Figure 6. Microphotograph of *C. tropicum* on hair strand after 60 days of degradation

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