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Research Article

BIODEGRADATION OF KERATIGENOUS WASTES BY DERMATOPHYTIC FUNGI WITH REFERENCE TO *TRICOPHYTON RUBRUM*

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ABSTRACT
Keratinophilic fungi are present in the environment with variable distribution patterns depending on availability of keratin wastes and hydrolyze insoluble keratin by virtue of their ability to produce keratinase. Present study involves investigations on degradation potential of *Trichophyton rubrum* using keratinous wastes of chicken animal and human origin. Reduction in the weight of substrates, change in the pH and estimation of the soluble proteins were investigated. Gradual reduction in the weight of substrate with increase in incubation time was observed which was more profound in chicken feathers when compared to the other two keratin substrates. pH of the medium changed to alkaline and amount of proteins also increased with incubation time. Increase in the protein content of the medium could be correlated with the extent of substrate degradation. Keratinase was assayed on keratin substrate prepared from chicken feathers and showed positive results.

Keywords*: Biodegradation, Keratin, Keratinophilous Fungi, Keratinase, Trichophyton Rubrum*

INTRODUCTION
Several millions of tons of feathers, animal and human hair wastes are generated every year and among others constitute the major source of environmental pollution. All these wastes of animal origin contain a large quantity of a stable, difficult to degrade protein that is keratin.
Keratins are insoluble fibrous protein found in hair, wool, feather, nail, horns, and others epithelial covering which are rich in beta helical coil linked through Cysteine bridges. Keratin molecules are organized with various other proteins and cementing substances in more or less keratin-rich structures. The high sulphur content of keratin is due to the presence of sulphur containing amino acids and the disulphide bonds between these amino acids make it resistant to most proteases.
Keratinases belongs to the class of hydrolytic enzymes, produced by several Keratinophilic fungi (Friedrich *et al.,* 1999; Soomoro *et al.,* 2007), they are serine or metallo proteases capable of degrading the structure forming keratin proteins more efficiently than other proteases. Using theses enzymes the keratin wastes can ultimately be converted into useful forms like feather meal, feedstuff, fertilizers, glues, and used for the production of amino acids and peptides.
Keratinophilic fungi are present in the environment with variable distribution patterns that depends mainly on availability of keratin, thus, Cattle shed, garbage dumps, birds' nests, barber's hair dumping areas, poultry sheds etc. are the preferred areas.
In natural environments these fungi are involved in recycling of the carbon, nitrogen and sulphur in α-keratins (Sharma and Rajak, 2003). A large number of fungi, including yeasts, dermatophytes and other moulds, exhibit 'keratinolytic' activity owing to production of keratinase which is an extracellular protease, and secretion of this enzyme appears to be induced by the presence of keratin in the substrate (Takiuchi *et al.,* 1984; Siesenop and Bohm, 1995).
Tricophyton rubrum is the most common organism causing animal dermatophytic infections involving the toe webs, nails, groins, soles, palms and hair. Keratinase in this fungus is expressed constitutively even in the stationary phase (Apodaca and Mckerrow, 1989).
The aim of the present study was to evaluate degradation potential of *Tricophyton rubrum* using various keratin substrates.

MATERIALS AND METHODS

Tricophyton rubrum, used in the present study was obtained from Microbiology laboratory of The Institute of Science, Mumbai. It was maintained on Sabouraud's agar medium.

Collection and Preparation of Substrate (Keratin Waste):

Human hair were collected from the hair salon, chicken feathers from the poultry shop and Goat hair used as animal hair were collected from the mutton shop.

Wastes were washed extensively with water and detergent, dried at 60ºC overnight, washed with chloroform: methanol (1:1, v/v) for removal of adhering lipids and dried at 60ºC, they were autoclaved and stored for future use under aseptic condition.

Keratinase Assay:

10gms of feathers (white chicken) were washed thoroughly and dissolved in 500ml (DMSO) Dimethylsulphoxide by heating at 100ºC for 2 hours. Protein was precipitated with cold acetone and suspended in 0.1 M phosphate buffer. Keratin so obtained was added to the mineral medium (0.06%) in Petri plates. 0.5ml of spore suspension was injected at the Centre of Petri dish and incubated at 37ºC for 3 days. Ability of the fungus to grow on keratin containing medium was considered as positive test for keratinase production.

Screening of Biodegradation Activity:

The experiments were conducted in 250 ml conical flasks. Spore suspension of test fungus was made in sterile distilled water.50mg of keratin in the form of hair/feathers was weighed and cut into small pieces (2cm in length). Keratin surface was washed with Teepol and sterilized with 3% ethanol. 50mg sterilized substrate was added to 20ml of phosphate buffer at pH 6.5 and was inoculated with 0.5ml of spore suspension and incubated at room temperature on a shaker at 100 rpm. The flasks without the fungus served as control. The flasks were harvested at weekly intervals. Reduction in the weight of substrate, changes in the pH of medium and proteins released were determined at weekly intervals. Morphology of control and degraded hair was studied under stereomicroscope and documented.

RESULTS AND DISCUSSION

Keratinase Activity:

Keratinase activity of *T. rubrum* was studied on mineral agar incorporated with soluble preparation of keratin from chicken feathers to ascertain its ability to grow on this medium by producing keratinase enzyme. The fungus showed positive growth after three days as against the control not containing any carbon and nitrogen source (plate 1). Incorporation of soluble keratin preparation enables a preliminary evaluation of fungal isolates that possessed keratinolytic activity (Sharma and Sharma, 2011), as it constitutes a universal source of C and N in comparison to native keratin (Wawrzkiewicz *et al.,* 1991). The results obtained are in agreement with that of Mushing *et al.,* (1997) and Sharma and Sharma (2011), who also reported appreciable keratinase activity of *T. rubrum* on mineral agar. Kumar and Kushwaha (2014), reported production of 19.57U/ml of keratinase by *T. rubrum* in medium containing whole chicken feathers after eight days of incubation.

Biodegradation Activity:

Biodegradation of three different keratin substrates viz. human hair, animal hair and chicken feathers by *T. rubrum* was studied.

Degradation results of these substrates by the test fungus are presented in table 1. There was an increase in the rate of degradation of all the three substrates in terms of weight loss by the fungus during incubation, however, maximum degradation was seen in chicken feathers with 70 % loss of weight after three weeks followed by animal hair (68%) and human hair (40%). Difference in the degradation rate of each keratin substrate was significant at $p<0.05$.

The amount of proteins released into the medium in general increased with increase in the time of incubation and varied with the source of keratin. The highest amount of protein (69µg/ml) was observed when feathers were used as substrate at three weeks and the least (33µg/ml) was detected in human hair at corresponding time (Table 2).

Table 1: Weight Remained of Keratin Substrates at Various Time Intervals (Initial Weight=50mg)

S. No.	Substrate	Weight Remained of Substrate(mg)/Incubation Time(Weeks)		
		1st	2nd	3rd
1.	Chicken feathers	42±0.05	28±0.01	15±0.03
2.	Animal hair	43±0.02	30±0.06	16±0.01
3.	Human hair	43±0.02	37±0.04	30±0.06

Values are means (n=3) ± SE, the results were considered significant when p <0.05

Table 2: Quantity of Proteins Released by the Test Fungus in Culture Medium Baited with Three Different Keratin Substrates

S. No.	Substrate	Proteins Released(µg/ml)/Incubation Time(Weeks)		
		1st	2nd	3rd
1.	Chicken feathers	31	62	69
2.	Animal hair	30	58	65
3.	Human hair	18	20	33

Values are mean of three readings (n=3) ± SE

Change in the pH of culture medium due to keratin degradation is presented in table 3. There was an increase in the alkalination of the medium during incubation in all the substrates tested. pH changed from 6.5-8.0 when chicken feathers were the keratin source, 6.5-7.8 when animal hair was used and 6.5-6.9 in the presence of animal hair.

Table 3: Change in pH of Culture Medium Due to Keratin Degradation by *T. Rubrum*

S. No.	Substrate	Change in pH/Incubation Time(Weeks)		
		1st	2nd	3rd
1.	Chicken feathers	7.1±0.03	7.5±0.02	8.0±o.03
2.	Animal hair	6.9±0.01	7.3±0.12	7.8±0.01
3.	Human hair	6.5±0.09	6.7±0.03	6.9±0.01

Values are mean of three readings (n=3) ± SE.

Experimental **Control**

Plate 1: Keratinase Assay on Mineral Agar after Three Days Incubation

Morphology of the degraded keratin was observed after three weeks under a stereomicroscope and the same is depicted in plate 2a-2c. Erosion of the keratin substrate by the test fungus was more evident in chicken feathers as compared to other two substrates.

Control Degraded

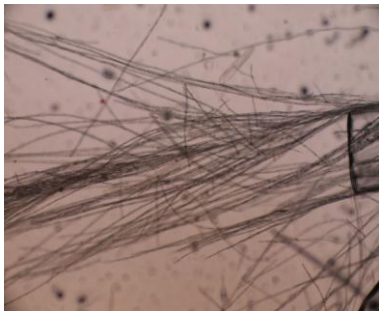
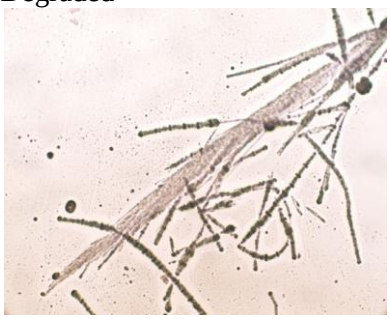

Plate 2a: Chicken Feathers

Control Degraded

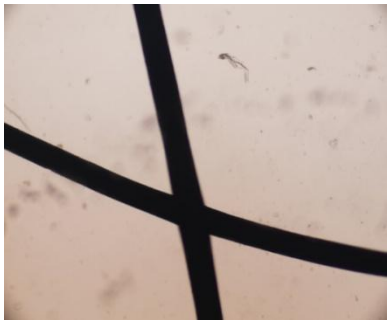


Plate 2b: Animal Hair

Control Degraded

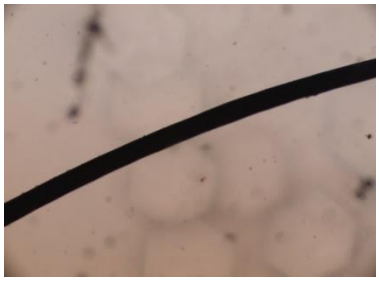


Plate 2c: Human Hair

Plate 2: Degradation of Keratin Substrates by T. *Rubrum* after Three Weeks

The present investigation showed different rates of keratin degradation by T. *rubrum* depending on the source of keratin. It was maximum in chicken feathers as indicated by substrate weight loss as well as by release of soluble proteins into the culture medium (Deshmukh and Agarwal, 1985). Degradation of human and animal hair by T. *rubrum* has also been reported by Sharma *et al.,* (2011), however, they obtained higher rate of degradation for human hair and lower for the animal hair.

Keatinolytic fungi are known to alkalinize culture media during degradation (Kaul and Sumbali, 1999). Our observations in this regard are in conformation with that of Hasija *et al.,* (1990) and Sharma *et al.,* (2011).

Conclusion

From present study, it can be concluded that showed a various response on degradation potential of *Tricophyton rubrum* used various keratin substrates. Keratinophilic in nature play an important role not only in pathogenicity but also in biodegradation of keratin substrates.

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