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SHORT COMMUNICATION

Study on *Microsporum gyspeum,* a fungus capable of biodegrading mammalian keratin

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Keratins are proteins with an extremely high molecular weight belonging to the category of structural fibrous proteins which are scientifically termed as scleroproteins. They are found to be almost impossible for digestion by the common proteolytic enzymes like pepsin and trypsin. They are also found to be insoluble in most of the solvents like dilute acids, alkalies, water as well as organic solvents. Keratinophilic fungi are found to be a group of highly specialized fungi which are capable of degrading this rigid and hard keratin in order to utilize it as a source of protein. The present research study is undertaken in understanding the keratinophilic ability of the fungus *Microsporum gypseum* in degrading human hair and horse hair.

Keywords: Fungi; horse hair; human hair; keratins; proteins

INTRODUCTION

Keratins are a type of proteins found in epithelial cells of vertebrates. They are usually present inside as well as outside of mammalian bodies. The phylum vertebrae usually have the alpha – keratin (Marchisio, 2000). These alpha –keratin type of proteins are found in hairs, nails, scales, claws, hooves, feathers, horns etc. which gives a rigid structure to these parts.They are resistant to digestion by pepsin and trypsin and insoluble in dilute acids, alkalies, water and organic solvents. Keratinophilic fungi are a group of highly specialized fungi that can degrade hard keratin and utilize it as a source of protein (Bentubo *et a*l. 2006; Sharma *et al*. 2020). Under natural conditions, the keratinized tissues are less suitable for microorganisms. Only certain highly specialized species of fungi can attack keratinous substrates which are found in polluted soil (Ali-Shtayeh, 2000) and waste water (Sharma *et al*. 2023).

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The fungi capable of degrading keratin are termed keratinolytic and these organisms play an important role in the degradation of keratinous substrates (Sharma and Rajak, 2003; Korniowicz-Kowalska and Bohacz, 2011). They digest keratin utilizing keratinolytic system that includes active alkalization of the substrates, the extracellular sulfitolysis of disulphide bonds and the proteolysis of keratin molecules. The study on keratinophillic fungi is gaining much importance as they can be used to process bioplastics, fertilizers, biogas, dehairing of leather and textiles etc. (Hassan *et al*. 2020; Bohacz and Kornillowicz- Kowalska, 2019). In the present study, an attempt has been made to study the ability of a keratinophilic fungus, *Microsporum gypseum*, to degrade human hair and horse hair.

MATERIALS AND METHODS

The keratin substrates used in the present study are human hair and horse hair. The substrates were sterilized with a chloroform-methanol mixture (1:1: v/v), renewed several times in 24 h, washed twice with glass distilled water and air dried. Mineral medium containing 1.5g of K_2HPo_4, 0.25g $MgSo_4$,

0.005g $ZnSo_4.7H_2O$, 0.025g $CaCl_2$, 0.005g FeSo4.7H_2O and 30g Dextrose per litre of distilled water (pH 6.5) was used in all the experiments. The inoculum comprised of a conidial suspension from the surface of 6 days old single spore cultures. The conidial suspension was obtained from culture tubes by brushing conidia in 5 ml of sterilized distilled water, and 2 ml of conidial suspension (300 conidia per ml) was added to each flask containing basal liquid medium. Each 100ml Erlenmeyer flask received 250mg of the sample. The cultures were incubated in stationary conditions at 28±2^{0}C. The treatments employed in the present research study are given below.

1. Keratin control was added 30 ml of mineral medium and 250 mg of the keratin substrate.
2. Fungus control was added 30 ml of mineral medium and 2 ml of fungal inoculum.
3. Test samples were added 30 ml of mineral medium, 250 mg of keratin substrate and 2 ml of fungal inoculum.

Determination of soluble proteins

After different incubation periods, the protein determinations from filtrates were carried out from the flasks of all three experimental sets. The filtrate from each flask was centrifuged at 4,000 rpm for 5 minutes, and the supernatant was assayed for protein using Folin ciocalteu reagent as described by Lowry *et al*. (1951) and Packer (1967). The developing colour was read at 660 nm on a spectrophotometer. BSA was used as the standard. The results of protein estimation were expressed as net values, i.e., the measured value in the test sample minus the sum of keratin and fungus controls values. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

In the present investigation, the ability of the Keratinophilic fungus *Microsporum gypseum* to degrade different keratin substrates –Human hair and Horsehair (Fig.1) has been studied under different incubation periods (day-wise, weekly). The net protein released by the *Microsporum gypseum* during the degradation of different keratin substrates under different incubation periods (day-wise and weekly) is tabulated in Table 1 and 2,respectively.

Table 1: Net protein released during the growth of *Microsporum gypseum* on different keratin substrates (Day-wise)

Incubation period (days)	Human hair Net protein (µg/ml) *	Horse hair Net protein (µg/ml) *
2	123	233.1
4	334	362.8
6	436	581.0
8	278	652.0
10	340	254.7

*Net protein released = Test sample – Sum of keratin control and fungus control The data is expressed in terms of standard error [Mean ± S.E (n=3)].

Table 2: Net protein released during the growth of *Microsporum gypseum* on different keratin substrates (Weekly)

Incubation period (Weeks)	Human hair Net protein (µg/ml) *	Horse hair Net protein (µg/ml) *
1	302	690
2	362	701
3	276	728
4	290	752

*Net protein released = Test sample – Sum of keratin control and fungus control The data is expressed in terms of standard error [Mean ± S.E (n=3)].

Observation of degradation of Human hair: (day-wise and weekly)

The perusal of Table 1 gives a picture of the amount of protein released from the human hair during the growth of *Microsporum gypseum* (day-wise). It has been observed that the amount of protein released increased from the 2nd day (123 µg/ml) and the highest was recorded on the 6th day (436 µg/ml).

However, it was noted that the amount of protein released decreased on the 8th day (278 µg/ml), followed by an increase on the 10th day (340 µg/ml). The values are presented in terms of standard error [Mean ± S.E (n=3)]. The results in Table 2 depict the amount of protein released from the human hair during the growth of *Microsporum gypseum* (weekly). It is evident from the results that the amount of protein released increased in the

first two weeks (302,362 µg/ml), followed by a decrease in the third week (276 µg/ml) and a marginal increase in the 4th week (290 µg/ml).

Observation of degradation of horse hair :(day-wise and weekly)

Table 1 gives a picture of the amount of protein released from horse hair during the growth of *Microsporum gypseum* (day-wise). There has been observed a steady increase in protein release from the 2nd day (233.1 µg/ml), and the highest was recorded on the 8th day (652 µg/ml).

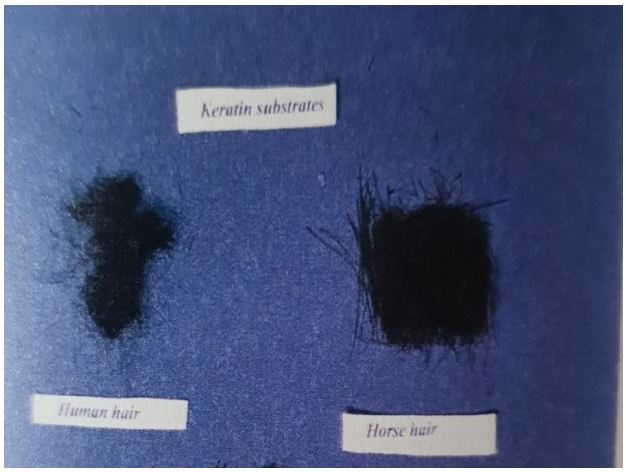

Fig. 1: Keratin substrates of Human hair and Horse hair

Interestingly, a sudden protein content decrease was recorded on the 10th day(254.7µg/ml).The data is expressed in terms of standard error [Mean ± S.E (n=3)]. The results presented in Table 2 gives an account of the amount of protein released from horse hair during the growth of *Microsporum gypseum* (weekly). The results reveal that the amount of protein release has also increased with an increase in the incubation period. A minimum (690 µg/ml) was recorded in the 1st week. Maximum (752 µg/ml) was recorded in the 4th week.

In the present investigation, the ability of *Microsporum gypseum* to degrade human hair and horse hair is studied from the 2nd to the 10th day and also from the 1st week to the 4th week. The net protein released due to the degradation by *Microsporum gypseum* from the components viz., human hair and horse hair has been calculated. The production of protein and growth of the fungi is stimulated by adding keratin substrates to the medium. These fungi grow on them and deteriorated them by releasing high amounts of protein which is in tune with the earlier research wherein the keratinophillic fungi are capable of degrading keratin containing hairs, feathers etc. and releasing proteins which are otherwise very difficult to degrade (Anbu *et al*. 2004; Bentubo *et al*. 2006).

Further, in the present study, it has been observed that horse hair degradation is more rapid than the human hair by the fungus, *Microsporum gypseum* . It was also observed that the amount of net protein released was not in direct proportion with the days of incubation as was it was evident that the day of degradation for the human hair was the 6th day while the day for degradation for horse hair, was the 8th day. In the earlier research studies, it was revealed that the days of degradation for different keratin substances differed with the type of keratin substance employed as well as the type of keratinophilic fungi employed. The earlier research workers found that kertain substances were degraded by fungi in sewage slude (Mushin *et al.* (2001), river beds (Vidal *et al.* (2000), hospital dust and soils at public places (Vidyasagar (2003) etc. Further, Maruthi *et al.* (2012) reported a highly potent keratinophilic fungi namely *Chrysosporium tropicum* was capable of degrading feathers and hair by releasing soluble protein into the medium. Maruthi *et al*. (2012) also reported that among the two substrates used, *Chrysosporium tropicum* had more effect on hair than that of feather.

It is an established fact that the keratinophillic fungi can be predominantly found in the forest soils, utility places of public, areas on and around markets, poultry sheds, cattle sheds, park areas, river beds, beaches, garbage dump areas, bird litter areas etc. (Kumawat *et al*. 2020; Gupta *et al*. 2012). This study of keratin degradation by the keratinophilic fungus *Microsporum gypseum* (day-wise and weekly) has revealed that keratin decomposition depends upon the type of added substrate and enzymatic system of the organism. Hence, intensive studies on the keratinases of these fungi will certainly contribute towards a better understanding of keratin decomposition.

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