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Study of Keratin Degradation by Some Potential Fungal Isolates from Soil

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ABSTRACT *Many of the microorganisms colonizing keratinic substances are not found keratinolytic in a nature and found secondary in succession during the process of keratin deterioration. The present study has been undertaken to know further about the growth and distribution of keratinophillic microorganisms in soil. Soil samples were collected from Barber shop [Suhagi & Gurandi], Chicken shop [Bhaisasur Road], Poultry farm [Gurandi] and Slaughter houses [Madartekri] of different places of Jabalpur district. Different strains of fungi were isolated from these soil samples and further screened for their keratin degrading capacity. Characterization of keratinase enzyme further proved their potential of keratin degrading capacity. 12 strains of fungi were isolated and screened for keratinophilic activity and among these 4 were found to be the potential keratinophilic fungi.*

Introduction

Keratins are the most abundant protein in epithelial cells of vertebrates and represent the major constituents of skin and its appendages such as nail, hair, feather, and wool. The protein chains are packed tightly either in [α–keratins] or in [β-keratins] structures, which fold into final 3-dimensional from [Kim 2007(1), Esawy et al. 2007(2), Krelpak et al. 2004(3)]. Keratins are grouped into hard keratins [feather, hair, hoof and nail] and soft keratins [skin and callus] according to sulphur content [Gupta et al. 2006(4)]. These proteins belonging to the scleropeptides group are compounds that are extremely resistant to the action of physical, chemical and biological agents. One of the main characteristics of keratins is that they have high mechanical stability and resistance to proteolytic degradation, which depends on the disulfide and hydrogen bonds, salt linkages and other crosslinkings [Korkmarz et al. 2004(5), Hoq et al. 2005(6)]. Therefore, keratinous material is water insoluble and extremely resistance to degradation by common proteolytic enzymes such as trypsin, papain and pepsin [Gupta et al. 2006(4), Hoq et al. 2005(6), Gradisar et al. 2005(7)].

World-wide poultry processing plants produce millions of tons of feather as a waste product annually, which consist of approximately 90% keratin feathers, represent 5-7% of the total weight of mature chickens. These feathers constitute a sizable waste disposal problem. Several different approaches have been used for disposing of feather waste including land filling, burning, natural gas production and treatment for animal feed. Most feather waste is land filled or burned which involves expense and can cause contamination of air, soil and water.

A group of proteolytic enzyme which is able to hydrolyze insoluble keratins more efficiently than other proteases is called keratinases produced by some microorganisms [Gradisar et al. 2005(7), Cai et al. 2008(8)]. Many keratinases from species of Bacillus [Hoq et al. 2005(6), Korkmaz et al. 2004(5), Joshi S.G. et al. 2007(9), Cai C. et al. 2008(8), Cortezi et al. 2008(10)], fungi [Kim et al. 2007(1), Friedrich J. et al. 1999(11), Soomoro et al. 2007(12)] and Actinomycetes [Esawy et al. 2007(2), Grandisar et al. 2005(7)] has been reported and some of them were purified and characterized.

Material and methods:

The present study has been undertaken to evaluate the potential Keratin degrading fungi. Soil samples were collected from Barber, Chicken shop, Poultry farm and Slaughter houses of different places of Jabalpur district. Different strains of fungi and bacteria were isolated from these soil samples collected and further screened for their keratin degrading capacity. Characterization of keratinase enzyme further proved their potential of keratin degrading capacity. Soil samples with hair as waste material were collected from Barber shop [Suhagi & Gurandi] and Slaughter house [Madartekri] in Jabalpur district. Soil samples were also collected from Chicken shop [Bhaisasur Road] and Poultry farm [Gurandi] where feather was used as waste.

i] Direct planting technique:-

Pieces of each hair samples [about 1 cm long] were plated directly on the surface of Sabouraud's Dextrose Agar medium supplemented with 40 µg/ ml streptomycin. The plates were incubated at 28°C ± 1°C and the growing moulds were examined at weekly intervals for 5 weeks.

ii] The soil plating technique:-

The bottom of the Petri dish was covered with double sterilized soil [autoclaved at 121°C for 30 min.]. Sterilized distilled water was added for moistening the soil and pieces of each sample [about 1 cm long] were placed on the soil surface. The plates were incubated at room temperature for about 3 weeks and examined at periodic intervals.

Screening for keratinophilic activity:

5 gm of feathers & 5 gm of hairs were weighed & autoclaved. In vials autoclaved feathers & hairs were placed & 2 ml of autoclaved SDA was added over these feathers & hairs. Loops full of different isolated fungal colonies were transferred in different vials and incubated at 28°C ± 2°C for 10 days. After 10days feathers & hairs were washed and dried in sun and then weighed. The difference in weights reveals the keratinophilic activity.

Characterization and identification of fungal isolates: -

Among the different fungal isolates, those exhibiting maxi-

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mum keratinase activities were subjected to morphological characterization and identified using the laboratory Manual of Mycology (Larone) and Dr Fungi.

Effect of temperature on keratinase:
The effect on temperature keratinase activity was, determined by the 20 µl keratinase [3mg ml 1 protein] to 1.5 phosphate buffer [100 mmol 1, pH 7.5] containing 15 mg powdered keratin and incubating at a range of temperature [26°C, 30°C, 34°C, 38°C] for 24 hrs. Peptide release was determined spectro-photometrically [600nm].

Effect of pH on keratinase:
The effect on pH keratinase activity was, determined by the 20 µl keratinase [3mg ml 1 protein] to 1.5 phosphate buffer [100 mmol 1, pH 7.5] containing 15 mg powdered keratin and incubating at a range of pH [6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0] for 24 hrs. Peptide release was determined spectro-photometrically [600nm].

Result and discussion:
By using baiting techniques [hairs and feathers] the keratinophilic fungi were isolated and identified. With the help of slide culture technique different types of fungus were isolated and identified under Motic Software. On screening for keratinophilic activity Fusarium solani, Aspergillus niger, Penicillium chrysogenum and Trichoderma harzianum proved to be the most potential keratinophilic fungus (Plate 1 &2). The same is expressed in table 1 &2.

The keratinophilic activities of several strains of fungus were evaluated out of which Penicillium chrysogenum showed maximum keratinophilic activity when feathers were used as substrate. Feathers contain over 90% of crude protein in the form of keratin. Feathers waste represents a potential protein alternative to more expensive dietary ingredients for animal feed. When hairs were used as substrates, keratinophlilc activity was evaluated by comparison of initially weight of hairs and after 10 days of incubation.

The different isolated fungal strains showed maximum activity in different temperatures such as Fusarium solani showed maximum keratinase activity at 36°C, Aspergillus niger at 24°C-28°C, Penicillium chrysogenum at 24°C and Trichoderma harzianum stain at 32°C. In Table-3 it was observed that the fungal keratinophilic enzyme was more active in temperature range of 28°C-34°C. From table 4 it is evident that the optimum pH for keratinase production is 8.0 – 9.0.

Table 1: Comparison of Keratin degradation [Hairs] by some potential fungal isolates.

FUNGI	INITIAL WEIGHT [HAIRS]	FINAL WEIGHT [AFTER 10 DAYS]
Fusarium solani	5 gm	4.06 gm
Aspergillus niger	5 gm	4.02 gm
Penicillium chrysogenum	5 gm	3.42 gm
Trichoderma harzianum	5 gm	3.50 gm

Table 2: Comparison of Keratin degradation [Feathers] by some potential fungal isolates.

FUNGI	INITIAL WEIGHT [Feathers]	FINAL WEIGHT [AFTER 10 DAYS]
Fusarium solani	5 gm	3 gm
Aspergillus niger	5 gm	4 gm
Penicillium chrysogenum	5 gm	1 gm
Trichoderma harzianum	5 gm	4 gm

Table -3: Effect of temperature on fungal isolates

TEMPERATURE	Fusarium solani [OD 600nm]	Aspergillus niger [OD 600nm]	Penicillium chrysogenum [OD 600nm]	Trichoderma harzianum [OD 600nm]
24°C	0.475	1.715	1.779	0.849
28°C	0.641	1.795	0.561	0.622
32°C	0.564	1.581	1.634	1.838
36°C	0.762	1.264	0.364	0.635

Table 4: Effect of different pH on keratinase activity of fungal isolates

pH	Fusarium solani [OD 600nm]	Aspergillus niger [OD 600nm]	Penicillium chrysoginum [OD 600nm]	Trichoderma harzianum [OD 600nm]
6.0	0.532	1.057	1.155	0.645
6.5	0.389	1.059	0.496	0.685
7.0	0.486	1.083	0.363	1.287
7.5	0.710	1.176	0.991	0.759
8.0	2.568	0.629	0.998	0.426
8.5	2.405	1.728	0.884	1.137
9.0	1.734	0.932	1.922	1.432

Plate 1 Showing feathers colonized by Fusarium solani

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Plate 2 showing colonization of Fusarium solani on hairs collected from barber shop.

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