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Extracellular keratinase of some dermatophytes, their teleomorphs and related keratinolytic fungi

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ABSTRACT

Seven species of Microsporum, 4 of Trichophyton and 1 Epidermophyton including 55 species belonging to 33 genera were studied for extracellular keratinase. The preliminary screening was done as their ability to develop clearing zone formation. The keratinase of Microsporum species ranged from 50-300ku/ml and of Trichophyton 100-140ku/ml while that of Epidermophyton was 20ku/ml.

Key words: Dermatophytes, teleomorphs, keratinolytic, keratinase.

INTRODUCTION

Dermatophytes develop their teleomorphs in Ascomycetes Onygenales and can survive in soil for very long period in the form of their hard ascoma, asci and other protective plectenchymatous fungal tissue. Dermatophytes looses their ability to produce macroconidia, hence their differentiation with anamorphs of other related fungi mainly *Chrysosporium*, *Malbranchea*, *Acremonium* etc. become difficult.

Keratinolytic fungi are a group of fungi that degrades keratin, and they include several of the most important dermatophytes and saprophytic species. Most of the keratinophilic fungi are soil inhabitants **(1,2)** has studied the degradation of peacock feathers, using the keratinases of 20 different fungi and have reported that some dermatophytes were most active. Keratinophilic fungi are important ecologically and recently have attracted attention throughout the world **(3)**. Keratinases are the key enzymes elaborated by keratinophilic fungi for the degradation of keratin **(4,5)**.

Keratinase producing microorganisms have ability to degrade chicken feathers, hair, nails, wool etc. Keratinases belong to the group of serine hydrolases that are capable of degrading keratin, a fibrous and insoluble structural protein extensively cross-linked with disulfide, hydrogen and hydrophobic bonds. The keratin chain of hair (hard keratin) is similar to that of the epidermis (soft keratin) as it is highly packed as an α-helix but differs from the latter in that it contains several fold higher amounts of cysteine. In feathers, the polypeptide chain assumes a β-confirmation, which is more readily hydrolysed than α-keratin **(6)**.

The hair from humans and animals and feather from birds which come to the soil either as dropped off or dead are affected by microbial decomposition. In the past few decades some studies on the decomposition of keratin in

submerged cultures appeared biodegradation of keratin by using *Chrysosporium* and other related fungi in submerged culture is reviewed **(7)** and scattered reports are available in literature. The process of keratin decomposition has also been found to be very fast in soil and it plays a very important role in energy transformation and nutrient cycling in soil **(2)**. Long term studies on the biology of *Chrysosporium*, and other scattered reports, revealed that its wide distribution is due to its antagonistic potential and ability to produce enzymes and other extracellular metabolites **(2)**. *Chrysosporium tropicum* produce amylase, urease, pectinase, keratinase, esterase lipase, leucine aryl amidase, cystine arylamidase, alpha galactosidase, alpha glucosidase, beta glucosidase, N acetyl glucosaminidase, alpha mannosidase **(8).** The most promising application of keratinase is in the production of nutritious, cost effective and environmentally benign feather meal **(6)**. Microbial keratinase can be used in the textile, cosmetic and leather industries and in medicine **(6,9,10)**.

MATERIALS AND METHODS

Isolation of keratinophilic fungi from soil

Fungi were isolated by the hair baiting method from the soil samples as reported earlier [**11**]

Screening of fungi for keratinolytic enzymes

Fungi were screened on solid medium milk agar plates. The fungi were inoculated on to plates and incubated for 10 days at 28±2^{0}C. Fungal strains that produced clearing zone in this were selected. The diameter of clear zone was measured to quantify activity. Keratinase activity of fungus was detected as a clear zone around the colony.

Submerged cultivation of fungi

Liquid media according Wawrzkiewicz *et al*. (12) was used for crude keratinase enzyme production. Chicken feathers were used as a source of keratin. 500mg of keratin source was added to the mineral salt medium: K2HPO4, 1.5 g/L; MgSO4.7H2O, 0.05 g/L; CaCl2, 0.025 g/L; FeSO4.7H2O, 0.015 g/L; ZnSO4.7H2O, 0.005 g/L. Erlenmeyer flasks containing 50 ml of mineral salt medium with keratin source were sterilized at 121°C for 15 min. Each flask was inoculated with 10 ml of 8 days old spore suspension. All variants were prepared in 3 replications. The cultures were harvested after 10 days. Aliquots of the culture medium were taken from the flask, filtered, and then centrifuged at 4000 rpm for 5 min. The supernatant was used as the crude enzyme (enzyme source).

Determination of keratinolytic activity

Keratinase activity was assayed by the modified method of Yu *et al*. (13). In brief, 20 mg of chicken feathers were suspended in 3.8 ml of 100 mM Tris– HCl buffer (pH 7.8) to which 200 µl of the culture filtrate (enzyme source) was added. The reaction mixture was incubated at 37°C for 1 h. After incubation, the assay mixture was dipped into ice-cold water for 10 min and the remaining feathers were filtered out. Then the absorbance of the clear mixture was measured at 280 nm (Shimadzu, 1800 UV– vis spectrophotometer). The keratinase activity was expressed as one unit of the enzyme corresponding to an increase in the absorbance value 0.1 (1 KU=0.100 corrected absorbance), 1 KU=keratinase unit.

RESULTS AND DISCUSSION

Seven species of *Microsporum*, 4 of *Trichophyton* and 1 *Epidermophyton* including 55 species belonging to 33 genera were studied for extracellular keratinase. The preliminary screening was done as their ability to develop clearing zone formation [Table 1].

Microsporum species showed keratinases ranging from 56-300 ku/ml. The keratinases of 4species of *Trichophyton* ranged from 100-140ku/ml. Teleomorph of *Microsporum* and *Trichophyton* namely *Nannizzia gypsea*, *Arthroderma simmi* and *Apinisia queenslandica* produced 60, 76 and 58 ku/ml keratinase. Anamorphic fungi related to dermatophytes exhibited very high range of keratinase. Among 8 species of *Acremonium* keratinase values ranged from 92-781ku/ml. Fifteen species *Chrysoporium* tested showed maximum value of 320ku/ml. *Ctenomyces serratus*, *Gymnoascus reessi*, *Trichoderma viride* and *Aspergillus* sp produced 310,, 312, 100 and 278 ku/ml keratinase. Keratinase is the key enzyme in fungal invasion of skin and its appendages [**14**] and have been mostly studied for *Trichophyton*, *Microsporum* and other dermatophytes [**12, 15-19**]. Other studies on keratinase of dermatophytes were carried out (**12,17,18,20**).

Maruthi et al (**21**) studied keratinase of *Chrysosporium tropicum* as 8.56ku/ml, venketesan et al found 77.65 ku/ml keratinase of *Microsporum gypseum* and 76.20 ku/ml in case of M. canis. Anbu et al ,2006 found highest keratinase in *Trichophyton mentagrophytes*. Kacinova et al (**22**) studied extracellular keratinase of *Chrysosporium tropicum* and *Trichophyton ajelloi* and optimize conditions of their production.

TABLE 1, KERATINASE OF SOME KERATINOLYTIC FUNGI

FUNGUS	SPECIES	ZONE MM	KERATINASE KU/ML
*Acremonium**	8*	55	92-781**
Alternaria alternata		25	73±06
Amauroascus kuehnii		50	76±09
*Aphanoascus**	3*	22	69-34**
Apinisia queenslandica		8	58±05
Arthroderma simii		20	40±05
*Aspergillus**	9*	21	33-278**
Auxarthron conjugatum		35	120±03
Blastomyces sp.		25	100±02
Botryotrichum piluliferum		22	45±04
Chaetomium globosum		50	56±05
Cladosporium macrocarpum		22	40±05
*Chrysosporium**	15*	50	115-320**
Ctenomyces serratus		50	310±06
*Curvularia**		0	20-22*
Epidermophyton floccosum		25	20±01
*Fusarium**		60	26-88**
Geomyces pannorum		0	26±02
*Gymnoascus**	2*	22	310-312**
Fusariella obstipa		15	ND
Geotrichum condidum		0	2±01
Histoplasma capsulatum		25	20±02
Humicola grisea		65	180±06
*Malbranchea**	6*	0	100-165**
*Microsporum**	7*	58	56-300**
Mucor sp.		55	16±02
*Myceliophthora**	2*	0	45-60**
Nannizzia gypsea		50	60±05**
*Paecilomyces**	3*	55	12-40**
*Penicillium**	6*	25	10-45**
*Trichophyton**	4*	20	100-140**
Trichoderma viride		45	100±10
*Verticillium**	2*	10	55-66**

**Represent number of species and **range of keratinase*

CONCLUSION

It is concluded that all the 55 fungi including dermatophytes, their teleomorphs produced sufficient amount of keratinase amounting to maximum 781ku/ml.

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