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# Incidence of Dermatophytes and Other Keratinolytic Fungi in the Soil of Amravati (India)

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**Abstract:** A total of one hundred fifty soil samples were screened for the presence of keratinophilic fungi and *Microsporum gypseum* (34%), *Trichophyton mentagrophytes* (22%), *Trichophyton rubrum* (14%), *Trichophyton tonsurans* (10%), *Microsporum canis* (10%), *Aspergillus fumigatus* (4%), *Aspergillus flavus* (4%) and *Aspergillus niger* (6%) were isolated and identified. The keratinolytic potential by release of protein from utilization of hair keratin was found to be 500 μg $mL^{-1}$ protein in *Microsporum gypseum*, 400 μg $mL^{-1}$ protein in *Microsporum canis*, 200 μg $mL^{-1}$ protein in each *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Trichophyton tonsurans*, 180 μg $mL^{-1}$ protein in each *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* after 60 days of incubation.

**Key words:** Dermatophytes, keratin degradation, protein extraction, Trichophyton, microsporum, aspergillus

## INTRODUCTION

In the kingdom Eumycota (true fungi), order Onygenales, have the keratinolytic members that occur in the soil as keratin decomposers. Some of them (the dermatophytes) are potential pathogens and can cause infection in the skin, hair, nail and scalp of mammals (Forbes *et al*., 2002). The pathogenicity of dermatophytes is directly linked to their ability to produce keratinase. Studies on keratinolysis revealed the crucial role of sulphitolysis in the degradation of cysteine resulting in the production of major end products sulphate and thiosulphate (as an unusual metabolite) in the medium containing wool and pure keratin (Hasija, 1999).

In India, a number of reports have appeared on distribution of keratinophilic fungi and related dermatophytes in the soil, which included the reports on isolation of fungi from soils of Jaipur and Mount Abu, Rajasthan (Garg, 1966), Mumbai, Maharashtra (Deshmukh, 2004), Chilka-Lake, Orissa (Ghosh and Bhatt, 2000), Mysore, Karnataka (Deshmukh *et al*., 2000), Damoh, Madhya Pradesh (Khanam and Jain, 2002) and Mussoorie (Deshmukh and Agrawal, 1985). The fungal isolates showed species of the genera *Chrysosporium*, *Ctenomyces*, *Microsporum*, *Trichophyton*, *Aspergillus*, *Penicillium*, *Fusarium* and *Uncinocarpus*. The keratinolytic potential (keratin degradation) of certain fungi e.g., *Cylindrocarpon lichenicola*, *Graphium cuneiferum*, *Microsporum gypseum*, *Microsporum fulvum*, *Scytalidium* species and *Fusarium solani* was studied by Malviya *et al*. (1992) and Oyeka and Gugnani, (1997). Tambekar and Agrawal (2002a, b and c) studied the keratinolytic potential, pathogenicity and *in vitro* and *in vivo* effect of garlic extract on *Trichophyton mentagrophytes*.

Since the prevalence of dermatophytes and keratinolytic fungi in soils of Amravati (India) has not been investigated, an attempt was made in the present study to find out the distribution of fungal flora by their isolation, identification and keratinolytic potential.

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## MATERIALS AND METHODS

### Collection, Isolation, Characterization and Identification of Fungal Isolates

A total of 150 soil samples were collected randomly from different localities in Amravati City, Maharashtra State, (India) over a period of four months from July to October 2006, in polythene bags and transported to laboratory for further analysis. The hair bait technique of Vanbreuseghem (1952) was used to isolate the keratinophilic fungi. Sterile petri dishes were half-filled with soil samples and moistened with water before being baited by burying sterile human hair in the soil. These dishes were incubated at room temperature and examined for fungal growth after five days and then daily until four weeks. When growth was observed, it was cultured on Sabouraud's dextrose agar medium supplemented with Chloramphenicol (50 mg $L^{-1}$) and cycloheximide (500 mg $L^{-1}$). The isolated fungi were identified on the basis of spore morphology, cultural characteristic and pigment formation on the reverse of slant (Forbes *et al*., 2002).

### Determination of Keratinolytic Potentials of Fungal Isolates

The hairs of 22 years old male were washed in solution of detergent and rinsed in distilled water. These were sterilized at 121°C for 15 min. The sterile hairs were defatted in mixture of chloroform and methanol (1:1) and washed twice with sterile distilled water. The air-dried hairs were cut into pieces and 1 g-weighed cut hairs were added in to 50 mL Basal salt medium in Erlenmeyer flask. Ten days old mycelial growth on Sabouraud's dextrose agar medium was scraped and inoculated in Basal salt medium in conical flask and incubated at room temperature for 60 days. The total protein was estimated according to the method described by Lowry *et al*. (1951) by using Bovine serum albumin as a standard and Folin-Ciocalteau as reagent. The controls were without inoculum or substrate used to compare the results.

## RESULTS AND DISCUSSION

A total of one hundred fifty samples was screened for the presence of keratinophilic fungi, out of which seventeen soil sample contained *Microsporum gypseum*, eleven samples contained *Trichophyton mentagrophytes*, seven samples contained *Trichophyton rubrum*, five sample each showed presence of *Microsporum canis* and *Trichophyton tonsurans*, three sample showed *Aspergillus niger* and two each samples showed presence of *Aspergillus flavus* and *Aspergillus fumigatus*. These isolates represent eight species of among the three genera. The frequency of occurrence of these fungi in different soil samples was noticed as, *Microsporum gypseum* (34%), *Trichophyton mentagrophytes* (22%), *Trichophyton rubrum* (14%), *Trichophyton tonsurans* (10%), *Microsporum canis* (10%), *Aspergillus fumigatus* (4%), *Aspergillus flavus* (4%) and *Aspergillus niger* (6%) (Table 1).

*Microsporum gypseum* (34%) was prominent among the species isolated in the present study. *M. gypseum* (13.6%) and *Chrysosporium indicum* (21.6%) were isolated from soils of Mysore. Particularly *Chrysosporium indicum* dominates the Indian soil flora because it is adapted to warmer condition in India but was not reported in the present study. *Microsporum gypseum* has been reported to be in higher number to *Chrysosporium indicum* in other Indian plane by various workers (Deshmukh *et al*., 2000). The other prominent species isolated was *Trichophyton mentagrophytes* (22%). It was also reported from costal habitat of Goa (Deshmukh and Agrawal, 1983) and it has been found in many Indian planes. *Trichophyton rubrum* (Vanbreuseghem, 1952) was third species according to its distribution in the present study (Fig. 1). *Trichophyton tonsurans* and *Microsporum canis*,the next species were distributed 10% each. Garg (1966) also reported these fungi from Jaipur and Mount Abu. The Microsporum *canis* was isolated from Mysore by Deshmukh *et al*. (2000).

Table 1: Percentage distribution and Keratin degradation by keratinophilic fungi in Amravati

| Fungal isolate | Percent distribution | Protein yield (μg $mL^{-1}$) |
|---|---|---|
| *Aspergillus flavus* | 4 | 180 |
| *Aspergillus fumigatus* | 4 | 180 |
| *Aspergillus niger* | 6 | 180 |
| *Microsporum canis* | 10 | 400 |
| *Microsporum gypseum* | 34 | 500 |
| *Trichophyton mentagrophytes* | 22 | 200 |
| *Trichophyton rubrum* | 14 | 200 |
| *Trichophyton tonsurans* | 10 | 200 |

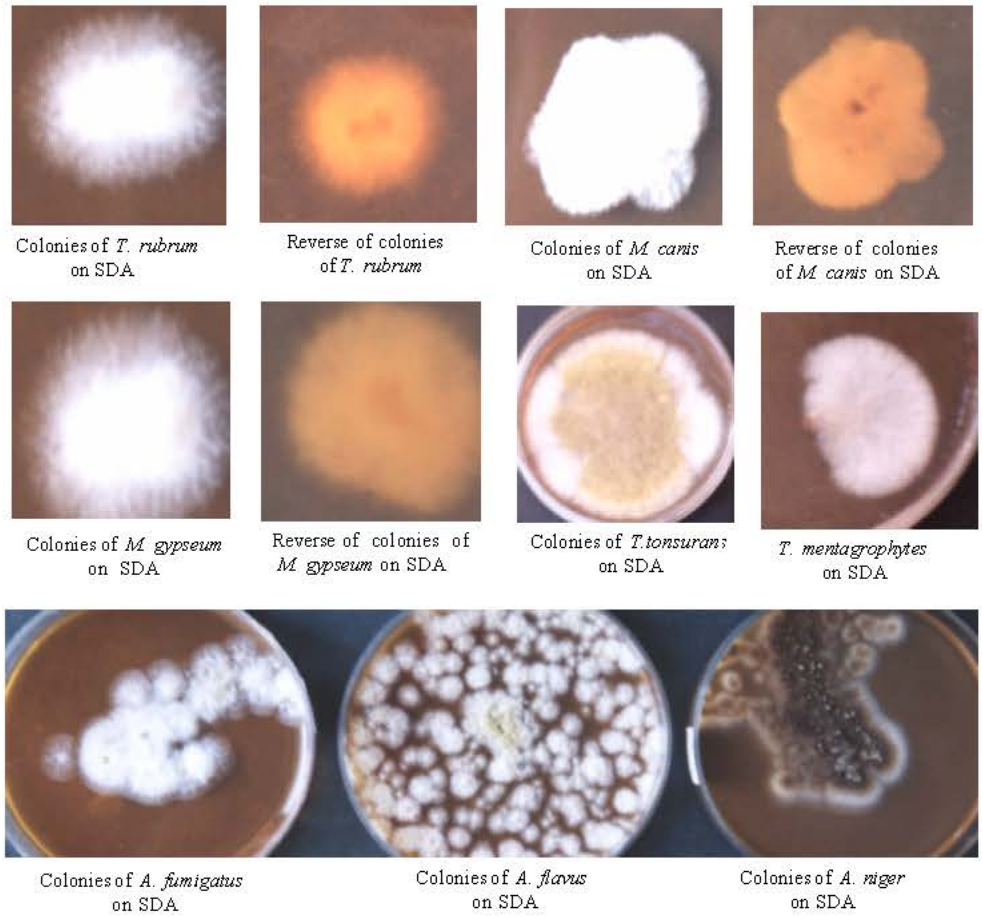


**Fig. 1: Keratinophilic fungi isolated from Amravati, Maharashtra State, India**

**In addition to well-known dermatophytes, some species of saprophytic fungi such as *Aspergillus flavus* (4%), *Aspergillus fumigatus* (4%) and *Aspergillus niger* (6%) were isolated in the present study which in supports of previous finding of Khanam and Jain (2002) from Damoh (MP, India). The keratinolytic potential of fungal isolates was determined by protein released in basal medium from utilization of hair keratin after sufficient time of incubation. *Microsporum gypseum* and *Microsporum canis* released 500 and 400 μg $mL^{-1}$ of protein, respectively. Malviya *et al.* (1992) reported the formation of 488 μg $mL^{-1}$ proteins from keratin degradation by *Microsporum gypseum* after 60 days of incubation. In this study the each *Trichophyton rubrum*, *Trichophyton mentagrophytes* and**

*Trichophyton tonsurans* released maximum of 200 μg $mL^{-1}$ protein and each *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* released maximum of 180 μg $mL^{-1}$ proteins after 60 days of incubation (Table 1).

The data obtained from this study adds the information on the flora of keratinophilic fungi of Amravati, Vidharbha region of Maharashtra State, India. It appears from this study that the keratinophilic flora of this area is somewhat different from that in other parts of India. This may be due to the high temperature in summer, humidity and temperate climatic conditions prevailing in this area. Thus the present study reveals the presence of important and common dermatophytes and keratinophilic fungi in Amravati, which are significant from central India and its ecological point.

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