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Occurrence of Keratinophilic Fungi in Soils of Ujjain

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ABSTRACT: Soil is well-known as a route to fungal infection in human. Recently, human exposure to potentially pathogenic fungi is threatening the health of people. Keratinophilic fungi in the soil are diverse group that depend on different factors, such as vertebrates and human activity. These fungi play vital task in decomposing keratinous debris of man and animals in nature. In order to study the prevalence of keratinophilic fungi present in soil of Ujjain (holy city), many soil samples were collected. These samples were screened using hair baiting techniques for isolation. Out of total samples, many of them (87.50%) were positive for growth of keratinophilic fungi. Present results show the occurrence of keratinophilic fungi in soils of different sites in Ujjain and may have a significant role in keratin degradation in the environment. Keratinophilic fungi are an ecologically important group of fungi that cycle one of the most abundant and highly stable animal proteins on earth - keratin. This article briefly explains how to isolate and identify them, the process of keratin degradation, and the ecological role of this important but unnoticed group of minute keratin cycling machines present in soil. We believe that Indian soil contains many more such fungi

KEYWORDS: keratinophilic fungi, soil, Ujjain, isolate, identify, degradation, environment, keratin, ecological

I. INTRODUCTION

Keratinophilic fungi are a group of fungi that colonize various keratinous substrates degrading them to components of low molecular weight. These include a variety of filamentous fungi comprising mainly hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a variety of non-dermatophytic keratinophilic fungi. Most species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur in soil as saprophytes and are termed geophilic, for example, *Microsporium gypseum* and *Trichophyton terrestre*. Non-dermatophytic keratinophilic fungi, including species of *Chrysosporium* and other genera of fungi, are known to occur as saprobes in soil; some of them are potential pathogens of humans and animals. Studies on keratinophilic fungi of soils of varying habitats in different countries have demonstrated frequent occurrence of *Chrysosporium* spp and dermatophytes like *Microsporium gypseum* complex (*M. gypseum* and *M. fulvum*), *Trichophyton terrestre*, and *Trichophyton ajelloi*. Other dermatophytes known to occur infrequently or sporadically in soil in some countries are *M. cookei*, *M. vanbreuseghemi*, and *T. gloriae*. *Trichophyton simii*, *T. mentagrophytes*, *M. nanum* and *M. persicolor*, well-known zoophilic dermatophytes, have also been frequently recovered from soil in some countries. [1,2]

Soil samples were collected from a depth of approximately 4-6 inches in zip polythene bags during December to February of the study period with the help of a stainless steel spatula, 6" inch long and 2" wide disinfected with 70% isopropyl alcohol each time before and after use. The polythene bags were sterilized with 70% isopropyl alcohol and left to dry for 3-4 minutes. Following this, the inside of each bag was rubbed with a sterile swab, which was then rolled on a plate of Sabouraud dextrose agar (SDA). [3]

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Microsporium gypseum

The SDA plates were incubated at room temperature for 4-5 days. No growth of molds occurred; however, in some of the plates single bacterial colonies were observed. Thus the sterility of the zip polythene bags was ensured for the purpose of this study. The collected soil samples were shortly transported to New Delhi CSIR. For determining the approximate pH of the soil samples, a small quantity of the sample was suspended in distilled water five times its volume, its pH was determined with the help of pH indicator strips, and then confirmed by testing with a pH meter.

Samples were processed in the Microbiology laboratory of New Delhi CSIR. For identification, pieces of human hair 0.6 to 1.6 cm in length (sterilized by autoclaving), were spread on the soil samples in sterile Petri dishes. Small quantities of sterile distilled water (10-15 mL) were poured on the hair-baited plates. The plates were incubated at room temperature on the laboratory bench under normal light. Sterile water was added periodically to provide moisture needed for fungal growth. Fungal growths appearing on hair baits after 2-4 weeks of incubation were microscopically examined and cultured on slopes of Mycobiotic agar to get pure cultures. Identification of the isolates was accomplished by studying the colonial and microscopical characters of the isolates in detail and comparing them with descriptions of suspected fungi in standard books and manuals.[4,5]

II. DISCUSSION

Keratinophilic fungi are mesophilic while a few have grown at 37°C such as *C. tropicum*, *Chrysosporium keratinophilum*, *C. queenslandicum*, etc. Nevertheless, geophilic dermatophytes grow best at 25–30°C which were identified. *Microsporuzri pypseim*, *Trichophyton ajelloi*, *T. terrestre* were also identified. A recurring existence of *Chrysosporium pannorum* and *Mortierella* spp. with exceptional incidence of *Trichophyton terrestre* and *Chrysosporium* sp. were found in Ujjain. Some varieties are thermotolerant, and recently, it was shown that they can adapt to adverse temperatures for survival. Season is quite anticipated with respect to dermatophytic flora of the soil, which has climatic differences resulting in change of temperature. *Keratinomyces ajelloi*, *Trichophyton Terrestre*, and *Microsporium gypseum* were found in soil samples during April and in August in Ujjain. [6,7]

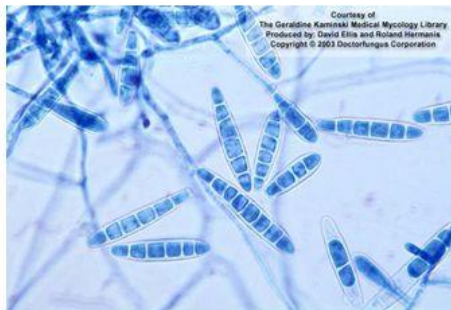
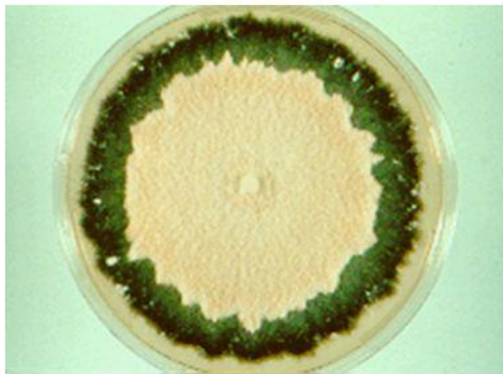
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Trichophyton ajelloi



It was studied that a plenty of keratinophilic fungi in carbonate field and chernozemic soils have greater humus substance in comparison to gray podzolic soils. Keratinophiles can decompose extremely compound carbon complexes in many culture media, a simple carbon source can be used and not influence their qualitative spreading but the findings on their quantitative dispersal may disclose fascinating truths. There was a better percentage of spore growing in *Arthroderma uncinatum* and *Ctenomyces serratus* at 90–100% RH. A higher number of keratinophilic fungi were described in soils with exceptional humus value. In all levels of soil profile extreme in the region with high humus content, there exist *M. gypseum* and *T. georgii*, while *T. oanbreuseqhemii* and its flawless state *A. gertleri* were primarily found in soils with low humus value. A repressive sodium chloride impact on the growth has been stated of dermatophytes. Growth was reserved by NaCl in *Microsporium*, *Epidermophyton*, *Trichophyton*, etc.[8,9]

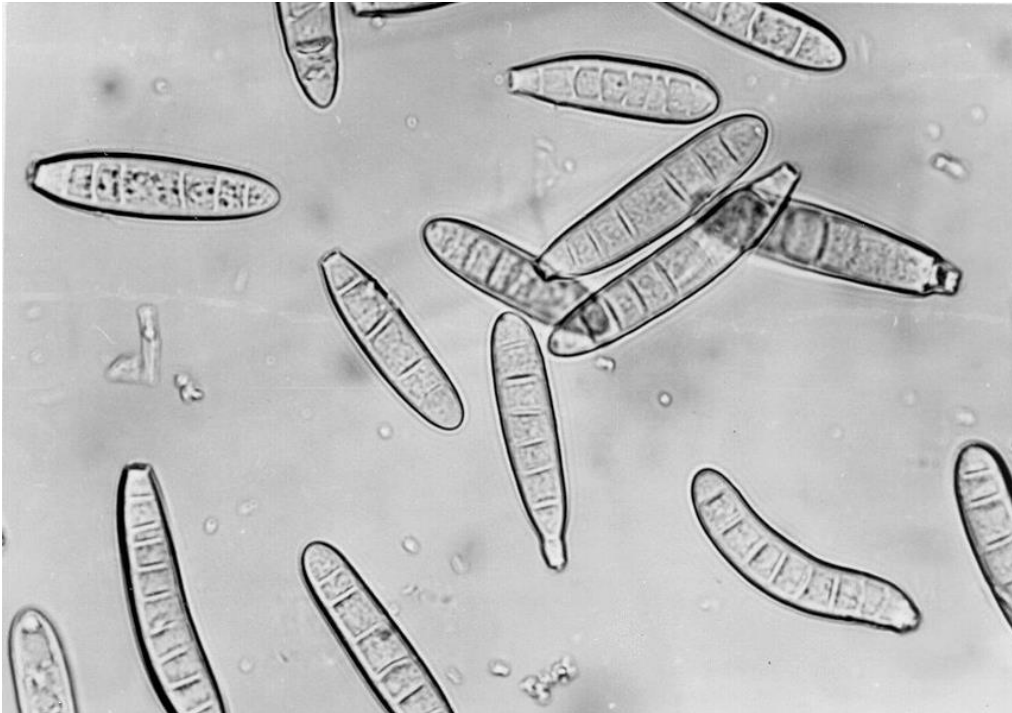
Microorganisms are described to produce keratinase as *Doratomyces microsporus*, *Alternaria radicina*, *Trichurus spiralis*, *Aspergillus sp.*, *Rhizomucor sp.*, *Absidia sp.*, etc. Keratin hydrolysis was the most active in *Verticillium tenuipes*, *Trichophyton equinum*, and *T. mentagrophytes* in peacock feathers areas where peacocks inhabited in Ujjain. *T. mentagrophytes*, *T. verrucosum*, and *Keratinomyces ajelloi* degraded hair, whereas only *T. gallinae* degraded chicken feathers where chickens inhabited in Ujjain.

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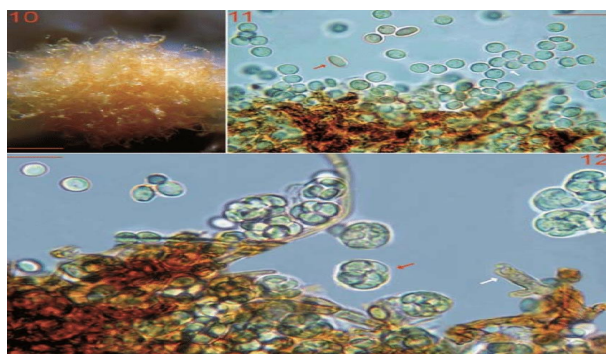
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Arthroderma uncinatum

Five keratinophilic fungi, i.e., *Chrysosporium indicum*, *Geotrichum candidum*, *Gymnoascoideus petalosporus*, *Scopulariopsis brevicaulis*, and *Talaromyces trachyspermus*, which grow on human hair in stationary culture, have been examined. Hair was studied on criteria of cysteine, cystine, inorganic sulfate, thiosulfate, total protein, keratinase, and change in alkalinity. *Gymnoascoideus petalosporus* showed degradation to remaining isolates when grown on human scalp hair. These were identified from Ujjain.[10,11]



Gymnoascoideus petalosporus

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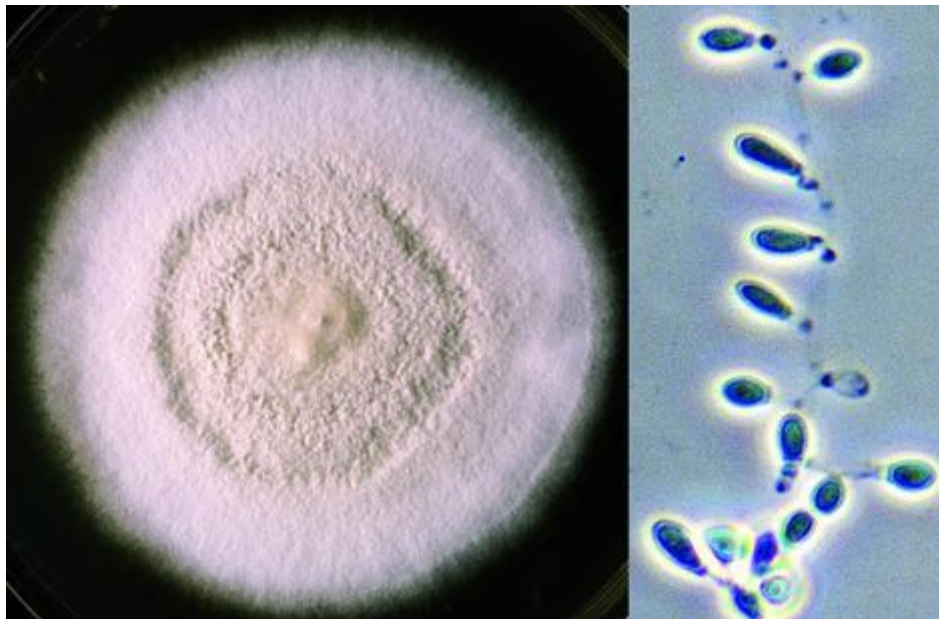
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III. RESULTS

The frequent occurrence of *Chrysosporium* as a geophilic keratinophilic fungus in Ujjain soil is in agreement with that recorded in surveys of keratinophilic fungi of soils in several other cities. Our knowledge of ecology and epidemiology of dermatophytes and the factors influencing their transmission has helped us understand better the natural history of dermatophytoses. Human infections due to the geophilic dermatophyte, *M. gypseum* complex are infrequently or rarely known, particularly in Ujjain. Comprehensive studies may reveal many more cases of skin and scalp infections due to *M. gypseum* in Ujjain.[12,13]

Microsporium gypseum complex was the most frequently isolated keratinophilic fungus being present in 50% of the samples.



Chrysosporium sp

The majority of the isolates of this species complex originated from soil samples collected from habitats of cows, goats, pigs, dogs and horses. The isolates of *M. fulvum* formed buff to pinkish-buff colored colonies with the reverse colorless to yellow-brown. Microscopically numerous, thin, rough-walled, and relatively longish and bullet shaped macroconidia. *Chrysosporium* was represented by 14 isolates, seven identified as *C. indicum*, four as *C. tropicum*, one as *C. keratinophilum*, and two as unidentified *Chrysosporium* sp. Two isolates were identified as *Sepedonium* sp. *Chrysosporium* species formed moderately fast growing, cream-colored colonies, dense or powdery at the center. Conidia were hyaline, smooth-walled sessile or on short protrusions or short branches. Species differentiation was done according to characteristics of conidia. *C. indicum* isolates were characterized by sub-hyaline, smooth, thin-walled, obovoid, ellipsoidal conidia, frequently elongated, less than 3 µm broad with a concave upper surface; intercalary, conidia were also observed. *C. tropicum* isolates formed pyriform, smooth-walled conidia of 6-8 µm. The conidia of the isolate of *C. keratinophilum* were subhyaline, thick-walled obovoid with conspicuous basal scars, smooth-walled or slightly rough-walled. *Sepedonim* sp. formed yellowish orange colored colonies; microscopically characteristic globose spiny macroconidia were observed.[14]

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IV. CONCLUSIONS

Dermatophytes are a unique group of fungi that infect keratinous tissue and are able to invade the hair, skin, and nails of a living host. This closely related group of organisms can be categorized into one of three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*.



Epidermophyton sp.

Species within these genera that do not invade keratinous tissue are by definition excluded from the dermatophytes. As with a number of fungi, dermatophytes may exhibit two phases in their life cycle: the anamorph state (imperfect or asexual phase) that is the state isolated in the laboratory, and the teleomorph state (perfect or sexual phase). The teleomorphs for all dermatophyte species have not yet been identified, but the generic name for both *Trichophyton* and *Microsporum* is *Arthroderma* (the obsolete name *Nannizzia* has been found to be identical). Dermatophytes are a closely related homogeneous clade of keratinophilic filamentous fungi and are associated with the stratum corneum of the skin, as well as hair and nails on the living host. They possess similar appearance, physiology, antigenicity, growth requirements, infectivity, and pathology. These fungi are classified in the anamorphic genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Some dermatophytes may reproduce sexually when the opposite mating types are crossed with each other. The single ascomycete genus *Arthroderma* accommodates the known sexual forms of all of the dermatophytes.[15]

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