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Isolation and Identification of Keratinophilic Fungi from Soil Samples of Different Animal Habitat of Ajmer District, India

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ABSTRACT

Thirty six soil samples were collected randomly from the 12 garbage dumping sites of Ajmer district. These soil samples are rich in pathogenic keratinophilic fungi including dermatophytes. These samples screened for presence of keratinophilic fungi using ''Hair baiting technique'' for isolation. Fungal growth appearing after 3-4 weeks of incubation at room temperature. Isolates were culture on mycological media and identify on the basis of colonial and microscopic feature. The keratinophilic fungal isolates were Aspergillus niger, Microsporum gypseum, Microsporum canis, Trichophyton tonsurans, T. mentagrophytes, T.equinum, T. rubrum, Chrysosporium sp. On the result concluded that these isolated fungi are pathogenic for animals and human beings.

Key words: *Keratinophilic fungi, Soil samples, Hair baiting technique, Ajmer district.*

INTRODUCTION

Keratinophilic fungi are ecologically an important group of fungi which are found in soil. During the past years, many researchers reported and isolated Keratinophilic fungi around the world. Keratinophilic fungi are involved in the breakdown of keratinaceous substrates and are the present in the environment worldwide. Keratinophilic fungi have ability to degrade keratin. Keratin is the cornified part of the epidermis of vertebrates, which includes feather, hair, nail, horn, wool etc. After the death of animals or insects the remain are added to the soil. A lot of substrate rich in keratin is also added to the soil. Keratin can be divided into soft and hard keratin depending on the cystine content in it. The molds capable of attacking keratinized tissues are termed as keratinophilic fungi. These belong to hyphomycetes and several other taxonomic groups hyphomycetes include dermatophytic fungi and a variety of non-dermatophytic keratinophilic fungi. Most of species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur soil as saprophytes and are termed geophilic, for example *Microsporum 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 keratinophilic fungi are called dermatophytes because of their capacity to parasitize keratinic tissues in human and animals.

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Dermatophytes are fungi which parasitize keratinized tissues in human and animal and causes dermatophytosis. The dermatophytosis, which is also known as cutaneous mycosis are infections of keratinized epidermal tissues of human beings. All the dermatophytes are keratinophilic in nature. Dermatophytes are one of the most frequent skin diseases of human, pets and livestock. The disease is widely distributed all over the world with various degrees and more common in men than in women.

On the basis of the morphology and sexual reproduction dermatophytes are included in arthroderma or in hannizza, two closely related genera within the family gymnoascaceae; order eurotiales in the ascomycotina recognized three genera of dermatophytes: *Microsporum*, *Trichophyton* and *Epidermophyton*; which are based on clinical rather than mycological observations.

Skin, Hair, Nail and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses. Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock.

A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, *viz. Microsporum canis, Trichophyton mentagrophytes, Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporum gypseum*.

MATERIAL AND METHODS

Collection of soil samples:

Thirty six soil samples were collected randomly from different animal habitat in the Ajmer District. Before collection of soil samples, superficial debris was removed from soil surface. Loosened soil (approximately 500g) has taken from the surface layer of each site to a depth of 2-5cm. Soils were collected in sterile plastic bags and sealed on the spot. Samples were brought to the laboratory and used immediately or stored overnight.

Isolation and Identification of keratinophilic fungi:

Keratinophilic fungi were isolated from different soils samples using ‘‘Hair baiting technique’’ The soil samples and moistened with sterile distilled water are baited by burying sterile keratinous baiting the soil. These dishes were incubated at room temperature (Period of 4 weeks).

Hair Baiting Technique

1.	Moist chamber were prepared using sterile Petri dishes and blotting paper.
2.	Sterile Petri dishes were half fill with soil sample.
3.	2-3cm short strands of sterilized defatted baits were spread over soil surface.
4.	10-15 ml of sterile water was added to the soil to facilitate germination of fungal spore.
5.	Petri dishes were then incubated at room temperature.
6.	Plates were examined periodically for the development of mycelium.
7.	Observed mycelium growth

After observing the mycelium growth on the baits, isolates were culture on Sabouraud’s Dextrose Agar (SDA) medium supplement with streptomycin (30mg/l). Isolated fungi were stained with lactophenol cotton blue and observed it under the phase contrast microscope.

Purification of fungi:

Macroscopic identification: For the macroscopic identification Sabouraud’s Dextrose Agar (SDA) was used based on morphology and cultural account of all isolated keratinophilic fungi. Their colony diameter and colour, texture examined.

Microscopic identification: Keratinophilic fungi were microscopically identified on the basis of morphological characteristics *viz.* conidia shape and size. Isolated fungi were stained with cotton blue and observed it under the phase contrast microscope.

Table: Colonial and conidial characteristics of isolated species of keratinophilic fungi

Fungus	**Fungus**	**Colonial characteristics**	**Conidial characteristics**
Anamorph	Teleomorph		
T. rubrum	-	Cottony to velvety, white to reddish surface, typically wine red reverse	Macro-conidia seldom seen pencil shaped; Micro-conidia drop shaped, abundant.
T. tonsurans	-	Powdery to velvet, white to yellowish or red brown surface.	Macro-conidia uncommon, pencil shaped; Micro-conidia abundant.
T. mentagrophytes	*A. benhamiae*, *A. vanbreuseghemii*	Powdery to cottony, Yellow cream to white surface, pale to red brown reverse.	Macro-conidia uncommon, club shaped, smooth; Micro-conidia drop shaped, abundant, produced mainly in dense tufts.
T. equinum	-	White to buff in color, suede-like to downy in texture	Macro-conidia thick or thin walled, clavate to fusiform. Micro-conidia spherical, pyriform to clavate or irregular shape.
M. canis	-	Flat to velvety thin, pale to yellow, with yellow reverse.	Macro-conidia thick walled, roughened and beaked: Micro-conidia drop shaped.
M. gypseum	*Gymnoasus gypseum*	Powdery to cottony, white to buff, pink to red yellow reverse.	Macro-conidia spindle shaped, smooth or rough, borne directly on hyphae. Micro-conidia drop shaped, clavate, and unicellular.
Chrysosporium sp.	-	Powdery or granular surface texture, white to tan to beige in color	Macro- conidia Sub-spherical, clavate, pyriform Micro- conidia spherical one-celled, occasional two celled.
Aspergillus niger	-	Yellow to white turning black with the formation of conidia.	Black globose conidia, conidiophore like as toiled brush, with its globose base at the end of the stalk.

RESULT AND DISCUSSION

The total soil samples collected from different animal habitats present in Ajmer district. Soil samples rich in organic matter are more suitable for keratinophilic fungi. Data on the distribution of these isolated keratinophilic fungi are given in Table 1. The data reveals that out of thirty six soil samples collected eight species of keratinophilic fungi were isolated from different animal habitat. In the present study most of keratinophilic fungi *viz. Aspergillus niger, Trichophyton equinum, T. tonsurans, Chrysosporium sp., Microsporum canis, M. gypseum, T. rubrum, T. mentagrophytes.*

Table 1: Distribution of keratinophilic fungi in animal habitat at Ajmer district

S.No.	Name of fungus	Sampling site											
		1	2	3	4	5	6	7	8	9	10	11	12
1.	*Aspergillus niger*	+	-	+	-	+	+	-	+	+	+	-	+
2.	*Chrysosporium sp.*	-	-	+	+	+	-	+	+	-	-	+	-
3.	*Microsporum canis*	+	-	+	-	-	-	+	-	-	-	-	-
4.	*Microsporum gypseum*	-	+	-	-	-	-	-	+	-	-	-	-
5.	*Trichophyton tonsurans*	+	-	+	-	+	-	-	-	-	+	-	-
6.	*Trichophyton equinum*	-	-	+	-	-	-	-	+	-	-	-	+
7.	*Trichophyton mentagrophytes*	-	-	-	-	-	-	+	-	-	-	-	-
8.	*Trichophyton rubrum*	-	-	+	-	-	-	-	+	-	-	-	-

Frequency of occurrence of the keratinophilic fungi (Table 2.) is in the order *Aspergillus niger* (66.67%), *Chrysosporium* sp. (50%), *Trichophyton tonsurans* (33.33%) are dominant and in minimum *T. mentagrophytes* (8.33%), *Microsporum canis* (25%), *M. gypseum* (16.67%), *T.equinum* (25%), *T. rubrum* (16.67%).

Table 2: Frequency occurrence of keratinophilic fungi in animal habitat

S.No.	Name of fungus	Number	% value
1.	*Aspergillus niger*	8	66.67%
2.	*Chrysosporium sp.*	6	50%
3.	*Microsporum canis*	3	25%
4.	*Microsporum gypseum*	2	16.67%
5.	*Trichophyton tonsurans*	4	33.33%
6.	*Trichophyton equinum*	3	25%
7.	*T. mentagrophytes*	1	8.33%
8.	*Trichophyton rubrum*	2	16.67%

Keratinophilic fungi have to ability to degrade keratin. Some keratinophilic fungi capacity to parasitize keratinic tissues in men and animals causes mycoses. A survey of literature reveals that keratinophilic fungi have been reported from different parts of the world. The present survey on distribution of keratinophilic fungi present in soils of different animal habitat is quite important because a number of domestic animals are known to carry dermatophytes on their skin coats without manifesting any sign of infection. The origin of several cases of human ringworm has been traced to animal harboring the etiologic dermatophytes species. The majority of the keratinophilic fungi though seldom involved in dermatophytoses, have the potential for it as one can see natural evolution from keratin utilizing soil saprophytes to association and finally invasion of cornified substrate in living animal and men. Study revealed that Asperg*illus niger*, *Chrysosporium* sp. are most dominant in animal habitat.

CONCLUSION

Keratinophilic fungi have included dermatophytic fungi and non- dermatophytic fungi. Dermatophytes are anamorphic hyphomycetes, keratinophilic fungi that parasitize keratinized tissue (hair, nails, and skin) of human and animals and cause dermatophytoses. These are the most frequent fungal infections worldwide. To identify the role of keratinophilic fungi in these cases knowledge of the presence of dermatophytic and non- dermatophytic fungi in soil is very necessary. According to present study *Aspergillus niger*, *Trichophyton* sp., *Microsporum* sp., *Chrysosporium* sp., are very common in soil and there is need for further Taxonomic and ecological investigation of these organism.

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