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ISOLATION OF KERATONOPHILLIC FUNGI AND RELATED DERMATOPHYTES FROM THE SOIL

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Abstract: Dermatophycoses are a group of closely related filamentous fungi that infect only superficial keratised tissue like skin, hair and nails. They are known as keratonophillic fungi, Tinea or ring worm fungi. These fungi occur in many natural and manmade habitats and utilize chiefly products of keratin decomposition.In our investigation various soil samples collected from different habitats were screened for the presence of keratophillic fungi. Soil samples were collected from the vicinity of animal houses, poultry farms, hospitals, barber shops, etc.From the soil samples examined 60% were found positive for the presence of keratophillic fungi and related dermatophytes.Among soil samples collected from a variety of barber shops 100% samples were recorded positive. Soil Samples of other habitats like poultry farm, hospitals, animal house were also found to be positive for keratonophillic fungi.

INTRODUCTION

Fungi had been recognized as causative agent of human disease earlier than bacteria. Fungi causing favus (***Trichophyton schonleinii***) and thrush (Candida ***albicans***) had been described as early as in1839. In spite of the earlier beginning the study of pathogenic fungi has received only scant attention in comparison with the study of other pathogens. This is probably due to relatively benign nature of common mycotic diseases. Fungal infection, however are common and some of them are serious and fatal. With the control of bacterial infections in developed countries fungal infections have assumed greater importance, Keratonophillic fungi along with dermatophytes are responsible for various cutaneous mycoses. Dermatophytes require keratin for growth. These fungi can cause different types of tinea in humans. The majority of the fungi producing diseases in human beings and animals exist freely in nature as soil saprophytes [Kumari GR,et al 2005].

The soils represent the main reservoir of fungi. Some soil fungi are potential pathogen to both human and animals. Soils that are rich in keratinous materials are the most conductive for the growth and occurrence of keratonophillic fungi. The potentially pathogenic keratonophillic fungi and allied geophilic-dermatophytic species are widespread worldwide.

The forest, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material are the best candidate for growth of keratinolytic and saprophytic fungi (Mohamed S Ali *et.al* 2000).Most fungi are soil saprophytes and human infections are mainly opportunistic. Modern advances I treatment such as antibiotics, steroids and immunosuppressive agents have led to an increase in opportunistic fungal infections.

Dermatophytes are spread by direct contact from infected people (Anthropophilic organisms), animals (Zoophilic organisms), and soil (geophilic organisms), and indirectly from fomites [Hainer 2005]. Several studies have been shown that soils are important sources of dermatophytes and keratonophillic fungi [Hedayati et al 2004]. The presence of dermatophytes in soil can be a reservoir for infection in human beings. In the recent years, many workers have reported the distribution of keratonophillic fungi and related dermatophytes in soils [Ramesh et al 1998; Papini et al 1998].To date, little epidemiological data on fungal flora of soil in this area has become available. The present paper reports the prevalence of dermatophytes and related keratonophillic fungi in the various locations in Warangal A.P India .

Material & Methods

Collection of soil samples

In the present investigation, various soil samples collected from different habitats were screened for the presence of keratonophillic fungi and related dermatophytic fungi. Soil samples were collected from the vicinity of animal houses, poultry farms,hospitals,barber shops, chicken centres. The soil samples were collected from the superficial layers with the help of a spatula in sterile polythene bags. All samples were carried to the laboratory and processed immediately for the isolation of keratophillic and related dermatophytes using baiting technique [Vanbreuseghem 1952; Rahul Sharma and Rajak 2003].

Isolation of keratonophillic fungi can also be done by the other techniques such as the dilution plate method or pour plate method although the hair baiting method is better as the keratinolytic ability is automatically checked if the fungus grows on the de-fatted natural keratin substrate. Once the fungus grows on the keratin substrate in the hair baited plate it can then be transferred onto agar media

as these fungi generally can grow on various artificial media.

Preparation of keratinic subsrates (Keratin baits)

The following substrates were used as keratin baits. Human hair, Nails, hen feathers, buffalo horn pieces. The samples were collected, washed with distilled water to remove the dust particles and then air dried. The were then cut into pieces and were soaked in diethyl ether for 24 hours. They were then sterilized by dry autoclaving at 15 lbs pressure for 15 minutes and are used as keratin baits.

Isolation of keratonophillic fungi by baiting technique

Approximately 50g of each soil sample was placed into a sterile Petri dish and baited with sterilized small pieces of hair. Each Petri dish was moistened with 5–10ml sterile distilled

water and incubated at room temperature for up to five weeks before being discarded. The growth was observed under the microscope . The isolates were then transferred to sabouraaud's dextrose agar (SDA) medium of the following composition (Peptone 10g, Dextrose 40g,Agar 20g, Distilled water 1000ml)

Results and Discussion

The results obtained are given in the Table 1 and the organisms isolated are given in Table 2

All the soil samples examined were positive for keratonophillic fungi. Among all the soil samples, the soil obtained from the barber shops exhibited maximum keratonophillic fungi followed by the soil collected from poultry farms and animal houses. The soil obtained near the hospitals only 2 samples exhibited the presence of keratonophillic fungi.

Table 1: Distribution of keratophillic fungi in the soil samples collected in Warangal

S.No	Source of soil sample	Number of soil samples examined	Number of samples found positive
1	Poultry farm	05	03
2	Animal houses	05	03
3	Barber shops	05	05
4	Near hospitals	05	02
5	Chicken centres	05	02

S.No	Fungi isolated	Poultry farm	Animal house	Barber shops	Hospitals	Chicken centres	Distribution (%)
1	*Aspergillus niger*	+	+	+	-	+	7.25
2	*Aspergillus flavus*	+	+	+	+	-	7.50
3	*Aspergillus fumigates*	-	-	+	-	-	6.50
4	*Mucor sps*	+	+	+	-	+	8.65
5	*Fusarium sps*	-	-	+	+	-	4.1
6	*Penicillium sps*	+	+	-	+	-	5.25
7	*Microsporum gypseum*	+	+	+	+	+	20.0
8	*Trichophyton rubrum*	+	-	+	-	+	6.5
9	*Epidermophyton floccosum*	-	-	+	-	-	4.5
10	*Microsporum sps*	+	+	+	+	+	12.5

Keratonophillic fungi are important ecologically and recently have attracted the attention throughout the world .They play a significant role in the natural degradation of keratinized residues (Sharma R, Rajak RC 2003, Fillipello MV, Fusconi A, Rigo S 1994, Fillipello MV 2000.), have many properties in common with dermatophytes and some can probably cause human and animal infections (Connole M 1990,Ali-shatayeh MS *et al*,1989, Filipello MV,*et al*1996, Spiewak R, Szostak W 2000, Spiewak R 1998, Restrepo A *et al* ,1976, Cano J,*et al* 1991). Keratonophillic fungi are presented in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. Reports on the presence of these fungi in different soil habitats from different countries, e. g., Egypt, Australia, Palestine, Spain, India, Kuwait, Ukraine and Malaysia, have indicated that this group of fungi are distributed worldwide(Mohamed S Ali et al 2000, Anbu P,*et al* 2004).

Table 2: Distribution of various fungi isolated from different soils in Warangal.

Keratonophillic fungi like to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof beak of the birds etc. They utilize keratin as carbon source (Cooke 1990). Keratin is highly insoluble protein having fibrous helical structure and numerous disulfide linkages which make it resistant to many proteases but is easily digested by keratinase enzyme [Grant and Long 1981].

The keratinous materials in or on soil are attacked by these keratonophillic microbes, therefore biodegradation takes place. Keratinases also provide the virulence to certain fungi such as dermatophytes to cause dermatophytoses or ringworm in human and animals [Dexter 1983; Fry L and Cornell 1985]. The data revealed that out of 25 samples, maximum number (05/25 ;100%) of keratonophillic fungi was isolated from soils of barber shops; followed by the soil samples from poultries farms (03/25; 75%) and from the soil samples from animal houses (03/25; 75%) . Isolation rates of keratonophillic fungi from soils from hospitals (02/25; 50%) and from soil isolated from chicken centres (02/25; 50%) were almost similar. The least number of keratonophillic fungi was isolated from hospital areas and chicken centres.

Out of the total isolates most of the isolates contained *Aspergillus niger* as the most common species (7.25%). The soil samples of poultry houses, animal houses, barber shops and hospitals contained *Aspergillus flavus* (7.50%).The soil isolated from barber shops contained *Aspergillus fumigates* which was not present in other isolates.(6.5%).*Mucor* sps was also identified in all the isolates expect the soil isolated near the hospitals.(8.65%).*Fusarium* sps was isolated only from soils isolated from barber shops and hospitals(4.1%).*Pencillium* sps was identified from the isolates of poultry farms,animalhouses and hospitals but not found in the isolates of soil from barber shops and chicken centres.

The keratonophillic fungi *Microsporum* sps and *Microsporum gypseum* was isolated from all the soil samples (20%, 12.5% respectively).*Trichophyton rubrum* was isolated from the soil samples of Poultry farms and barber shops only.(6.5%).*Epidermophyton flocossum* was isolated from barber shops (4.5%).

Figure 1: A and B Colonies showing *Epidermophyton flocossum*,*C and D* Colonies showing *Microsporum sps and E* Colony showing *Trichophyton rubrum*

Shukia *et al* reported that geophilic *M. gypseum* was only isolated from soil in all the cases. [Shukia 2003] believed that increasing temperature and decreasing humidity lead to providing a less favourable condition for the growth of *M. gypseum* in the soil. *M. gypseum* is a common geophilic dermatophyte widely distributed in soil globally. It causes ringworms of scalp and glabrous skin in human and animal [Mohamed Ali *et al* 2000]. Irshad 2007 reported that that *Aspergillus niger* is the most prevalent keratonophillic fungus and also dominant species that isolated from 51 soil samples of five different regions like fertile lands, animal herds, slaughter houses, poultries and barbers' shops. Isolation rate of keratonophillic fungi including *A. niger* was higher in soil samples collected from the farm lands and poultries.

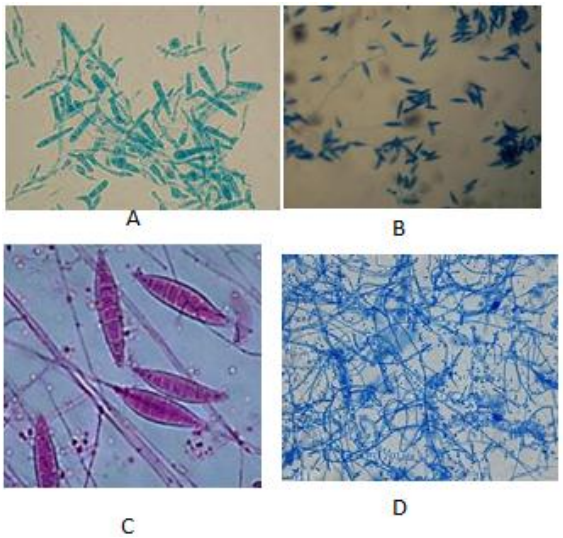

Figure 2: Observations under microscope of A. *Epidermophyton floccosum* B. *Microsporum gypseum* C. *Microsporum sps* .and D. *Trichophyton rubrum*

Aspergillus flavus was the second dominant species in soils of Gorgan (19.5%) and Gonbade Kavus (19%) areas(Moallaei *et al* 2006). Velasco Benito *et al* 1979 reported the presence of prevalence of *Trichophyton verrucosum* and *Epidermophyton floccosum* was strikingly high in relation to other fungi. Youssef YA *et al* 1992 reported the isolation of keratonophillic fungi by "ToKaVa" hair baiting technique. 22 species belonging to 6 genera were isolated viz.: *Chrysosporium tropicum, C. indicum, C. keratinophilum, C. queenslandicum, C. merdarium, C. anamorph of Arthroderma curreyi, C. pannicola, C. lobatum, C. anamorph of Renispora flavissima, C. pseudomerdarium, Microascus mangini, Malbranchea gypsea, reesii, Coccidioides immitis, Microsporum gypseum, Mr. distortum Mr. audouinii, Mr. fulvum, Trichophyton mentagrophytes, T. terrestre, T.*

verrucosum and Epidermophyton floccosum.

Conclusion

Hence in the present investigations keratonophillic fungi have been isolated in different types of soil in Warangal and they have been identified.

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