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KERATINOPHILIC FUNGI FROM THE SOIL OF DISTRICT, JAMSHORO, SINDH, PAKISTAN

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Abstract

Soil is well known to support the ongoing existence of keratinophilic fungi and potential sources of infection for humans and animals. Keratinophilic fungi from the soil of District Jamshoro, Sindh were investigated at five sites viz., LMUHS, Super highway, near pitaro, Phulalli head, and Kotri station from June 2005 to May 2006. Forty soil samples were collected from surface, 10, 20, and 30cm depth to determine the prevalence of Keratinophilic fungi and dominant species. The Keratinophilic fungi were studied by soil dilution plate method and baiting techniques. Eleven species of Keratinophilic fungi viz., *Aspergillus niger* (31.59%), *A. flavus* (21.40%), *A. fumigatus* (2.82%), *A. candidus* (11.55%), *A. ustus* (2.35%), *A. wentii* (5.26%), *A. nidulans* (2.05%), *Microsporum gypseum* (8.60%), *M. canis* (5.99%), *Cunninghamella echinulata* (4.10%), *C.elegans* (4.23%) were identified. Higher numbers of species were recovered from LUMHS as compared to other sites; isolates were in moderate frequency.

Introduction

The soil constitute one of the most complex of microbial habitats in which many fungi complete their entire life cycle. Different soils have specific fungus floras, but the majority of species found in them are cosmopolitan, (Ainsworth & Sussman, 1968). Some soil fungi are potential pathogen to both human and animals. Soils that are rich in keratinous materials are the most conducive for the growth and occurrence of keratinophilic fungi (Moallaei & Zaini, 2006). Keratinolytic mycoflora love to grow and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof, beak etc. They utilize keratin as carbon source (Cooke, 1980). Keratinophilic fungi are important ecologically and present in the environment with variable distribution patterns and cause human and animal mycoses (Mohamed *et al*., 2000). Most cutaneous infections are the work of homogeneous group of keratinophilic fungi known as dermatophytes (Boni, 1998). The dermatophytes have the capacity to invade keratinized tissue of the body including skin, hair and nails (Irene Weitzman 1995). The dermatophytes have been divided into three ecological groups: geophiles, zoophiles and anthropophiles (Rippon, 1982). The prevalence of dermatophytes varies according to geographical location, season or living conditions and the manipulation to which the susceptible animal or human is exposed (Boyanowski, 2000). However, in general, they occur more commonly in countries with a hot and humid climate (Cavalcanti, 2003). These fungi have also been reported from the soil of Pakistan (Masih *et al.*, 1971). However, the soil of District Jamshoro has not been investigated for keratinophilic fungi. Therefore, hygienic and ecological interests have led us to study the keratinophilic mycoflora from Jamshoro, where farmers, tourists, patients, students and animals spend a large proportion of their time and may be exposed to pathogenic fungi. This paper reports the prevalence of keratinophilic fungi from the soil of district Jamshoro, Sindh, Pakistan.

Materials and Methods

Forty soil samples were collected from various depths (surface, 10, 20 and 30cm) of different sites of the Jamshoro district. These sites include LMU, S. Highway, near pitaro, Phullali head and Kotri Station. Two samples were collected at each depth. The samples were kept in sterile, tightly closed polyethylene bags and transferred immediately to the laboratory. Two major techniques have been used for the qualitative and quantitative isolation of these fungi from soil: Soil dilution plate method (Waksman, 1922) and Baiting Technique (Vanbreuseghem, 1952) were applied. For selective isolation of keratinophilic fungi from soil about half fill sterile Petri dishes with the soil samples were baited by short (2-3cm) strands of sterilized human healthy hairs, nails and cow skin over the surface of the soil. The baited soils were moistened with 10-15 ml of sterile distilled water and incubated at 28^{o}C for three to four weeks. Ten Petri dishes were used for every soil sampling site. The plates were periodically examined for the development of mycelium on the baits. The invaded baits were inoculated on Sabourauds Dextrose Agar (SDA) with 0.1% chloramohenicol in plates for obtaining the cultural growth. After colonies developed, species of the cultured fungus were identified based on its macroscopic and microscopic morphology (St-Germain & Summerbell, 1996). Identification was performed according to Gilman (1945), Domesch *et al*., (1980) and Kane (1997).

Result and Discussion

Out of a total of 2336 colonies of keratinophilic fungi isolated, a total of 11 species were obtained from 40 soil samples from the five sites; LMU, S. Highway, near Pitaro, Phullali head and Kotri station. *Aspergillus niger* (31.59%) was in the highest frequency, *A. flavus* (21.40%) and *A. candidus* (11.55%) were recovered in a moderate frequency followed by *Microsporum gypseum* (8.60%), *A. fumigatus* (2.82%), *A. ustus* (2.35%), *A. wentii* (5.26%), *A. nidulans* (2.05%), *M. canis* (5.99%), *Cunninghamella echinulata* (4.10%) and *C. elegans* (4.23%) recovered in a low frequency (Table 1). The highest number of colonies per soil unit belonged to *A. niger* in Kotri station followed by *A. flavus* in LMUHS. Overall, in different sites; LMU had the highest frequency of keratinophilic fungi followed by Kotri Station and Phullali head. Super Highway appeared to be the lowest in the total count of keratinophilic fungi (Table 1).

Keratinophilic fungi are present 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 (Anbu *et al*., 2004). The study revealed that the total number of species isolated decreased with increased sampling depth. A greater number of species and colonies were isolated on baiting technique than on dilution plates (Fig. 1).

Table 1. Distribution of Keratinophilic fungi isolated from soil samples of different sites of Jamshoro Distt. Sindh, Pakistan.

Species	LUM		Super Highway		Near Pitaro		Phullali		Kotri station		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
Aspergillus niger	186	28.09	108	33.33	141	40.63	116	30.60	187	29.96	738	31.59
A. flavus	134	20.24	96	29.62	27	7.78	112	29.55	131	20.99	500	21.40
A. fumigatus	19	2.87	03	0.92	16	4.61	0	0	28	4.48	66	2.82
A. candidus	87	13.14	09	2.77	0	0	85	22.42	89	14.26	270	11.55
A. ustus	33	4.98	0	0	10	2.88	07	1.84	05	0.80	55	2.35
A. wentii	28	4.22	0	0	12	3.45	36	9.49	47	7.53	123	5.26
A. nidulans	0	0	30	9.25	18	5.18	0	0	0	0	48	2.05
Microsporum gypseum	61	9.21	53	16.35	28	8.06	0	0	59	9.45	201	8.60
M. canis	42	6.34	25	7.71	44	12.68	23	6.06	06	0.96	140	5.99
Cunninghamella echinulata	35	5.28	0	0	22	6.34	0	0	39	6.25	96	4.10
C. elegans	37	5.58	0	0	29	8.35	0	0	33	5.28	99	4.23
Total	**662**		**324**		**347**		**379**		**624**		**2336**	

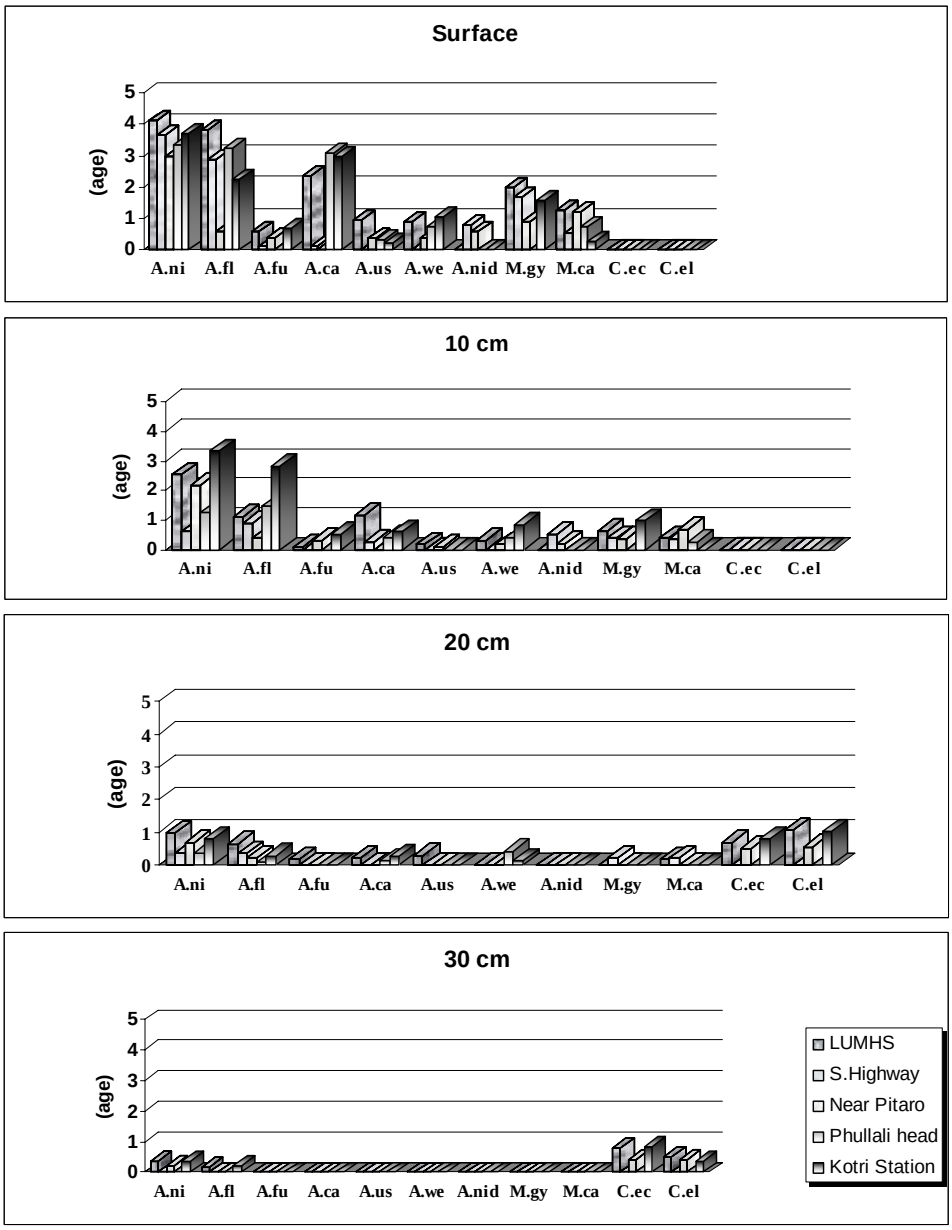

Fig. 1. Distribution and frequency of keratinophilic fungi at different depth. *A. ni= A. niger, A .fl= A. flavus, A. fu= A. fumigatous, A. ca= A. candidus, A. us= A. ustus, A. we= A. wentii, Anid= A. nidulans, M. gy= M. gypseum, M. ca= M. canis, C. ec= Cunninghamella echinulata, C. el= Cunninghamella elegans*.

Aspergillus niger was the most dominant species in soil of Pitaro (40.63%) and S.highway (33.33%). *A. niger* is regarded as a pathogen (Padhye,1982). It can cause otomycosis (Austwick, 1965). *A. niger* can also produce allergic reactions in humans (Edwards, 1977). *Aspergillus* produce a variety of fungal metabolites, termed mycotoxins (Ueno & Ueno, 1978). *A. flavus* was the second dominant species in soil of LUM (20.24%). This species is present in soil of various areas (Sarquis, 1990). The species, also occurs in external ears and involved in otitis (Jesenka *et al*., 1992). On the other hand this species is a potential mycotoxin producer (Richardson, 2003). *A. fumigatus* causes skin, eye and ear infections (Bodey, 1989). *A. candidus* is involved in a wide range of human infections: otomycosis (Yasin *et al*., 1978), and onychomycosis (Nasreen *et al*., 2006). *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, 2000). Ringworm of hair, skin and nail is caused by *M. canis* (Nasreen *et al*., 2006). *Cunninghamella* is a filamentous fungus found in soil (De Hoog, 2000) *C. echinulata* and *C. elegans* are known as human and animal pathogens (Sutton, 1998). Since there was no evidence of any study on mycoflora of Jamshoro district, the present investigation was carried out for the detection of keratinophilic fungi in soil of five different sites of Jamshoro district. Presence of keratinophilic species with strong keratinolytic activity were generally found due to high and variety of population level in soil of Jamshoro. On the other hand, some of the species that showed weak or moderate keratinolytic activity were also found to be among the most dominant components of keratinophilic fungal communities of these habitats. The present study appear to be the first report concerning isolation of keratinophilic fungi from soil samples in studied area in Jamshoro, Sindh.

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