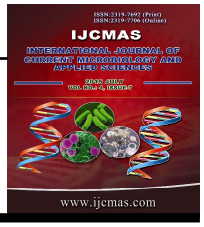
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 229-237

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 229-237**
http://www.ijcmas.com



Original Research Article

Isolation of Keratinophilic Fungi from Soils Samples of Agricultural Fields of Saharanpur (U.P), India

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Keywords

Keratinophilic fungi, Pathogenic, Spread plate method, Dermatophytes

A B S T R A C T

The soils samples from agricultural fields in Saharanpur Village (U.P) are rich in pathogenic keratinophilic fungi including dermatophytes. This study deals with the isolation and characterization of keratinophilic fungi from different soils samples of animals' habitat and agricultural fields in Saharanpur Village (U.P). Eight different fungi were isolated out of twenty two soils samples using a spread plate method. These fungal isolates were *Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton equinum, Microsporum gypseum, Microsporum canis, Microsporum audouinii, Microsporum cookei.* Our result concluded that these isolated fungi are pathogenic for animals and human beings. Keratinophilic fungi play an important role in degradation of keratin and they are known to cause superficial cutaneous infections. Present results show the occurrence of keratinophilic fungi in soils of different sites in Saharanpur villeges and may have a significant role in keratin degradation in the environment.

Introduction

The soils represent the main reservoir of fungi. Some soil fungi are potential pathogen to both humans and animals. The potentially pathogenic keratinophilic 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 *et al*, 2000). Therefore hygienic and ecological interests have led us to study the keratinophilic mycoflora of farm yards and forests, where farmers, tourists, and animals spend a large proportion of their time and may be exposed to pathogenic fungi (Mahdavi Omran, 1991). This would help us to know the distribution and occurrence of dermatophytes and other keratinophilic fungi and risk of human dermatophytosis in those regions, which could have a role in degradation of keratinous material as an industrial point of view (Nasery Bande Gharaey, 1992). Dermatophytes are a group of fungi that cause infections of skin, hair and nails. They include three genera namely *Epidermophyton*, *Microsporum* and *Trichophyton*. Keratin substances which are important natural material, occurring in

nature mainly in the form of hairs, wools, feathers, horns, hooves, nails, skin and other cornified appendages constitutes natural baits for these fungi (Khanam and Jain, 2002). Soil is the main reservoir of different types of fungi and some of them are pathogenic to both humans and animals. Soil is also a good source of keratinophilic fungi and the probability of the incidence of such fungi increases manifold if it is rich in keratinous materials (Marchisio, 2000). Keratinophilic fungi, also known as dermatophytes, are mostly pathogens of humans and other animals but also have the ability to live saprotrophically. Every keratinophilic fungus has the potential to cause infection and tissue invasions (Simpanya, 2000). Both dermatophytes and non-dermatophytes can colonize and invade skin, nails and hair **(**Deshmukh *et al.*, 2008). The first discovery of keratinophilic fungi from soil was by hair baiting technique, the most common method used for qualitative and quantitative, isolation of these fungi from soil. These fungi are natural colonizers of keratin substances. Some are keratinolytic and play an important ecological role in decomposing α- keratins, the insoluble fibrous protein. Because of tight packing of hair polypeptide chains in α- helix structures and their linkages by disulphide bonds, they are poorly biodegradable (Kunert *et al.*, 2000). This study because of having a subtropical climate and geographic diversity leading to the area suitable for wisely distribution these fungi.The present paper reports the prevalence of keratinophilic fungi & other fungi in with soil pH. The various areas of Agricultural fields in Saharanpur areas (U.P)

Materials and Method

Collection of soil samples

A total 22 soils samples were collected from different areas of animals habitat and agricultural fields in Saharanpur Village (July 2014 to March 2015). Different soils samples were collected in sterile polyethylene bags and brought to the laboratory for further microbiological analysis.

Physical properties of soils

The collected soils samples for the were analyzed for the following physical-chemical parameters like pH, temperature, electronic conductivity, total dissolve solid, salinity, dissolve oxygen, chemical oxygen demand and chloride content.

The pH, electrical conductivity, total dissolved solids, salinity parameters were analyzed by using soils analysis kit and the other parameters viz. dissolved oxygen, chemical oxygen demand and chloride content were analyzed by adopting standard procedures.

Isolation of keratinophilic fungi from soils

The keratinophilic fungi were isolated from different soil samples using 'hair baiting technique' (Vanbreuseghem 1952). The keratinolytic nature of these fungi makes it possible to isolate them from soil by implanting human hairs, nails, pig hairs, goat hairs, buffalo hairs, sheep hairs, cow hairs, dog hairs, horse hairs.

In this technique sterile petri plates were half filled with soil and short strand of sterilized defatted hairs, nails, were spread over the surface of soil. 10-12 ml. sterile water was added to petriplates for the facilitation of fungal spores to germinate. Now the Petri plates were incubated at 20-25°C in dark for 3-4 weeks. After vigorous growth inoculums was placed over the Sabourauds Dextrose Agar media.

Purification of fungi

Macroscopic identification

For the macroscopic identification different selective media were used. Sabouraud's Dextrose Agar (SDA), Trichophyton Agar, Dextrose Agar and Rice media were used for the growth and sporulation of different fungi.

Microscopic identification

Morphological characteristics were examined under microscope. Slide culture technique was adopted for the identification of fungi with lacto phenol cotton blue.

Results and Discussion

The soils samples collected from different animal habitats and agricultural fields in Saharanpur villages. The results of isolation of keratinophilic fungi are presented in table 2. The data reveals that out of twenty two soils samples collected, eight species of keratinophilic fungi were isolated from different animal habitats and agricultural fields with different keratin substrates such as human hairs, pig hairs, buffalo hairs, goat hairs, sheep hairs, horse hairs, dog hairs, cow hairs and human nails. In our present study, the maximum numbers of keratinophilic fungi were isolated from agricultural fields. These habitats contain also lots of keratin debries. The collected soils samples were analyzed for the physi-chemical parameters were represented in table 1 and found that maximum numbers of fungi were present in the range of pH 6.92 to 8.22. In the present study most of the isolated keratinophilic fungi viz *Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton equinum, Microsporum gypseum, Microsporum canis, Microsporum audouinii, Microsporum cookei*. The results are given in table 2. In this study the maximum keratinophilic fungi was found in agricultural fields because due to the presence of wild and domestic animals in agricultural field serving as a reservoir of organisms. *Microsporum gypseum, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis,* were isolated from agricultural fields and *Microsporum audouinii, Trichophyton equinum* isolated from animal habitats. *Trichophyton tonsurans,Trichophyton mentagrophytes, and Microsporum gypseum* was found to be the most common keratinophilic fungi isolated from slightly alkaline pH soils.

Several investigations have been done in various part of India during last years and showed that rich variety of keratinophilic fungal flora exists in the soils (Simpanya and Baxter, 1996). Therefore present investigation carried out for study of biodiversity in the distribution of keratinophilic fungi from different soil samples of agricultural fields of Saharanpur area. Its temperature even 43°C in summer and has high humidity during monsoon season. All these conditions favour the higher incidence of keratinophilic fungi in Saharanpur. In present work, *T. tonsurans* was most predominant fungi. Similar results were observed by (Ramesh and Hilda, 1999) who also done survey study of keratinophilic fungi in primary school and public parks. In present study, a survey study of keratinophilic fungal flora of Saharanpur district with particular reference of soils pH was carried out and reported *T. tonsurans* as the most predominant fungi and *T. mentagrophytes* as second predominant fungi from pH 7.19 to 9.2. *T. mentagrophytes* species was known to be polymorphic. *M. gypseum* fungi existing in a geophilic, zoophilic or anthropophilics state.

Table.1 Physico- chemical parameters of collected soils samples from various habitats and agricultural fields

s.no	soils samples	pH	Temp. (C°)	EC (ms)	TDS (mg/l)	Salinity (mg/l)	D.O (mg/l)
1.	Sonaarjunpur	7.09	31.3	114.1	65.0	0.391	21.2
2.	Salempur	7.18	19.3	0.242	154.1	0.0.421	22.1
3.	Sadhalihariya	7.16	30.3	0.216	142.4	0.421	21.3
4.	Rampur	7.04	31.2	79.9	52.3	04.31	20.1
5.	Pilkhani	7.36	33.0	22.3	78.2	0.435	22.3
6.	Naurangpur	7.21	33.0	5.64	4.18	0.532	4.4
7.	Kanjoli	8.24	30.0	0.663	0.433	0.157	73.0
8.	Pahansu	7.21	33.0	5.64	4.18	0.532	4.4
9.	Jandhera	7.19	33.0	5.61	3.03	0.251	75.1
10	Bahankla	6.92	33.0	8.95	4.99	0.981	102.0
11.	Dariypur	7.34	31.0	98.7	65.0	0.297	20.2
12.	Nandpur	7.93	32.0	o.444	0.411	0.335	25.2
13.	Tapri	7.71	32.5	0.808	0.534	0.153	15.4
14.	Charro	8.01	32.7	0.511	0.334	0.386	15.6
15.	Khatka heri	8.06	32.8	6.12	3.89	1.88	14.4
16.	Nanauta	7.71	32.8	10.39	20.26	0.305	20.2
17.	Ambheta	7.77	32.9	0.516	338	0.352	103.0
18.	Nagal	7.84	33.0	0.458	0.296	0.339	60.3
19.	Khudana	7.59	33.0	0.576	0.389	0.422	44.0
20.	Luharli	7.71	33.0	0.728	0.467	0.522	75.0
21.	Jagrolly	7.63	33.0	0.791	0.529	0.618	18.6
22.	Nakur	7.76	33.0	0.847	0.547	0.630	3.3

Keratinophilic fungi were isolated from different soils samples using ‘hair baiting technique’

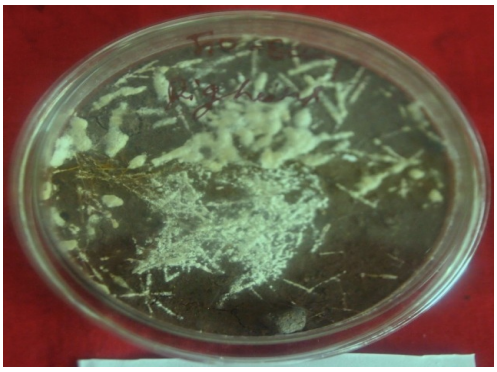


Table.2 Isolation of keratinophilic fungi from soils samples of various habitats of agricultural fields in Saharanpur villages by using different keratin substrates

S/no	Source of soils samples	Keratin substrate for the Isolation of fungi									Probable Genera
		hairs									
		Buffalo	humans	horses	dog	pig	sheep	cow	goat	nails	
1.	Sonaarjunpur	+	+	-	-	-	-	-	-	-	*Trichophyton tonsurans*
2.	*Salempur*	+	-	-	-	-	-	-	-	-	*Trichophyton mentagrophytes*
3.	Sadhalihariya	+	-	-	+	-	-	-	-	-	*Microsporum canis*
4.	Rampur	+	-	-	-	-	-	-	-	-	*Trichophyton tonsurans*
5.	Pilkhani	+	-	-	-	-	-	-	-	-	*Trichophyton tonsurans*
6.	Naurangpur	-	-	-	+	-	-	-	-	-	*Microsporum canis*
7.	Kanjoli	+	-	+	-	-	-	-	-	-	*Trichophyton equinum,*
8.	Pahansu	+	-	+	-	-	-	-	-	-	*Trichophyton equinum*
9.	Jandhera	+	-	-	-	-	-	-	-	-	*Microsporum gypseum*
10.	Bahankla	-	-	-	-	+	-	+	+	-	*Trichophyton tonsurans*
11.	Dariypur	-	-	-	-	-	+	-	-	-	*Trichophyton mentagrophytes*
12.	Nandpur	-	-	-	-	+	-	-	-	-	*Microsporum audouinii*
13.	Tapri	-	-	-	-	-	-	-	+	-	*Microsporum gypseum*

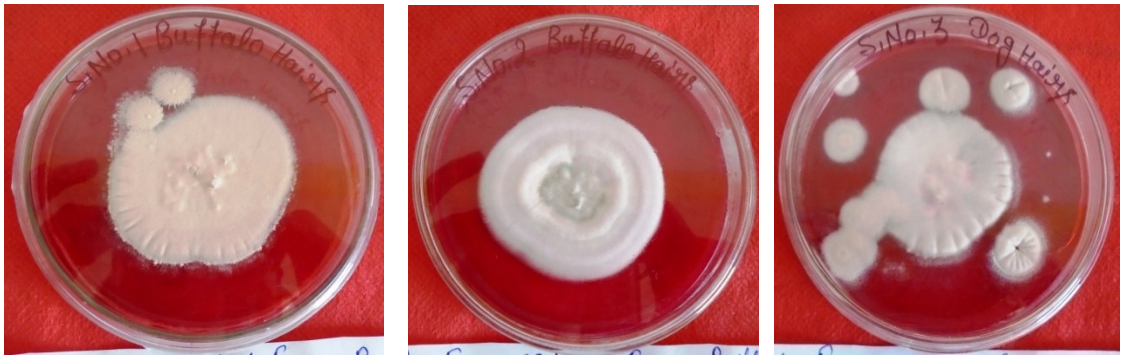
+ = Positive result (Growth observed), - = Negative result (No growth observed)

Figure.1 Collection of soils samples from various habitats of agricultural fields in Saharanpur Village

Soil samples from agricultural fields

Soil samples from animal habitats

Figure.2 Identified Keratinophilic fungi from Saharanpur areas (U.P)



T. tonsurans *T. mentagrophytes* *M.canis*

T. equinum

M. gypseum

T. rubrum

Microsporum cookie

Microsporum audouinii

T. mentagrophytes has been a major causative agent of human superficial mycosis (Ogbonna and Pugh, 1987; Anbu *et al.*, 2004) and also reported higher incidence of *T. mentagrophytes* from soil samples in the present study. This indicates that soil harbors a variety of keratinophilic fungi most of which are seldom involved in infection but have the potential to cause infection. There is natural evaluation of keratin–utilizing soil saprophytes (geophilic species) to associate with and finally invade thickly cornfied substrates in living animals (zoophilic species) and human beings (anthropophilic species). Adaptation to parasitic existence has resulted in a reduced ability to produce spores, which are abundant in soil inhabiting species. *T. rubrum* is an anthropophilic species. Sundaram and Subramanian (1986) isolated *T. rubrum* along with 16 species of human pathogenic fungi from the soil of Madras City by baiting, dilution plate and soil plate method. Jain and Sharma (2011) also reported *T. rubrum* from Jaipur soil. Keratinophilic fungi preferred slightiy acidic to alkaline soils. During present investigation soil pH range varied from 7.19 to 8.24. Most of the fungi were isolated from neutral to slightly soil pH. No keratinophilic fungi were reported below pH 6.0. The results was achieved by Meinhoof, et al and strongly suggested that highly acidic soil were mostly a poor source of keratinophilic fungi and mostly fungi were isolated slightly neutral pH range from 6.92 to 8.24. It's interesting to note that the maximum number of fungi was recorded from soils of gardens and play grounds. The most frequently isolated keratinophilic fungi in this study were *T. tonsurans and T. mentagrophytes.* The high prevalence of

these fungi from these soils explain that, hair of human and animals and feather from birds which come to the soil either as dead or dropped off, serves as substrates and are subjected to microbial decomposition. Keratinophilic fungi play a significant role in the natural degradation of keratinized residues. Sharma and Rajak (2003) obtained 641 isolates from 125 soil samples of parks from Uttar Pradesh indicating soils of park to be a rich source of many keratinophilic fungi. During present investigation all the soils samples of agricultural fields was found most suitable for the occurrence of all most keratinophilic fungi the animal habitats. However higher incidence of keratinophilic fungi was found near textile agricultural fields and animals habitats. Soil rich in kerationous material is most common in India and other tropical countries due to environment factors such as heat and humidity. All these factors and personal hygiene are involved in flourishing of fungal infections and causing fungal diseases. The present investigations can aware common people who directly or in directly associated with these habitats to observe health regulation to control and prevent fungal disease.

It is clear from present investigation that soils of Saharanpur villages are ideal environment for the keratinophilic fungi. This could be attributed to the high organic debris and keratinous substrates present in these soils. However the isolation of fungi was not uniform as it depends on organic matter. Organic matter content of soils is one of the major factors affecting the presence of keratinophilic fungi in soils. Thus these fungi which are human, animal pathogens could be considered as bioindicators of environmental pollution with keratinous substrate and can pose risk of human and animal mycoses.

Acknowledgement

The authors are thankful to Dr. Panckaj Garg, Advisor, Jayoti Vidyapeeth Women's University for providing laboratory and technology facilities used in this work.

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