



Biodiversity of Keratinophilic Fungal Flora in University Campus, Jaipur, India

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Abstract

Background: Soil is well known to support the transient or ongoing existence of keratinophilic fungi and potential source of infection for human and animals

Methods: Samples were collected from 67 sites of university campus like PG study centers, playgrounds, gardens, hostels, administrative blocks, library, bank, canteen and road side for the estimation of keratinophilic fungi using the hair baiting technique.

Results: Totally, 192 isolates belonging to 14 genera and 21 species were reported. Soil pH range varies from 6.5 to 9.0 pH. Most of the fungi isolated from neutral to slightly alkaline soil. *Chrysosporium tropicum* (20.83%) was the most predominant fungi reported from all sites. *Trichophyton mentagrophytes* (15.10%) was the second most commonly reported fungi. *Chrysosporium indicum* (11.45%), *T. simii* (9.37%), *C. evolecanii* (8.83%) *T. terrestre* (4.68%) and *Cephalophora irregularis* (4.68%) were frequently reported. *Microsporium audouinii*, *Paceliomyces* sp., *Cladosporium* sp. and *Sporothrix schenckii* were isolated for the first time from Jaipur.

Conclusion: Road sides were found most suitable for the occurrence of all most all keratinophilic fungi. Higher incidence of keratinophilic fungi was found in hostel sides followed by road sides, PG study centers and play grounds.

Keywords: Keratinophilic fungi, Playground, Soil, India

Introduction

Keratinophilic fungi include a variety of filamentous fungi mainly comprising hypomycetes and several other taxonomic groups. Hypomycetes include dermatophytes and a great variety of non-dermatophytic filamentous fungi. These fungi occurred abundantly in the superficial soil layer of landfills and surrounding and distributed worldwide (1-7). 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.

They occur on cornfield debris in the soil and degrade hard keratin and keratinous material. Therefore they play an important ecological role

in decomposing such residue (8, 9). Keratinophilic fungi are generally considered as soil saprophytes (10). Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. The species of keratinophilic fungal group have been divided into three categories according to their natural habitats. Anthropophilic, when human being are the natural hosts; Zoophilic, when a variety of animals act as natural hosts; Geophilic, when the soil is the natural habitat. Most of the keratinophilic fungi are not dermatophytes but soil inhabitants. Keratin decomposition in soil leads to an increase in carbon, and nitrogen ratio in soil. They are therefore fast growing nonpathogenic keratinophilic fungi

which it is proposed, should be utilized for the recycling of keratin in soil and may be exploited for their biotechnological potential in industry. Many of them are closely related to the dermatophytes having properties in common with them and cause human and animal infection (11-14). In our previous work (15) we carried out a survey of keratinophilic fungal flora of Jaipur district with particular reference to soil pH and found that road side and garden soil were the most suitable sites for the almost all keratinophilic fungi. During that study we took few samples from PG study center.

These studies promoted us to explore biodiversity distribution of keratinophilic and dermatophytic fungal flora from world famous Rajasthan University Campus, Jaipur District in order to promote the knowledge of students and people to observe health regulations to control and prevent fungal disease.

Material and Methods

University of Rajasthan is the oldest university in Indian state of Rajasthan. It was set up on 8th Jan 1947 as the University of Rajputana and was renamed to its current name in 1956. University campus is situated in the heart of Jaipur City which is popularly known as Pink city. A total 67 soil samples were collected from PG study center, Playgrounds, Hostels, Garden, Library, Bank, Administrative blocks, Canteen and Road side from August 2010 to November 2010. Most of the samples were collected in the morning (8-12 am). Surface soil was collected with the help of a sterilized spoon. Each plastic bag was labeled indicating the date and site of collection. Soils were sandy (92%) in nature containing 3-4 % slit and clay. These samples were then tightly closed to maintaining original moisture and kept in the culture room at a temperature of $28 \pm 2^\circ\text{C}$ as per the need. To.Ka.Va hair baiting technique (16) was followed for isolating the fungi. Twenty five gram of soil sample was taken in a separate sterilized plate of Petri from each sample. Sterilized distilled water was added to provide moisture to the soil. Bits of sterilized human hair and animal hair (cow,

buffalo, dog) were used as baits. The hair were scattered uniformly only on wet soil. Each plate of Petri was incubated at $28 \pm 2^\circ\text{C}$ for 3-4 weeks in culture room.

Isolation, purification and identification of fungi

The baited cultures were examined after 3-4 weeks for the development of any fungal growth on the hair and nail bait. For fungal examination, a small portion of fungal growth was picked up with the help of a sterilized needle, mounted on a slide under covered glass containing a drop of sterilized distilled water or any other staining solution, and examined under a microscope. Isolation, purification and identification were done on Sabouraud dextrose agar media fortified with chloramphenicol (0.05mg/ml) and cycloheximide (0.5 mg/ml). Identification of fungal culture was done on the bases of colony characters, pigmentation, different media study and by their morphological and physiology characteristics according to the procedure described earlier (17, 18).

Metzer's digital pH meter was used to detect the pH of soil solution. For this purpose, a small amount of soil (1 gm) was shaken with double distilled water (5 ml) and the mixture was allowed to stand for about 30 minutes. pH of distilled water was also detected before addition of soil sample. Electrode was inserted in the soil solution and reading was noted. This procedure was followed for each soil sample.

Results

Data incorporated in Table 1 shows the prevalence and distribution of keratinophilic fungal flora of university campus. Out of 67 samples examined from different sites 65 were found positive in fungal growth. A total of 192 isolates belonging to 14 genera and 21 species were reported in present investigation.

Chrysosporium tropicum (20.83%) was found to be the most common keratinophilic fungi isolated from all sites. *Trichophyton mentagrophytes* (15.10%) was the second most common fungi.

Table 1: Keratinophilic fungal flora of University Campus

	<i>T. rubrum</i>	<i>T. simii</i>	<i>T. terrestre</i>	<i>T. mentagrophytes</i>	<i>C. tropicum</i>	<i>C. indicum</i>	<i>C. evolceanui</i>	<i>Gymnoascus reessii</i>	<i>Microsporium gypseum</i>	<i>M. audouinii</i>	<i>Cephalophora irregularities</i>	<i>Histoplasma capsulatum</i>	<i>Cladosporium</i> sp.	<i>Fusarium</i> sp.	<i>Alternaria</i> sp.	<i>Paceliomyces</i> sp.	<i>Curvularia</i> sp.	<i>Torula</i> sp.	<i>Sporothrix schenickii</i>	<i>Nocardia</i> sp.	Unidentified species	Total	Total soil samples	+ve soil samples
1. P.G. study center	-	2	3	3	7	3	-	1	-	1	3	1	-	1	-	-	-	1	-	-	4	30	14	12
2. Hostels	1	6	-	10	14	6	3	2	3	-	3	-	1	-	-	-	-	-	1	2	3	55	16	16
3. Play Ground	1	3	3	5	5	3	2	-	1	-	1	-	2	-	-	1	-	-	-	-	-	28	7	7
4. Garden	-	-	1	1	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	5	3	3
5. Library	1	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2	2
6. Bank	2	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	2	2
7. Administrative blocks	-	2	-	1	2	-	2	-	1	-	-	1	-	-	-	-	1	-	-	-	2	12	5	5
8. Canteen	-	-	-	2	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	5	2	2
9. Road side	2	5	2	7	7	6	7	-	2	-	2	1	1	1	1	-	1	-	-	-	3	48	16	16
	7	18	9	29	40	22	16	3	7	1	9	3	4	2	2	1	1	1	1	2	13	91	67	65
%	(3.64)	(9.37)	(4.68)	(15.1)	(20.8)	(11.4)	(8.33)	(1.56)	(3.64)	(0.52)	(4.68)	(1.56)	(2.08)	(1.04)	(1.04)	(0.52)	(0.52)	(0.52)	(0.52)	(1.04)	(6.77)			(95.83)

Among all these fungal species *Microsporium audouinii* (0.52%), *Cladosporium* sp. (2.08), *Paceliomyces* sp. (0.52%) and *Sporothrix schenckii* (0.52%) were isolated for the 1st time in Jaipur. Among all habitats, road side were found most suitable site for the occurrence of all most all keratinophilic fungi. However the higher incidence of these fungi was reported in hostel site.

Some of the soil samples yields a single species and some yielded a mixed growth of two or more than two species of keratinophilic and dermatophytic fungi. (i) *Chrysosporium tropicum* + *T. terrestris*,

(ii) *C. tropicum* + *Cephalophora irregularis* and (iii) *C. tropicum* + *T. mentagrophytes* + *C.indicum* were the most commonly isolated fungal mixture. During the study of soil pH (Table 2) pH range varies from 6 to 9.5. Most of the fungi were isolated from 7.5-8.0 pH range soil samples. *C.tropicum* reported from 7.0-9.0 pH soil samples but higher incidence was seen at 8.5 pH soil samples. Single isolate of *M.audouinii* was isolated from 7.0 pH while *Paceliomyces* sp. at 8.5 pH, *Cladosporium* sp. at 8.0-8.5 and *Sporothrix schenckii* at 8.5 pH soil sample.

Table 2: Keratinophilic fungi and soil pH

	Species	pH of soil samples					
		6.5	7.0	7.5	8	8.5	9
1.	<i>Trichophyton simii</i>	1	-	4	8	7	1
2.	<i>T. terrestris</i>	-	1	2	8	1	1
3.	<i>T. verrucosum</i>	-	2	2	3	-	1
4.	<i>T. rubrum</i>	-	-	2	1	-	-
5.	<i>Chrysosporium tropicum</i>	-	1	7	13	6	2
6.	<i>C. indicum</i>	1	2	3	6	4	-
7.	<i>C. evolecanii</i>	-	-	2	5	2	1
8.	<i>Microsporium gypseum</i>	-	-	1	2	-	1
9.	<i>M. audouinii</i>	-	1	-	-	-	-
10.	<i>Gymnoascus reessii</i>	-	1	-	1	-	-
11.	<i>Histoplasma capsulatum</i>	-	1	-	-	1	-
12.	<i>Cladosporium</i> Sp.	-	-	-	3	1	-
13.	<i>Fusarium</i> Sp.	1	-	-	-	-	-
14.	<i>Alternaria</i> Sp.	-	-	-	2	-	-
15.	<i>Paceliomyces</i> Sp.	-	-	-	-	1	-
16.	<i>Curvularia</i> Sp.	-	-	-	1	-	-
17.	<i>Torula</i> Sp.	1	-	-	-	-	-
18.	<i>Sporothrix schenckii</i>	-	-	-	-	1	-
19.	<i>Nocardia</i> Sp.	-	-	1	-	1	-
20.	<i>Cephalophora irregularis</i>	2	2	2	1	-	-
21.	Unidentified species	1	-	-	6	-	-

Discussion

The keratinophilic fungi include true fungi that vigorously degrade keratin as well as some more important pathogenic and non pathogenic dermatophytes (3,8,10,14). The composition of keratinolytic fungal communities in the soil differed from that observed for a highly populated and walked area and displayed distinct seasonal variations. Several investigations have been done in

various parts of India during the last two decades and showed that a rich variety of keratinophilic fungal flora exists in the soils of the studied area (8,10,19-23). However, there was no evidence of any study on university or higher education centers. Therefore, the present investigation was carried out for the study of biodiversity in the distribution of keratinophilic fungi in the soil of the university campus of the world famous Rajasthan University where the students from all over the world come for higher studies. Jaipur,

the capital of Rajasthan, is a highly populated city. Its temperature exceeds even 46 ° c in summer and has high humidity during monsoon season. All these conditions favour the higher incidence of keratinophilic fungi in Jaipur. In present investigation, *C. tropicum* (20.83%) was the most predominant fungus followed by *T. mentagrophytes* (15.10%) and *C. indicum* (11.45%). Similar results were observed by Ramesh and Hilda (21) during the survey of keratinophilic fungi of primary schools and public parks.

Out of 45 soil samples studied, incidence of *C. tropicum* was 62.2%. Sharma and Sharma (23) also seen the maximum incidence of *C. tropicum* (26%) followed by *C. indicum* (22%) and *T. mentagrophytes* (16%) during the survey of keratinophilic and dermatophytic fungal flora in the schools and college playground soils of Jaipur. Jain and Sharma (15) carried out a survey of keratinophilic fungal flora of Jaipur district with particular reference to soil pH and reported *C. tropicum* as the most predominant fungus (46.08%) and *T. mentagrophytes* as second predominant fungus (24.88%) from pH 6.5 to 9. *T. mentagrophytes* species is known to be polymorphic fungus existing in a geophilic, a zoophilic or an anthropophilic state. *T. mentagrophytes* has been a major causative agent of human dermatophytes. Nowadays anthropophilic species named as *T. interdigitale*. This fungus is also found frequently on apparently asymptomatic animal skin. Ogbonna (24) and Anbu et al. (5) also reported higher incidence of *T. mentagrophytes* from soil samples. This indicates that soil harbors a variety of keratinophilic fungi, both dermatophytes and non dermatophytes, 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 cornified substrates in living animals (zoophilic species) and men (anthropophilic species). Adaptation to parasitic existence has resulted in a reduced ability to produce spores, which are abundant in soil inhabiting species.

Incidence of *T. simii* and *C. evolecanui* was 9.37% and 8.33%, respectively. In our previous work (15) *C. evolecanui* was re-isolated after 23 years from

Jaipur area. Present investigation also indicates the survival and existent of *C. evolecanui* in Jaipur area at pH 7.5 to 9.0.

T. rubrum is an anthropophilic species. Sundaram and Subramanian [25] 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 (15) and Sharma and Sharma (23) also reported *T. rubrum* from Jaipur soil. In present study *T. rubrum* (3.64%) was isolated from hostels, play ground, library, bank and road side. *M. gypseum* is a common geophilic dermatophytes widely distributed in soil globally (26). *M. gypseum* causes ring worm of scap and glabrous skin in human and animals. In this study prevalence of *M. gypseum* (3.64%) was reported in hostel soil followed by road side.

One isolate of *M. audouinii* was reported in PG study center at pH 7.0. *M. audouinii* is an anthropophilic fungus causing non-inflammatory infection of scalp and skin especially in children. These fungi can also survive in the soil as resting spores and germinate when suitable condition arises. Majority of fungi producing disease in men and animals exist freely in nature as soil saprophytes and gain entrance into body through abrasion, implantation or inhalation. P.G study centers are highly populated and walked places where thousands of students studied. Infection can easily transmitted from one person to another through frequent handling of articles, files, papers and hand shake. In our previous work (15) *M. audouinii* was reported from bird habitat soil sample from Jaipur district. We also reported one case of *Tinea capitis* suffering from *M. audouinii*. This work is under consideration in another journal.

Among all other related fungi, *Cephalophora irregularis* (4.68%) was abundantly reported in university campus like PG study center, hostel, road side and play ground.

Keratinophilic fungi mostly preferred slightly acidic to alkaline soil (27). During present investigation soil pH range varied from 6.5 to 9.0. Most of the fungi were isolated from neutral to slightly alkaline soil pH. No keratinophilic fungi were reported below pH 6.0 and above 9.0. The same was achieved by Meinhof et al. (28) and Ka-

ben (29). They strongly suggested that highly acidic soils were mostly a poor source of keratinophilic fungi. During present investigation all the soil samples of hostels, playground, garden, library, bank, administrative blocks, canteen and road side were positive for fungal growth. Road side was found most suitable for the occurrence of all most all keratinophilic fungi. However higher incidence of keratinophilic fungi was found in hostel side followed by road side, PG study center and play ground. Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic and dermatophytic fungi.

The fungal infection of human beings is more 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 investigation can aware the students, teachers and all other people who directly or indirectly associated with university campus to observe health regulation to control and prevent fungal disease.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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