

Isolation and identification of keratinophilic fungi from soil of college campus, Bhopal

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Abstract

The soil constitutes one of the most complex microbial habitats in which many fungi complete their entire life cycles. Soil microflora plays a pivotal role in the evaluation of soil conditions and in stimulating plant growth. Microbes are beneficial as they increase the soil fertility. During our investigation, soil samples were collected from an underground pit in Sri Sathya Sai College for Women, Bhopal that was specifically created for dumping keratin rich substances like human hairs, nails etc. The samples were screened for presence of keratinophilic fungi using hair baiting technique for isolation. Out of the 17 species which were isolated 9 were keratinophilic. The genus *Aspergillus* had 4 species including *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Aspergillus flavus*. The genus *Alternaria* had 2 species including *Alternaria alternata* and *Alternaria tenuissima*. Single species of *Humicola fuscoatra*, *Cladosporium cladosporioides* and *Penicillium chrysogenum* were also isolated. Out of these *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus flavus* were isolated from hair and nails. *Alternaria tenuissima*, *Alternaria alternata* and *Humicola fuscoatra* were isolated from hair. *Cladosporium cladosporioides* was isolated only from nails. This shows that these respective isolated fungi have good potency in degrading keratinous waste. Further studies are needed for developing good inoculate for safe management of keratinous waste.

Key Word: Human hair waste, human Nail Waste, Keratinophilic Fungi, soil, Bhopal

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INTRODUCTION

Numerous microbes are present in our environment mostly bacteria and fungi¹. The soil constitutes one of the most complex microbial habitats in which many fungi complete their entire life cycle². Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth. Microbes are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soils³. The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. Fungi are an important component of

the soil microbiota typically constituting more than bacteria, depending on soil depth and nutritional condition⁴. Soil is rich in pathogenic and non-pathogenic keratinophilic fungi⁵. Keratinous substances which are important natural material, occurring in nature in the form of hairs, wools, feathers, horns, hooves, nails, skin and other cornified appendages, constituting natural baits for these fungi. Keratinophilic fungi represent an important component of soil microflora where they decompose highly resistant keratin, a proteinaceous substrate. This unique group may exist and proliferate activity in soil under favourable condition, particularly in places, where they can utilize various forms of keratin⁶. Keratinolytic fungi occur in many natural and manmade habitats. These fungi exist in communities together with keratinophilic fungi that have weaker affinity to keratin and utilize chiefly the products of its decomposition. Keratinophilic fungi are ecologically important group of fungi that decompose one of the most abundant and highly stable animal proteins on Earth, keratin, which they use as a nutrient substrate for growth.

Common Habitats of Keratinolytic Fungi

- Cattle shed

- Garbage
- Barbers hair dumping area
- Public places like parks, school, marketplace, etc
- Poultry sheds

In 1952, with invention of the technique of the isolation of soil fungi, studies on keratinophilic fungi started and soil proved to be the natural reservoir of these fungi¹. The aim of the present investigation was to report the distribution of keratinophilic fungi from selected area of college in order to promote the knowledge among students and public, how to control and prevent fungal diseases.

MATERIEL AND METHODOLOGY

Collection of Samples: In our work keratinophilic fungi were isolated from soil of hostel area where hairs and nails were dumped by students of Sri Sathya Sai College for Women, Bhopal. The samples were collected from topmost part of the soil (5cm depth) with sterile spatula and were collected inside sterile polythene bags from month November 2015 to October 2016. These samples were brought to the laboratory and processed immediately and stored at room temperature for further studies.

Isolation of fungi from the soil sample by soil dilution plate method: 1gm of soil sample was suspended in 10 ml of distilled water to make dilution series (10⁻¹ to 10⁻⁵). Dilution of 10⁻³, 10⁻⁴ and 10⁻⁵ were used to isolate fungi. 1ml of microbial suspension of each concentration were added to sterile Petri plates containing 15 ml of SDA (Sabouraud's Dextrose Agar) with 1% streptomycin solution for preventing bacterial growth. The Petri plates were then incubated at 28°C. The plates were observed regularly for three days³. Pure cultures can be prepared and identified morphologically. For further identification these isolated species were sent to NCFT New Delhi (National Centre for Fungal Taxonomy).

Isolation and Identification of keratinophilic fungi from soil sample: The human hair and nails were obtained from barber's shop. Hairs and nails were washed thoroughly in three changes of distilled water. They were air dried and were defatted by soaking in diethyl ether for 24 hrs. They were thoroughly washed again with distilled

water and were completely dried in hot air oven at 60°C for 24 hrs.

Hair and Nail Baiting: The modified hair baiting method was used for the isolation of keratinophilic fungi⁷. A weight of 50g of each sample was transferred to a sterile Petri plate and pieces of sterilized and defatted human hairs and nail was spread over the soil in the plate. Add 10-15 ml of sterile water to the plates to facilitate germination of fungal spores. Incubate the plates at room temperature (20°C–25°C) in the dark for 4-6 weeks. Remove hair and nail fungal growth to take inoculum and place it on SDA medium. After one or more week, check the colonies and identify the fungus. Pure cultures can now be prepared and identified by NCFT (National Centre for Fungal Taxonomy).

RESULTS AND DISCUSSION

During the investigation 17 species were obtained by soil dilution method presented in Table 1. Then, the samples were screened for the presence of keratinophilic fungi using hair bait technique for isolation. Isolated fungi were identified on the basis of colony and morphological characterization. Out of 17 fungal species, 9 were reported as Keratinophilic. The genus *Aspergillus* had 4 species including *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, and *Aspergillus flavus*. The genus *Alternaria* had 2 species including *Alternaria alternata* and *Alternaria tenuissima*. Single species of *Humicola fuscoatra*, *Cladosporium cladosporioides*, and *Penicillium chrysogenum* were also isolated. Table 2 shows that *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus flavus* were isolated from hair and nails, both while *Alternaria tenuissima*, *Alternaria alternata*, *Penicillium chrysogenum* and *Humicola fuscoatra* were isolated from hair only. *Cladosporium cladosporioides* was isolated only from nails. It appears from this study that *Aspergillus* is the most prevalent keratinophilic fungus. Other isolated fungi have also good potency in degrading keratinous waste. In Table 3 cultural characteristics and microscopic morphology of isolated keratinophilic fungus on SDA medium were shown.

Table 1: Monthly distribution of fungi isolated from soil sample from Nov 2015 to Oct 2016.

Sr. No	Name of Isolated Fungi	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Total	%
1	<i>Aspergillus fumigatus</i>	2	0	0	1	0	0	0	1	2	1	0	0	7	2.35
2	<i>Aspergillus niger</i>	1	1	0	1	1	0	3	0	2	2	0	1	12	4.03
3	<i>Aspergillus terreus</i>	1	2	0	0	0	2	0	6	3	4	1	0	19	6.38
4	<i>Aspergillus flavus</i>	0	0	0	1	1	2	0	0	7	3	4	2	20	6.71
5	<i>Cladosporium cladosporioides</i>	1	0	0	1	0	0	3	7	3	1	0	4	20	6.71
6	<i>Alternaria alternata</i>	0	0	0	1	2	1	4	2	5	2	0	2	19	6.38
7	<i>Alternaria tenuissima</i>	1	2	0	0	1	3	6	0	2	4	3	4	26	8.72
8	<i>Humicola fuscoatra</i>	0	1	0	0	0	0	3	0	1	3	2	1	11	3.69
9	<i>Penicillium chrysogenum</i>	1	0	0	0	0	2	2	1	3	2	5	2	18	6.04
10	<i>Penicillium glabrum</i>	4	2	1	1	0	1	0	0	1	3	0	0	13	4.36
11	<i>Curvularia pallescens</i>	2	4	2	0	2	0	3	2	0	0	2	0	17	5.70
12	<i>Aspergillus oryzae</i>	1	5	1	2	4	1	2	4	2	2	1	2	27	9.06
13	<i>Emmericella nidulans</i>	3	0	0	0	1	2	0	1	2	0	1	1	11	3.69
14	<i>Penicillium expansum</i>	4	2	1	2	1	0	1	0	1	2	2	1	17	5.70
15	<i>Penicillium oxalicum</i>	0	0	4	5	0	2	1	0	6	1	0	0	19	6.38
16	<i>Aspergillus parasiticus</i>	2	1	2	3	1	4	2	2	5	2	0	0	26	8.72
17	<i>Curvularia lunata</i>	1	0	1	0	4	3	0	3	0	2	0	2	16	5.37
	Total	24	20	12	18	18	23	30	29	42	37	23	22	327	

Table 2: Keratinophilic Fungi Isolated On Human Hair Bait and Human Nail Bait

Sr. No	Name of Isolated Fungi	Human Hair Baits	Human Nail Baits
1	<i>Aspergillus fumigatus</i>	+	+
2	<i>Aspergillus niger</i>	+	+
3	<i>Aspergillus terreus</i>	+	-
4	<i>Aspergillus flavus</i>	+	+
5	<i>Cladosporium cladosporioides</i>	-	-
6	<i>Alternaria alternata</i>	-	-
7	<i>Alternaria tenuissima</i>	+	-
8	<i>Humicola fuscoatra</i>	-	-
9	<i>Penicillium chrysogenum</i>	+	-

+ = Keratinophilic fungi present on human hair and nail baits

- = Keratinophilic fungi absent on human hair and nail baits

Table 3: Cultural Characteristics and Microscopic Morphology of fungi

Sr. No	Isolated keratinophilic fungi	Growth medium used	Cultural characteristics	Microscopic Morphology
1.	<i>Aspergillus fumigatus</i>	SDA	Wooly to cottony, smoky grey-green color, unevenly spread	Hyphae are septate and hyaline
2.	<i>Aspergillus niger</i>	SDA	Dense granular, globose, dark black thin walled	Conidiophores are smooth walled, hyaline or turning dark
3.	<i>Aspergillus terreus</i>	SDA	Velvety, green color	small conidiophores
4.	<i>Aspergillus flavus</i>	SDA	Cottony black, white	Thread like branching filaments known as hyphae
5.	<i>Cladosporium cladosporioides</i>	SDA	Velvety, olivish brown, black	Blastic conidia, pigmented and conidiophores: erect, and form tree like conidial structure
6.	<i>Alternaria alternata</i>	SDA	Grey to olive Brown	Muriform, beaked conidia produced in acropetal chain
7.	<i>Alternaria tenuissima</i>	SDA	White color colony, cottony appearance	Conidia are smooth walled
8.	<i>Humicola fuscoatra</i>	SDA	Thin or floccose, grey to dark brown and smooth	Dark holoblastic conidia
9.	<i>Penicillium chrysogenum</i>	SDA	Blue-green or grey green, with white edge	Septate, hyaline hyphae, conidiophores branched

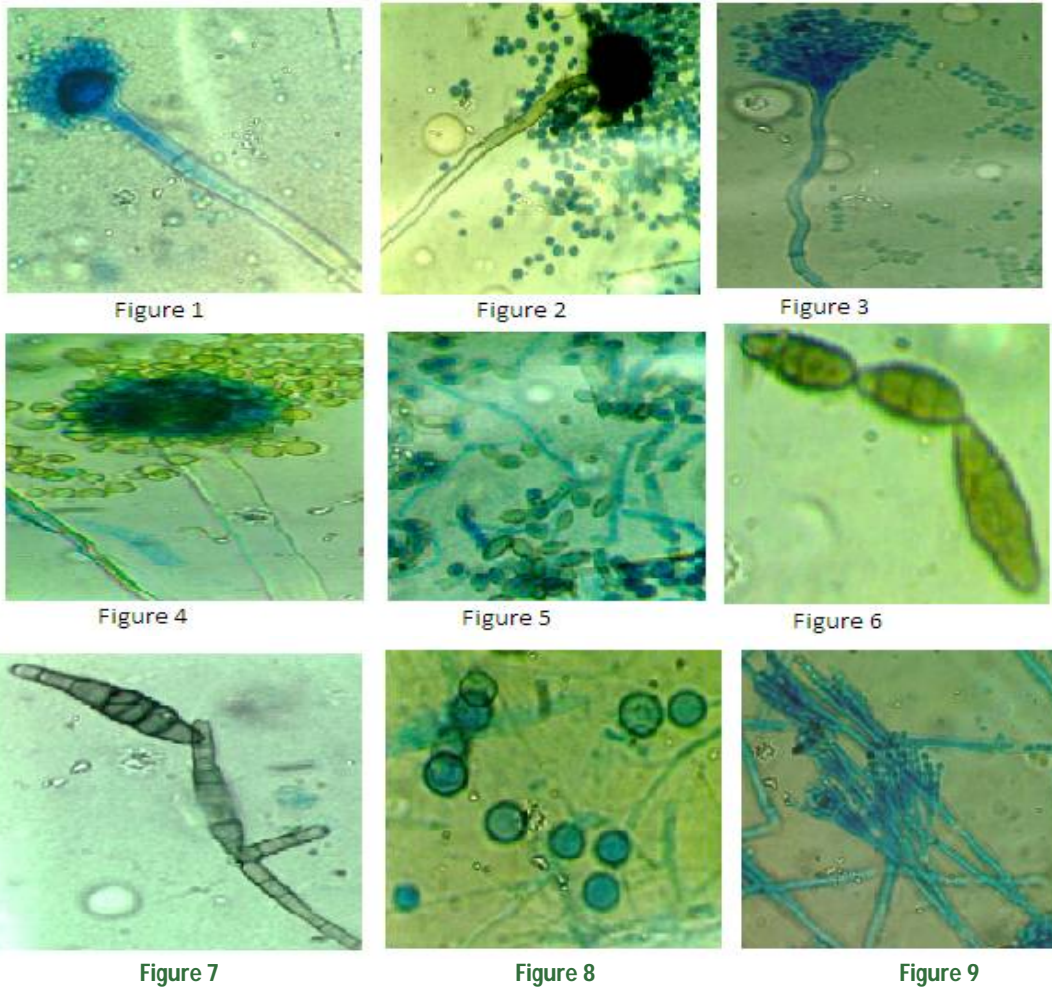


Figure 1: *Aspergillus fumigatus*; **Figure 2:** *Aspergillus niger*; **Figure 3:** *Aspergillus terreus*; **Figure 4:** *Aspergillus flavus*; **Figure 5:** *Cladosporium cladosporioides*; **Figure 6:** *Alternaria alternate*; **Figure 7:** *Alternaria tenuissima*; **Figure 8:** *Humicola fuscoatra*; **Figure 9:** *Penicillium chrysogenum*

Keratinophilic fungi are attracting lot of attention around the world because they play an important role in the decomposition of keratin substrates and could be used in bioremediation of such type of waste and waste contaminated sites. In India, numbers of reports have appeared concerning the presence of keratinophilic fungi from various habitats⁸. Previously, in Jaipur, keratinophilic fungi *Chrysosporium tropicum* (20.83%) was the most predominant fungi reported. *Trichophyton mentagrophytes* (15.10%) was the second most commonly reported fungi. *Chrysosporium indicum* (11.45%), *T. simii* (9.37%), *C. evolceanui* (8.83%) *T. terrestre* (4.68%) and *Cephalophora irregularis* (4.68%) were frequently reported. *Microsporium audouinii*, *Paceliomyces* sp., *Cladosporium* sp. and *Sporothrix schenckii* also reported⁹. In the Gwalior region of India keratinophilic fungi were isolated and characterized from different public park soils. Two

keratinous fragments, human hair and nails were used for the growth of fungi by the hair-baiting technique. *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton equinum*, *Microsporium gypseum*, *Microsporium nanum*, *Microsporium audouinii* and *Aspergillus niger* were isolated⁵. From piggery soil of Jharkhand, India reports the Keratinophilic fungi were isolated in which maximum percentage of *Aspergillus niger* (12.06%) and *Fusarium oxysporium* (10.34%) was found¹⁰. Some of the other fungi were also isolated from soils of Hyderabad by hair baiting technique *C. zonatum*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium moniliforme*, *F. solani*, *Aphanoascus fulvescens* etc¹¹. Several reports have indicated that *Aspergillus* species are among the most prevalent keratinophilic fungi in the soils. *A. niger*, was one of the dominant fungus in the garbage waste soil of Ranchi and *A. flavus*, also

isoalted which is reported to have kaeatinase activity. *Penicillium chrysogenum* was the most prevalent sapeophyte isolated during their study on kaeratinophilic fungi¹². In which *Aspergillus* and *Penicillium* were the most predominant saprophytic genera. Hair and nail samples collected from students of Benisuef house of keratinophilic fungi isolated from hairs and nails of students included *Aspergillus*, *Penicillum*, *Candida albicans*, *Rhizopus stollenifer*, *T.harzianum*, *Chrysosporum* and *Microsporium*¹³. Also most of these fungi were isolated previously from hairs of large mammals or feathers of poultry and human¹⁴. The study clearly indicates the varied distribution of Keratinophilic fungi in soil of college campus.

CONCLUSION

Various microbes are present in the environment and invade tissues which are capable of breakdown keratin protein. Keratinophilic fungi are important ecologically and recently have attracted attention throughout the world. They play a significant role in the natural degradation of keratinized residues. The present investigation can aware the students, teachers and all other people who directly or indirectly associated with college campus to observe health regulation to control and prevent fungal diseases.

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