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Identification of Keratinophilic Fungi in Urban Waste and Cattle Field Soil of Kanpur, India for Environmental Pollution Management

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Kanpur is a city which has huge number of leather product units and leather processing plants. These units are one of major contributors of keratinous waste and produces keratinous material as waste in the form of hairs, hides, dermis. During the present study 83 keratinophilic fungi were isolated from 40 soil samples of urban waste and cattle field habitat of various localities. From 20 samples of urban waste, 44 keratinophilic fungi were isolated, 39 fungi recorded from Cattle field. The frequency of genera *Chrysosporium* was recorded in urban waste (29.54%) and cattle field soil (20.51%). Maximum (13.83%) frequency was recorded in the case of *Chrysosporium indicum* in urban waste.

Keywords: Keratinophilic fungi; keratin waste; dermatophytes; Chrysosporium.

1. INTRODUCTION

Soils are natural reservoir of keratinophilic fungi due to presence of keratinous waste materials which is most suitable for the growth [1]. Keratinophilic fungi are present in the environment with in consistent al location which depends on as human and animal presence [2].

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The existence of keratinophiles in the soil is also influenced by the presence of other microbes namely the bacteria and actinomycetes and fungal components which exert an antagonistic effect on keratinophilic fungi [3-5].

Soil of various habitats of Kanpur city were studied for occurrence of keratinophilic fungi; indoor environment [6-7], house dust [8-9], birds [10], house sweeping dust, sand [11-13], potted indoor plant [14], parks [15] aquatic habitats [16] and sewage slug [17] Few other recent reports of occurrence of keratinophiles from India and other parts of Worlds are Hilly soil [2], Ladak [18], Sewage sludge of Vishakhapatnam [19], Vidarbha region of Maharashtra [20], poultry site of Shivamogga Karnataka [21], predatory birds [22], Parks of Jaipur [23]. Climate and keratinous waste in environments make Kanpur city appropriate study area. Kanpur is city situated at the bank of river Ganga and famous for their leather-based industry. Leather product units and leather processing plants are one of major contributors of keratinous waste and produces keratinous material as waste in the form of hairs, hides, dermis. Soil of Kanpur is thoroughly screened for these fungi in other habitats but particular emphasis was not given to the urban waste and cattle fields. The objective of this study to isolate new strains of these fungi.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples and Keratinous Substrate

A total 40 soil samples were collected from 20 each from cattle fields and urban waste soil were taken from superficial soil layer depth not exceeding 2 to 3 inches with a sterilized plastic spoon in sterilized polythene bags. Human hair, Chicken feather, Human nail, Horn and cattle hair were washed and cut into pieces of 1-2 cm autoclaved for 15 min at 15 lbs pressure and used as keratinous substrates.

2.2 Isolation Methods

Ten to twenty gm of soil from each collected sample was collected in pre sterilized poly bags. Petri dishes and moistened with 10 ml of sterilized distilled water by hair baiting method [24] (Fig 1A). These samples were baited by keratinous substrates. Human, horse, and buffalo hair, human nails, chicken feathers and cow horns were used as keratinous substrate. When fungal colony observed on bait it is isolated on Potato dextrose agar and Sabouraud's dextrose agar (Fig. 1B) and maintained as culture in tube, water cultures and dry herbarium are also maintained.

2.3 Purification and Identification of Fungi

In the Petri dishes, when a fungal colony was seen for the first time, it is transferred to other dishes for purification. To ensure the purity of cultures, all the isolated cultures studied are derived from a single spore raised through the dilution method. After ensuring the complete purity of cultures, the descriptions are made. Measurement for each fungus is taken by culturing it on a suitable medium. Identification of the isolated fungi will be confirmed with the help of literature available in this department and secured through the courtesy of various mycologists from India and abroad. Living cultures were deposited in DST sponsored Germplasm Centre for Keratinophilic Fungi (GPCK), Department of Botany, Christ Church College, Kanpur.

Fig. 1. A. Growth of *Chrysosporium indicum* B. Growth of *C. indicum* on Potato dextrose agar

For quantitative analysis, following parameters were considered to estimate fungal population.

Distribution (%) =

$$\frac{\text{Number of samples in which species occurred}}{\text{Total number of samples examined}} \times 100$$

Frequency of isolation (%) =

$$\frac{\text{Number of strains of a given species}}{\text{Total number of fungal strains}} \times 100$$

3. RESULTS AND DISCUSSION

During the present study, 40 soil samples collected from the different habitat of various localities yielded 83 keratinophilic fungi. Isolated fungi were morphological identified (Fig. 2).

Isolated strains belong to 21 genera and 45 species. Results of the incidence of keratinophilic fungi are given in Table 1. From 20 samples of urban waste, 44 keratinophilic fungi were isolated, 39 fungi recorded from Cattle field (Fig. 3 & 4).

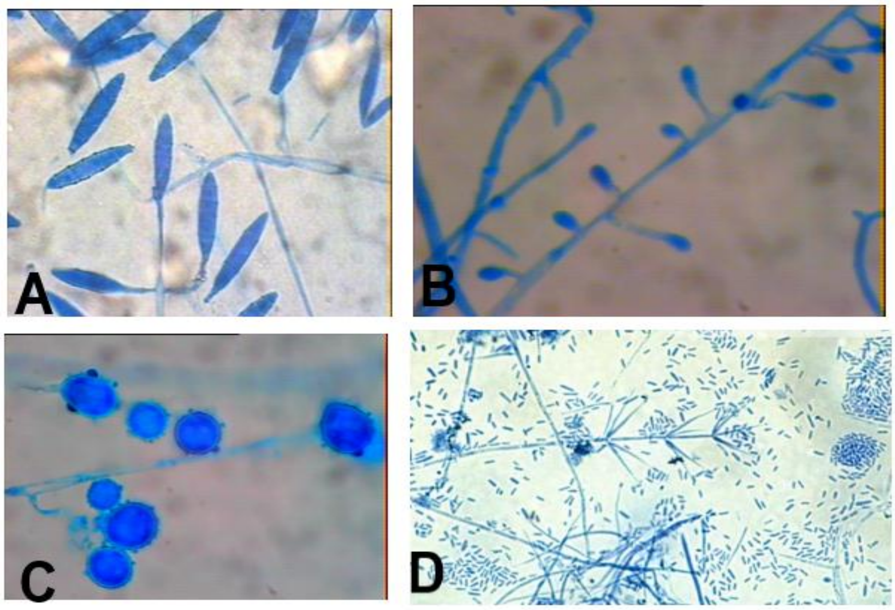

Fig. 2. Conidia X 1000 A. *Microsporum gypseum*, B. *Chrysosporium tropicum*, C. *Ctenomyces serratus*, D. *Verticillium* sp.

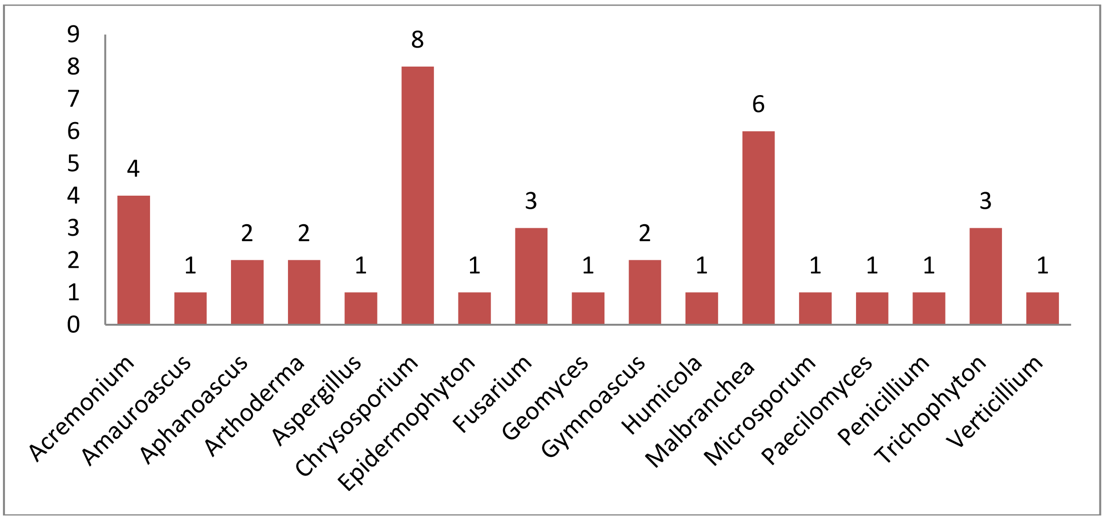

Fig. 3. Number of fungi in cattle field soil

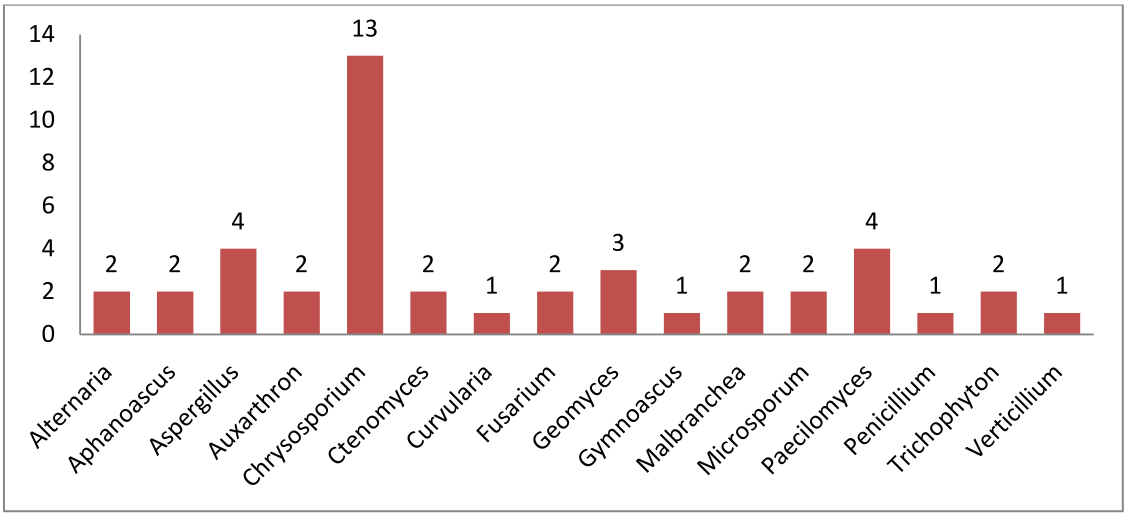

Fig. 4. Number of fungi in urban waste soil

Auxarthron conjugatum (anamorph of *Malbranchea* sp.) showed its highest percentage of distribution in urban waste. *Arthroderma simii* (anamorph of *Trichophyton simii*) showed its maximum distribution in urban waste. Among all perfect forms, the maximum percent frequency was recorded in urban waste. The fast-growing nondermatophytic keratinophilic fungi isolated from keratinous substrates and found in various habitats. *Alternaria alternata* was recorded in urban waste. Five species of *Aspergillus* was isolated during the present study *Aspergillus sparus* was isolated from street sweeping soil with 10.52% distribution. *Aspergillus sydowii* revealed the 5.56%% distribution in urban waste. *Penicillium* was next dominant genus among fast growing keratinophilic fungi. *Penicillium griseofulvum* was isolated from three habitats. Two species of *Fusarium* belonging to 8 isolates were isolated from various habitats. *Fusarium oxysporum and Fusarium proliferatum* exhibited same pattern of distribution and isolated from both habitats.

Urban waste represents polluted field soil while sand represents water habitats. Urban waste and sand allow the growth of several dermatophytes and non dermatophytic keratinophilic fungi. Urban waste and street sweepings are polluted habitats as comparison to normal habitats. Street sweepings are a component of municipal solid waste. The urban waste was found to be rich in keratinolytic fungi and the genera *Chrysosporium* predominated among the isolates. In sweepings, *Epidermophyton* and *Microsporum* were predominated. The quantitative and qualitative composition in the sweepings was associated with pH, the content of heavy metals and particle size [25]. The diversity of keratinophilic fungal communities in field soils and waste water habitats was studied b [26]. Cattle field samples were rich with a high content of keratin in the form of cattle hairs, horns. [27] Isolated *Aphanoascus terreus*, *Apinisia queenslandica*, *Chrysosporium indicum*, *Chrysosporium lucknowense*, *Chrysosporium tropicum*, *Chrysosporium queenslandicum* from cattle soil. All the habitats discussed above are hygienic and epidemiological importance. However, in reports many fungi are used for feather waste utilization for biofertilizers [28-34].

Out of forty-three keratinophilic fungi, twenty-one isolates of *Chrysosporium* were observed as dominating fungi. *Chrysosporium queenslandicum* was isolated from two habitats. *Chrysosporium pannicola* was found in the soil of urban waste. *Chrysosporium sulphureum* was restricted in its distribution and isolated from cattle field soil. The frequency of *Chrysosporium* recorded in various habitats was as follows: urban waste (29.53%) and cattle field soil (17.94 %). A Maximum (15.90%) frequency was recorded in the case of *Chrysosporium indicum* in urban waste (Table 2). The frequency of *Microsporum* recorded in urban waste (10.00 %), cattle field (5.00%). *Trichophyton ajelloi* was isolated from soils collected from urban waste.

Trichophyton rubrum was isolated from cattle field was 10.00 % in its distribution. In some soil samples keratinophilic fungi also developed ascostoma of their corresponding anamorph. *Aphanoascus terreus* (anamorph of

Chrysosporium indicum) was isolated from urban waste where it was 5.00 % in its distribution. *Aphanoascus fulvescens* (anamorph of *Chrysosporium* sp.) showed 5.00 % distribution in urban waste while *Aphanoascus keratinophilus* (anamorph of *Chrysosporium keratinophilum*) showed the 10.00 %, distribution in cattle field soil.

Table 1. Nondermatophytic keratinophilic and related fungi from Cattle field and urban waste habitats

Habitat	Locality	Fungus
Cattle Field	CF1	*A keratinophis* GPCK 3765, *P. griseofulvum* GPCK 3624
	CF2	*C. indicum* GPCK 3627
	CF3	*M.pulchella* GPCK3625, *A.simii*GPCK 3724, *G.pannorum* GPCK 3626
	CF4	*F. oxysporum* GPCK3723, *M.gypsea* GPCK 3629
	CF5	*C. tropicum* GPCK 3628
	CF6	*F. oxysporum* GPCK3574, *M. chrysosporoidea* GPCK 3764
	CF7	*Epidermophyton* sp. GPCK 3557
	CF8	*G.reessii* GPCK 3715, *T. mentagrophytes* GPCK 3763
	CF9	*A. recifei* GPCK 3510, *T. rubrum* GPCK3722, *A. mutates* GPCK 3716
	(CF10)	*A. keratinophilus* GPCK3721, A. *sydowii* GPCK 3737
	(CF11)	*C. indicum* GPCK 3631, *G. reessii* GPCK 3556
	(CF12)	*M. pulchella*GPCK3514, *A. simii*(GPCK 3555)
	(CF13)	*A. strictum* GPCK3717, H. *griesa* GPCK 3720
	(CF14)	*C. indicum* GPCK 3719, *P. javanicus* GPCK 3593
	(CF15)	*M. pulchella* GPCK 3632, *C. sulphureum* GPCK3596
	(CF16)	*M. canis* GPCK 3509, *C. indicum* GPCK 3714
	(CF17)	*A. strictum* GPCK 3743, *T. rubrum* GPCK 3713
	(CF18)	*F. proliferatum* GPCK3513, *F. oxysporum* GPCK 3633
	(CF19)	*C. tropicum* GPCK 3642, *Verticillium* sp. GPCK 3762
	(CF20)	*C. indicum* GPCK 3507, *M. aurantiaca* GPCK 3551
	(UW1)	*C. indicum* GPCK 3552, *V.* sp. GPCK 3634
Urban Waste	(UW2)	*C. tropicum* GPCK 3520, *G. pannorum* GPCK 3535
	(UW3)	*C. tropicum* GPCK 3508, *A. conjugatum* GPCK 3554
	(UW4)	*Ct. serratus* GPCK 3553, *A. flavipes* GPCK 3590
	(UW5)	*C. indicum* GPCK 3643, *P. javanicus* GPCK 3712
	(UW6)	*C. zonatum* GPCK 3641, *A. terreus* GPCK 3761
	(UW7)	*M. pulchella* GPCK 3576, *T. ajelloi* GPCK 3635
	(UW8)	*C. indicum* GPCK3640, *A.terreus* GPCK 3759
	(UW9)	*C. tropicum* GPCK 3511, *G. reessii*GPCK 3760
	(UW10)	*P. javanicus* GPCK 3639, *A.candidus* GPCK 3592
	(UW11)	*A. alternata* GPCK 3512, *C. indicum* GPCK 3638*, F. oxysporum* GPCK 3636
	(UW12)	*C. indicum* GPCK 3644, *Paecilomyces sp.* GPCK 3637
	(UW13)	*G. pannorum* GPCK 3756, *C. tropicum* GPCK 3757
	(UW14)	*A. sparse* GPCK 3645, *A. conjugatum* GPCK 3706, *P. fusisporus* GPCK 3526
	(UW15)	*C. pannicola* GPCK3742, M. *gypseum* (GPCK 3738)
	(UW16)	*A. fulvescens*GPCK 3700, *C. indicum* GPCK 3711
	(UW17)	*C. indicum* GPCK 3741, *T. terrestre* GPCK 3538
	(UW18)	*C. lunata* GPCK 3740, *Malbranchea* sp., *P. chrysogenum* GPCK 3701
	(UW19)	*G.pannorum* GPCK 3758, *M.equinum* GPCK 3703, *Ct. serratus* GPCK 3646
	(UW20)	*A. alternata* GPCK 3739, *F.oxysporum* GPCK 3702

Table 2. Distribution (percent) and frequency of nondermatophytic and other related fungi in cattle field and urban waste

S No.	Fungus	Distribution %		Frequency	
		UW	CF	UW	CF
	Num. of samples	20	20		
	Num. of samples positive	20	20		
	% Occurrence	100	100		
1	*Acremonium recifei*	0.00	10.00	0	5.12
2	*Acremonium strictum*	0.00	10.00	0	5.12
3	*Alternaria alternata*	10.00	0.00	4.54	0
4	*Amauroascus mutatus*	0.00	5.00	0	2.56
5	*Aphanoascus fulvescens*	5.00	0.00	2.27	0
6	*Aphanoascus keratinophilus*	0.00	10.00	0	5.12
7	*Aphanoascus terreus*	5.00	0.00	2.27	00
8	*Arthoroderma simii*	0.00	10.00	0	5.12
9	*Aspergillus candidus*	5.00	0.00	2.27	00
10	*Aspergillus flavipes*	5.00	0.00	2.27	00
11	*Aspergillus sparsus*	0.00	0.00	2.27	00
12	*Aspergillus sydowii*	0.00	5.00	00	2.56
13	*Aspergillus terreus*	5.00	0.00	2.27	00
14	*Auxarthron conjugatum*	10.00	0.00	4.54	00
15	*Chrysosporium indicum*	35.00	25.00	15.90	10.20
16	*Chrysosporium pannicola*	0.00	0.00	2.27	00
17	*Chrysosporium sulphureum*	0.00	5.00	00	2.56
18	*Chrysosporium tropicum*	20.00	10.00	9.09	2.56
19	*Chrysosporium zonatum*	5.00	0.00	2.27	00
20	*Ctenomyces serratus*	10.00	0.00	4.54	00
21	*Curvularia lunata*	5.00	0.00	2.27	00
22	*Epidermophyton* sp	0.00	5.00	00	2.56
23	*Fusarium proliferatum*	0.00	5.00	00	2,56
24	*Fusarium oxysporum*	10.00	10.00	4.54	5.12
25	*Geomyces pannorum*	15.00	5.00	6.82	2.56
26	*Gymnoascus reessii*	5.00	10.00	2.27	5.12
27	*Humicola griesa*	0.00	5.00	00	2.56
28	*Malbranchea aurantiaca*	0.00	5.00	00	2.56
29	*Malbranchea chrysosporoidea*	0.00	5.00	00	2.56
30	*Malbranchea gypsea*	0.00	5.00	00	2.56
31	*Malbranchea pulchella*	5.00	15.00	2.27	7.69
32	*Malbranchea* sp.	5.00	0.00	2.27	00
33	*Microsporum canis*	0.00	5.00	00	2.56
34	*Microsporum equinum*	5.00	0.00	2.27	00
35	*Microsporum gypseum*	5.00	0.00	2.27	00
36	*Paecilomyces javanicus*	10.00	5.00	4.54	2.56
37	*Paecilomyces fusisporus*	5.00	0.00	2.27	00
38	*Paecilomyces* sp.	5.00	0.00	2.27	00
39	*Penicillium chrysogenum*	5.00	0.00	2.27	00
40	*Penicillium griseofulvum*	0.00	5.00	00	2.56
41	*Trichophyton ajelloi*	5.00	0.00	2.27	00
42	*Trichophyton mentagrophytes*	0.00	5.00	00	2.56
43	*Trichophyton rubrum*	0.00	10.00	00	5.12
44	*Trichophyton terrestre*	5.00	0.00	2.27	00
45	*Verticillium* sp.	5.00	5.00	2.27	2.57

4. CONCLUSION

The keratinolytic fungi can be the bioindicators of environmental pollution with waste. Fungal indices also show the contamination hazard related with pollution of the environment with potential fungal pathogens. Given these findings, it can be concluded that urban waste and cattle fields are rich in keratinophilic fungi as well as dermatophytes. Therefore, cleanliness actions should be taken to control the spread of these fungi in the environment and check fungal infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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