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SHORT COMMUNICATION

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Keratinophilic fungi in soil of potted plants of indoor environments in Kanpur, India, and their proteolytic ability

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Abstract One hundred nine isolates of 10 genera representing 20 species of keratinophilic fungi were isolated from soil of planted earthen pots in indoor environments of 15 localities of Kanpur, India, by the hair baiting technique. *Microsporum gypseum*, *Trichophyton vanbreuseghemii*, and *Botryotrichum piluliferum* were found to release 698.66, 512.99, and 519.99 μg/ml net protein, respectively, whereas *Arthroderma cuniculi* released the minimum, 107.99 μg/ml. All other isolates were keratinolytically active.

Key words India · Keratinophilic fungi · Potted plants

Kanpur claims importance as the largest and oldest industrial city within the largest state of north India, with a population of more than 5 000 000. Geographically, Kanpur is situated on the right bank of River Ganges at latitude 26°26′ N and longitude 80°22′ E. It is in the semiarid region of north India, and there are no surrounding hills. The soil in Kanpur is impregnated with salts. The climate is dry and hot (50°C maximum) in summer, changing to warm and humid (95% maximum) at night, and dust storms are common. Industrial development of the city as well as urban growth are totally unplanned and haphazard, and domestic animals such as cows, buffalos, dogs, cats, and pigs have no separate places and are intermingled in most of the thickly populated localities, which has confounded the problem of pollution and health hazards. Human exposure to fungi in occupational settings, homes, and the outdoor environment where fungi may be naturally occurring has not been directly investigated to date. During recent years, attention has been drawn to the possible health risk of handling microorganisms. Identifying places where people are exposed to these organisms is important for assessing the risk factors. It is now recognized that exposure of airways to microorganisms in occupational environments is associated with a wide range of adverse health effects with major health impact (Madsen et al. 2007). Li and Kendrick (1995) found the greatest concentration of indoor aeromycota in living rooms in 15 residents in Canada. The dust of indoor environments in Jordan and Saudi Arabia was reported to be rich in keratinophilic fungi (Ali-Shtayeh and Asad Al-Sheikeh 1988; Bokhary and Parvez 1995). Airborne keratinophilic fungi in Torino, Italy, play an important role in decomposing keratin, and 19 of 36 fungal species were keratinolytically active in air (Filipello et al. 1994). Kushwaha (1995) also isolated keratinolytic fungi from indoor dust of a museum in Spain. Summerbell et al. (1989) documented that potted plants in hospitals act as reservoirs of pathogenic fungi.

Planted pots are used inside houses (bathrooms, bedrooms, family kitchens, living rooms), hotels, and offices, which are continuously contaminated with spores and hyphal fragments. We did not find earlier records of keratinophilic fungi in potted plants in indoor environments of India. A survey for keratinolytic fungi in soil of planted earthen pots, used for the growing of plants in houses, was made. The soil used in the pots comes from different sources and harbors a wide variety of keratinolytic fungi. This study was confined to those fungi present in the soil of pots that colonize keratin. Keratinolytic fungi were isolated from soils collected from potted plants placed in 15 different houses (Table 1). Five soil samples were collected from every house and baited with a thin uniform layer of sterilized human, horse, and cow hairs, nails, and feathers previously cut into 5-mm pieces. These samples were incubated at 28° ± 2°C and observed for any growth of mycelium. The fungi were isolated as and when they appeared on these baits. The percent frequency of the isolated fungi and number of isolates were recorded (Table 1).

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Table 1. Occurrence (%) of keratinophilic fungi in potted plant soil in different houses in India

Fungus[a]	House														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acremonium sp.	20 (1)	0	0	0	40 (2)	40 (2)	20 (1)	0	20 (1)	20 (1)	60 (3)	20 (1)	20 (1)	20 (1)	60 (3)
Acremonium implicatum	0	0	0	20 (1)	0	0	0	0	0	0	0	0	0	0	0
Acremonium hennbertii	0	0	0	20 (1)	0	0	0	0	0	20 (1)	0	0	0	0	0
Aphanoascus terreus	0	0	0	0	0	0	0	0	0	20 (1)	0	0	0	0	0
Aphanoascus keratinophilus	0	0	0	0	0	0	0	0	0	0	0	20 (1)	20 (1)	20 (1)	0
Arthroderma cunniculi	0	0	0	0	40 (2)	0	0	0	0	0	0	0	0	0	0
Botryotrichum piluliferum	0	0	20 (1)	0	0	0	0	0	0	0	0	0	0	0	0
Chrysosprium indicum	0	0	40 (2)	40 (2)	20 (1)	0	40 (2)	40 (2)	60 (3)	80 (4)	0	80 (4)	0	0	60 (3)
Chrysosporium keratinophilum	40 (2)	40 (2)	0	0	0	0	20 (1)	0	20 (1)	0	20 (1)	0	20 (1)	20 (1)	0
Chrysosporium queenslandicum	0			0	0	40 (2)	0	0	20 (1)	0	20 (1)	0	40 (2)	40 (2)	0
Chrysosporium pannicola	0	0		0	20 (1)	20 (1)	0	60 (3)	0	0	20 (1)	0	0	0	0
Chrysosporium sulfureum	0	0	0	0	20 (1)	20 (1)	0	0	0	0	0	0	0	0	0
Chrysosporium merdarium	0	0	20 (1)	0	0	0	0	0	0	20 (1)	0	0	0	0	0
Chrysosporium zonatum	0	0	0	0	0	0	0	0	0	0	0	0	20 (1)	0	0
Chrysosporium tropicum	0	0	0	0	0	0	0	0	0	0	20 (1)	0	0	20 (1)	0
Ctenomyces serratus	0	0	0	20 (1)	0	0	0	0	0	0	0	0	0	0	0
Malbranhea pulchella	60 (3)	80 (4)	0	40 (2)	20 (1)	0	40 (2)	0	0	20 (1)	40 (2)	0	20 (1)	0	0
Microsporum gypseum	80 (4)	0	40 (2)	60 (3)	0	0	0	0	20 (1)	20 (1)	0	0	0	0	0
Trichophyton vanbreseghmii	0	0	0	0	0	0	0	0	0	0	0	0	0	20 (1)	20 (1)
Verticillium tenuipes	0	0	0	0	40 (2)	0	20 (1)	0	0	0	0	0	0	0	0
No. of isolates	10	6	6	10	10	6	7	5	7	10	9	6	7	7	7

[a] Data are percent frequency of the fungus; the number of isolates is shown in parentheses

Frequency % = total number of samples in which species occurred/total number of samples examined × 100

For study of the frequency of keratinophilic fungi on different baits, a separate experiment was set. All the 75 soil samples were pooled, and this soil was distributed into five Petri dishes: one each for human hair, horse hair, cow hair, human nails, or feathers. Ninety pieces of these baits were put in one Petri dish and the percent frequency of the fungi that occurred on the baits was recorded (Table 2). The isolated fungi were cultured on Sabouraud's dextrose agar (SDA) and potato dextrose agar (PDA). Fungi were identified using their micro-morphological characters on SDA and PDA. *Acremonium* spp. were identified following Guarro et al. (1997) and de Hoog and Guarro (1995); *Microsporum gypseum* and *Trichophyton vanbreuseghemii* were identified following de Hoog and Guarro (1995); *Aphanoascus* spp. and *Arthroderma* spp. were identified following Currah (1985); and *Malbranchea pulchella* by Sigler and Carmichael (1976). *Chrysosporium* spp. were identified and compared as given by van Oorschot (1980) and Kushwaha (2000) and *Botryotrichum piluliferum* as described by Kushwaha and Agrawal (1976). The identification of some of the isolated species was confirmed by J. Guarro (personal communication).

The subcultures of isolated fungi were deposited in the Germplasm Centre for Keratinophilic Fungi, Kanpur, India (GPCK), Indian Type Culture Collection, New Delhi, India (ITCC), and Faculty of Medicine, University Rovira i Virgili, Reus, Spain (FMR). Substrate decomposition and protein released to the medium from human hair was monitored by following Lowry et al. (1951) and Nigam and Kushwaha (1992). Protein determinations from filtrates were carried out from flasks at 1-, 2-, 3-, and 4-week intervals. The developing color was read at 660 nm on a Shimadzu UV-VIS spectrophotometer. Freshly prepared human albumin serum was used as the standard. The results of protein determination were expressed as net values, i.e., the measured value in the test sample minus the sum of values of keratin and fungus controls of 113 isolates. The maximum and average values were also recorded (Table 3). All the experiments were carried out at in triplicate at 28° ± 2°C with appropriate controls.

Ten genera representing 20 species were isolated from 75 samples using human, horse, and cow hairs, human nails, and feathers as baits (see Table 1). The fungi isolated here are identified on the basis of micromorphological characters as most of them can be distinctly differentiated. However, use of characteristics for species distinction in dermatophytes (Simpanya 2000) may be reconfirmed by using molecular data (Gräser et al. 2000). Phylogeny of 57 species of the genus *Chrysosporium* and related fungal taxa has also been described (Vidal et al. 2000). Phylogeny of other several Onygenalean fungi is reviewed by Guarro and Cano (2002) and Sugiyama et al. (2002).

Maximum percent occurrence in 15 houses was of *Chrysosporium indicum*, followed by *Malbranchea (Mal.) pulchella* and *Acremonium* sp. *Acremonium* species colonized all baits used (see Table 2). *Acremonium (Ac.) implicatum*, *Ac. hennebertii*, *Aphanoascus* (*Ap.) terreus*, *Botryotrichum piluliferum*, *Chrysosporium merdarium*, *Ctenomyces serratus*, *Microsporum gypseum*, and *Trichophyton vanbreuseghemii* could only colonize the hair baits used. These fungi showed maximum occurrence on fragments of human and cow hairs and bird feathers in 15 localities, which probably indicates their efficiency to parasitize humans, animals, and even birds. The range of keratinolytic activity as expressed by protein released by all the fungi is quite high, from a maximum of 698.66 to a minimum of 107.99, showing their keratinolytic potential. The average values of protein released by different isolates also ranged from 103.95 to

Table 2. Frequency (%) of keratinophilic fungi on different keratinous substrates in pot soil

	Baits used				
	Human hair	Horse hair	Cow hair	Human nail	Feather
Acremonium sp.	27.27	12.5	33.33	25	15.38
Acremonium implicatum	4.54	0	0	0	0
Acremonium hennebertii	9.09	0	0	0	0
Aphanoascus terreus	0	0	6.66	25	0
Aphanoascus keratinophilus	5.54	0	0	0	0
Arthroderma cuniculi	0	0	6.66	25	3.84
Botryotrichum piluliferum	4.54	0	0	0	0
Chrysosporium indicum	36.36	0	13.33	25	42.3
Chrysosporium keratinophilum	0	37.5	6.66	0	11.53
Chrysosporium queenslandicum	0	25	13.33	0	15.38
Chrysosporium pannicola	4.54	0	6.66	25	16.38
Chrysosporium sulfureum	0	0	13.38	0	0
Chrysosporium merdarium	4.54	0	0	0	0
Chrysosporium zonatum	0	12.5	0	0	0
Chrysosporium tropicum	0	0	0	0	7.69
Ctenomyces serratus	4.54	0	0	0	0
Malbranchea pulchella	27.27	50	26.66	0	11.53
Microsporum gypseum	40.9	0	0	0	7.69
Trichophyton vanbreuseghemii	9.09	0	0	0	0
Verticillium tenuipes	0	0	20	0	0

Data are percent frequency of the fungus on different baits
Frequency (%) = total number of samples in which species occurred/total number of samples examined (95) × 100

Table 3. Proteolytic ability of keratinophilic fungi isolated from pot soil

Isolation species	Isolation no.	Strain no. producing maximum value[a]	Protein released (μg/ml)	
			Maximum	Average
Acremonium sp.	17	GPCK 658	494.33	260.71
Acremonium implicatum	1	FMR 6212	187.33	187.33
Acremonium hennebertii	2	FMR 6213	195.33	190.49
Aphanoascus terreus	1	GPCK 659	416.99	416.99
Aphanoascus keratinophilus	3	GPCK 642	466.55	359.06
Arthroderma cuniculi	2	GPCK 660	107.99	103.95
Botryotrichum piluliferum	1	GPCK 643	519.99	519.99
Chrysosporium indicum	23	GPCK 639	300.33	178.05
Chrysosporium keratinophilum	9	ITCC 4731	397.33	235.06
Chrysosporium queenslandicum	8	GPCK 656	217.00	169.74
Chrysosporium pannicola	6	GPCK 671	354.32	172.32
Chrysosporium sulfureum	2	GPCK 676	336.32	280.65
Chrysosporium merdarium	2	GPCK 902	126.66	115.33
Chrysosporium zonatum	1	ITCC 4732	236.32	236.32
Chrysosporium tropicum	2	GPCK 647	355.66	280.99
Ctenomyces serratus	1	FMR P131	253.99	253.99
Malbranchea pulchella	16	GPCK 686	314.33	205.1
Microsporum gypseum	11	GPCK 693	698.66	334.08
Trichophyton vanbreuseghemii	2	GPCK 904	521.99	470.27
Verticillium tenuipes	3	FMR 6216	265.32	210.65

[a] GPCK, Germ Plasm Centre for Keratinophilic Fungi, Kanpur, India; ITCC, Indian Type Culture Collection, New Delhi, India; FMR, Faculty of Medicine, University Rovira i Virgili, Reus, Spain

519.99 μg/ml, as represented by *Ar. cuniculi* and *B. piluliferum*. The latter fungus released the maximum amount of net protein into the medium.

The presence of highly keratinolytic fungi with a pathogenic potential inside houses is of considerable importance for human health. The fungi isolated and reported here were not reported earlier from planted pots of domestic environments in India. Nigam and Kushwaha (1990) isolated 28 keratinophilic fungi from house dust in Kanpur, India. The distribution of *Chrysosporium* spp. and other keratinophilic fungi with diverse species was recently reviewed (Kushwaha 2000). *Chrysosporium (Ch.) keratinophilum*, *Ch. pannorum*, *Ch. tropicum*, *Microsporum gypseum*, *Ar. cuniculi*, and *Verticillium psalliotae* were reported from indoor environments of Jordan (Ali-Shtayeh and Asad Al-Sheikh 1988), *Ch. indicum* from Saudi Arabia (Bokhary and Parvez 1995), and *Ch. carmichaelii*, *Ch. indicum*, *Ch. keratinophilum*, *Ch. pannicola*, *Ch. pseudomerdarium*, *Ch. queenslandicum*, *Ch. sulfureum*, *Ch. tropicum*, *Ch. xerophilum*, and 7 *Malbranchea* species were isolated from air of Torino, Italy. The pathogenic potential of *Chrysosporium* species was reviewed by Kushwaha (2000). Kumari et al. (2005) reported 46 non-keratinophilic fungi from 40 soil samples, and Gugnani and Shrivastav (1972) also reported *Geotrichum*, *Fusarium*, and other pathogenic fungi from soil. Recently Sarma and Borthakur (2007) isolated non-dermatophytic molds from patients and explored the possibility of occupational exposure from soil.

Indoor soil-inhabiting keratinophilic fungi have received little attention, but their occurrence in outdoor samples is known (Filipello 1986; Li and Kendrick 1995). Their presence in hospitals and potable waters is also noted (Arvanitidou et al. 1999). Sharma et al. (2008) isolated 82 strains of *Microsporum* from central India. They found *M. persicolor* to be preponderant in soil, and *M. fulvum* and *M. gypseum* were also reported by means of molecular detection. However, there was no correlation between genotype, geographic location, or habitat. Deshmukh et al. (2008) screened 80 soil samples from Bahrain and isolated *Ap. fulvescens*, *Ap. punsolae*, *Ch. indicum*, *Ch. tropicum*, *Ch. zonatum*, *Spiromastix warcupii*, *M. gypseum*, and *Trichopyton mentagrophytes* with 8.75%, 20.00%, 2.50%, 2.50%, 3.75%, 1.25%, 3.75%, and 2.50% occurrence, respectively. Greif and Currah (2007) isolated 1687 fungal strains representing 65 species in arthropods, including keratinophilic Onygenales. Soomro et al. (2007) isolated 253 strains belonging to 11 species and 8 genera from outdoor soil of Pakistan, including *Chrysosporium asperatum*, and *Aspergillus niger* was most dominant. Hedayati et al. (2004) isolated 13 fungi from soil samples of potted plants. In their study, *Penicillium* sp., *Acremonium* sp., *Paecilomyces* sp., *Cladosporium* sp., and *Aspergillus* spp. were predominate, showing 50%, 20%, 11.9%, 3.7%, and 3.1% frequency of occurrence, respectively. They have also isolated *M. gypseum*, *M. cookie*, and *Chrysosporium* sp. by the hair baiting technique. Staib et al. (1980) isolated *A. niger* from the soil of potted African violets that were kept in a living room and reported potted plant soil was a reservoir of pathogenic fungi. Ulfig (2000) and Ulfig et al. (2006) demonstrated the effect of the open-air drying of sewage sludge and showed that *M. gypseum* and *Pseudallescheria boydii* required special attention from an epidemiological point of view. Deshmukh (2002, 2004) also isolated 15 and 10 species, respectively, from soil of Kerala, India, and feathers of pigeons in Maharastra, where *Ch. indicum* occurred at 20.25% in soil and 24% in feathers. Bowman et al. (2007) found fungi to cause fatal dermatitis in reptiles. Thirty-four

species of keratinophilic fungi were recovered from feather dumping soil of Chennai, India, including the *Ctenomyces* state of *Ar. tuberculatum* and *T. mentagrophytes*. The latter fungus showed strong keratinolytic activity, with 2.7 keratinase units (Ku)/ml (Anbu et al. 2006). The soils of cultivated indoor plants may be significant reservoirs of certain keratinophilic fungi in houses because indoor plant soils have been linked to cases of dermatomycosis and other diseases.

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