An Overview of Quick-Witted Vacuum Cleaner Tape Technique towards Cataloguing Keratinophilic Fungi from Floor Dust Samples of Student Hostels

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In this work, we report a quick-witted vacuum cleaner tape technique for sampling of keratinophilic fungi from floor dust samples in hostel rooms. As substantiated by partial identification through routine mycological methods and monographs, the isolates recovered belonged to four major keratinophilic fungi viz. HTP4 (*Epidermophyton* spp), HTP2 (*Microsporum* spp), HTP7 (*Chrysosporium* spp) and HTP6 (*Trichophyton* spp). It was fascinating to note that the above isolates were predominantly present in floor dust samples of student hostels. An overview of enzymatic potential of these isolates revealed that isolates HTP2 (*Microsporum* spp), HTP3 (*Chrysosporium* spp) and HTP7 (*Chrysosporium* spp) displayed both amylase and protease activity whereas isolate HTP4 (*Epidermophyton* spp) displayed protease activity. Such studies serve as a decisive inventory towards developing guidelines for maintaining health and hygiene among inhabitants in student hostels and further to make them receptive towards infections caused by these fungi. This technique provides a superior tool for air sampling and documenting the microbial load of floor dust of these locations.

Key words: Keratinophilic fungi, Student hostels, Dermatophytes, Vacuum cleaner tape technique.

Keratinophilic fungi are a group of fungi that degrade keratinous substrates into lowmolecular weight compounds by the process of sulphitolysis.^{1,2} These fungi release substances which breakdown disulphide bonds of cysteine present in keratin. The fungi then release proteolytic enzymes that cleave the partially denatured protein. The enzymes responsible for keratin degradation are referred to as keratinases and disulphide reductases. These fungi also produce many extracellular enzymes viz. amylase, lipase, protease and keratinases which have varied industrial applications. There are many studies reported for ecological screening of various molds and keratinophilic fungi from different niches. The enzymatic potential of these fungi are also elucidated in some of these papers.^{2,3,4,5,6,7,8,9} Besides this, recent reports on isolation of other metabolically important molds also indicate the role of significant quicker techniques towards isolation of various groups of fungi.^{10,11} The objective of this study was to use a rapid technique to isolate keratinophilic fungi from floor dust samples of student hostels and further cataloguing their enzymatic potential.

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MATERIALS AND METHODS

Sample collection, isolation and its preliminary characterization

An indigenously developed vacuum cleaner tape technique was introduced for collection of 10 dust samples from 10 rooms of student hostels (Table 1) in Birla Institute of Technology, Mesra, Ranchi, India during the year 2010-2011. In this technique, a small vacuum cleaner was used to accumulate floor dust samples in filter papers. These samples were sealed by locally available Sellotape (pressure-sensitive adhesive tape) and transferred aseptically onto Petri dishes containing potato dextrose agar (PDA) and Sabouraud's Dextrose Agar (SDA). The petri dishes were incubated for 48 hrs at 28°C and were observed for growth of fungi. The isolates were sub-cultured and allowed to grow on keratin baits (hair, nails and feathers) using Hair Baiting Technique as recommended by Vanbreuseghem.² The isolated colonies of fungi were cultured on Sabouraud's dextrose agar supplemented with cycloheximide and chloramphenicol and then stained by lactophenol cotton blue (LPCB) wet mount preparation towards its further identification on the basis of their microscopic and macroscopic morphology. 12,13,14

Preliminary screening of enzymes

Preliminary screening for amylase production from the fungal isolates (HTP1 to HTP10) was carried out by starch agar plate assay on standard media.¹⁵ The various constituents of media used were 1.5 g yeast extract, 2.0 g soluble starch, 0.5 g peptone, 1.5 g NaCl and 15.0 g agar dissolved in 1.0 liter MiliQ water. The prepared media was autoclaved. The plates were then incubated for 72 hours and the inoculated plates containing media supplemented with starch were confirmed by flooding the plates with Gram's iodine. For protease screening, the fungal isolates were streaked on casein agar medium (0.5% of casein, 0.5% of glucose, and 2% of agar (w v-1), pH 7.0) and incubated at 28°C for 78 hrs. Enzyme activity was indicated by the formation of a clear zone around colonies after precipitation with 1 M HCl solution.¹⁶

RESULTS AND DISCUSSION

As apparent from Table 1, 10 isolates (HTP1 to HTP10) were recovered from floor dust samples of student hostels. Furthermore, enzymatic activity was also seen by few of them. The enzymatic potential of these isolates divulged that isolates HTP2 (*Microsporum* spp), HTP3 (*Chrysosporium* spp) and HTP7 (*Chrysosporium* spp) displayed both amylase and protease activity whereas isolate HTP4 (*Epidermophyton* spp.) displayed protease activity. The enzymatic potential of these groups of fungi can be utilized for various industrial applications. Moreover, it is also manifested that these species belong to dermatophyte group of fungi, which may cause

S. No.	Isolate	Site of isolation (Room/Hostel no.)	Partial characterization	Amylase activity	Protease activity
1	HTP 1	F91/H9	UN	No (-)	No (-)
2	HTP 2	S45/H9	Microsporum spp	Yes (+++)	Yes (+++)
3	HTP 3	G22/H8	Chrysosporium spp	Yes (+)	Yes (++)
4	HTP 4	F121/H9	Epidermophyton spp	No (-)	Yes (++)
5	HTP 5	F115/H9	UN	No(-)	No (-)
6	HTP 6	G30/H8	Trichophyton spp	No(-)	No (-)
7	HTP 7	F110/H9	Chrysosporium spp	Yes (+++)	Yes (+++)
8	HTP 8	S22/H9	NG	No (-)	No (-)
9	HTP 9	G33/H9	UN	No (-)	No (-)
10	HTP 10	F52/H9	NG	No (-)	No (-)

Table 1. Details of isolates and their enzymatic activity

Abbreviations: UN: Unidentified; NG: No growth observed

(+++): Excellent; (++): Good; (+): Fair, (-): No activity

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S. No.	Regimen code	Advisory details
1	RAI (Legs/Feet)	Use rubber slippers while using common bath areas and washrooms.
2	RAI (Legs/Feet)	Dry feet and other parts of the body properly.
3	RAI (Rooms)	Allow ample sunlight to enter the rooms.
4	RAI (Personal hygiene)	Avoid sharing of towels, hairbrushes and combs.
5	RAI (Rooms)	Clean rooms properly.
6	RAI (Room walls/living area)	Prevent formation of wet, damp zones where microbial activity thrives.
7	RAI (Rooms/living area)	Initiate disinfection plans twice a year.
8	RAI (Administrative)	Appoint hostel staff in-charge for cleanliness and hygiene.
9	RAI (Personal hygiene)	Take proper care and attention when symptoms of infections are seen.
10	RAI (Personal hygiene)	Wash bed linen often to get rid of fungal spores.

Table 2. Regimen advisory for inhabitants (RAI)

skin infections (mycoses) and can be noticeably detrimental for inhabitants in these areas. Hence, an obligatory regime of awareness has to be created. For this purpose, a ten point Regimen advisory for inhabitants (RAI) was developed which can be a competent tool for the well being of inhabitants (Table 2).

CONCLUSION

It is known that keratinophilic fungi and some related dermatophytes are a threat to human health and a few studies have highlighted the presence of keratinophilic fungi in hospitals and other public places.^{2,17,18,19} The occurrence of these fungi in student hostels has previously been unstudied. Our study shed light on the common occurrence of these fungi in student hostels. Moreover, the self-optimized vacuum cleaner tape technique was an effective and low-cost air sampling method for keratinophilic fungi. Four different species of keratinophilic fungi were found in student hostels, which accentuate the considerable incidence of these fungi and the potential of fungal infections in students (Table 1). This study was also instrumental in increasing awareness among students on the potential incidence of fungal infections and developing effective guidelines to avoid the occurrence and spread of such infections in hostels (Table 2). The accomplishment of this technique also paves a new area of sampling in these locations and documenting the diversity of air micro-flora for healthy living of inhabitants.

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