

Full Length Research Paper

A descriptive study of keratinophilic fungal flora of animal and bird habitat, Jaipur, Rajasthan

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Accepted 30 May, 2012

Keratinophilic fungi occur abundantly in the superficial soil layer of landfills and their surrounding. Forty seven soil samples of animal (37 samples) and bird (10 samples) habitats from different localities of Jaipur District were collected for the estimation of keratinophilic fungi using the hair baiting technique. Seventy five isolates belonging to 14 genera and 20 species were reported. Soil pH range varies from 6.5 to 10.5. But most of the fungi (33.33%) were isolated from neutral soil (pH 7.0). *Chrysosporium tropicum* (25.33%) was the predominant fungi isolated from both habitats soil. This was followed by the predominance of *Trichophyton terrestre* (12%), *Trichophyton mentagrophytes* (9.3%), *C. indicum* (5.33%), *Actinomyces* sp. (6.67%), and *Nocardia* sp. (6.67%) in both habitats. Interestingly, *Exserophilum* sp., *Microsporium audouinii*, *Trichophyton verrucosum* were isolated for the first time from Jaipur India.

Key words: Dermatophytes, *Trichophyton*, *Microsporium*, *Chrysosporium*, soil fungi.

INTRODUCTION

Keratinophilic fungi include a variety of filamentous fungi mainly comprising of hyphomycetes and several other taxonomic groups. Hypomycetes include dermatophytes and a great variety of non dermatophytic filamentous fungi. 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 (Fillipello, 2000; Sharma and Rajak, 2003). Newly emerging non-dermatophytic keratin colonizing fungi which actively invade and degrade keratin are now the subject of investigation because of their medical importance and their ability to decompose slowly and act as a reservoir for pathogenic fungi. Keratin decomposition in soil leads to an increase in carbon to nitrogen ratio of soil. They are therefore fast growing non-pathogenic keratinophilic fungi which, it is proposed, should be utilised for the recycling of keratin in soil and

may be exploited for their biotechnological potential in industry (Kaul and Sumbali, 1999). Keratinophilic fungi are generally considered as soil saprophytes (Ajello, 1953). In India, open school play grounds, public parks and public places are often invaded by animals such as cows, bullocks, birds, dogs, pigs, cats and rats. These transient animals leave organic residues, which may contaminate the soil with keratinaceous debris and possibly propagules of keratinophilic fungi including fungal pathogens. Many of them are closely related to the dermatophytes having properties in common with them and cause human and animal infection (Gupta et al., 1993; Patwardhan and Dave, 1999; Sarma and Borthakur, 2007; Spiewak and Szostok, 2000). These fungi occurred abundantly in the superficial soil layer of landfills, their surrounding and distributed worldwide (Anbu et al., 2004; Deshmukh, 2004; Sarma and Borthakur, 2007; Simpanya and Baxter, 1996; Singh et al., 1990; Zaki et al., 2005). The geographical distribution of keratinophilic fungi in soil samples depend a large number of factors like site of soil collection, climatic and edaphic factors of that area, organic material, pH and

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moisture of soil (Bhadauria and Sharma, 2001; Gupta and Garg, 1991; Kaul and Sumbali, 1998).

The present communication deals with the occurrence of keratinophilic fungal flora particularly in animal and bird habitats of Jaipur District. For animal habitats we strictly selected those site where animal like, dog, cat, cow, buffalo, pig and horse are found abundantly like, kennels, dairy and byre, pen, stable and road side from different areas of Jaipur. Similarly for birds habitats soil samples were collected from sites where only birds are abundantly found like bird section of zoo, garden, poultry farm and temples of different areas.

MATERIALS AND METHODS

Collection of soil samples

A total 47 soil samples were collected randomly from animal habitats (kennel, byre pen, stable and road sides) and bird habitat (poultry farm, temples, garden and bird section of zoo) of Jaipur from August 2008 to November 2008. Most of the samples were collected in the morning (8:00-12:00 am). Soils were sandy (92%) in nature containing 3-4% clay and slit. Surface soil was collected with the help of a sterilised spoon. Each plastic bag was labelled indicating the date and site of collection. 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 (Vanbreuseghem, 1952) was followed for isolation of the fungi. Twenty five gram of soil sample was taken in a separate sterilised petriplate from each sample. Sterilised distilled water was added to provide moisture to the soil. Bits of sterilised human hair and animal hair (cow, buffalo, dog) were used as baits. The hair were scattered uniformly only on wet soil. Each petriplates 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 human 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 medium fortified with chloramphenicol (0.05 mg/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 by Conant et al. (1959) and Forbes et al. (2002) study.

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

RESULTS

A total of 75 isolates belonging to 13 genera and 20

species were reported in present investigation. Out of 37 soil samples of animal habitats, 31 were found positive for fungal growth. While in bird habitats all sample were found to be positive for keratinophilic fungi (Table 1). *Chrysosporium tropicum* (25.33%) was found to be the most common keratinophilic fungi in both habitats. *Trichophyton terrestre* (16.28%) was the second most common fungi in animal habitat followed by *Nocardia* sp. (11.62%), *Aspergillus* sp. (9.30%), *Actinomyces* sp. (9.30%), *T. mentagrophytes* (6.98%) and *T. simii* (6.98%). While in bird habitat *T. mentagrophytes* (12.50%) and *Cephalophora irregularis* (12.90) were the second most common fungi. *Cephalophora irregularis* was completely absent in all animal habitat samples.

Histoplasma capsulatum (4.65%), *Ctenomyces serratus* (2.32%), *Exserophilum* sp. (2.32%) and *Botrytis* sp. (2.32%) were isolated only from animal habitat soil. Likewise *T. verrucosum* (3.12%), *M. gypseum* (3.22%), *M. audouinii* (3.12%), *Cladosporium* sp. (3.12%) and *Gymnoascus* sp. (3.12%) were isolated only from bird habitat site. Among all these fungal species *Exserophilum* sp. (pH 8.5), *T. verrucosum* (pH 7.0), *M. audouinii* (pH 7.0), *Cladosporium* sp. (10.5 pH) and *Botrytis* sp. were isolated for the first time in Jaipur.

During the study of soil pH (Table 2), pH range varies from 6 to 10.5. It was observed that soils of animal habitat site (pH 7 – 10.5) are more alkaline as compared to bird habitat (pH range 6.5 – 8.0). These keratinophilic fungi preferred 7.0-8.5 pH range while mostly isolated from neutral pH. Dog (*Canis familiaris*), Cat (*Felis catus*), Pig (*Artiodactyla suidae*), Cow (*Bos primigenius*), Buffalo (*Bubalus bubalis*) and Horse (*Equus ferus caballus*) were commonly seen in animal habitats. In bird habitats Pigeon (*Crocopus phaenicopterus*) and House sparrow (*Passer domesticus*) were abundantly found. In some site Crow (*Splendens vieilloi*), Peacock (*Pavo cristatus*), Parrot (*Pyrrhura frontalis*) and common mynah (*Acridotheres tristis*) were also reported.

DISCUSSION

The keratinophilic fungi include true fungi that vigorously degrade keratin as well as some more important pathogenic and non pathogenic dermatophytes (Ajello, 1953; Gupta and Garg, 1991; Singh et al., 1990). The composition of keratinolytic fungal communities in the soil differed from that observed for a highly populated and walked area and displayed district seasonal variations. Several investigation have been done in various part of Rajasthan, India during last 2 decade and showed that a rich variety of keratinophilic fungal flora exists in the soils of studied area (Iyer and Williamson, 1991; Singh et al., 1990). However, there was no evidence of any study of comparative micro flora of animal and bird habitats.

Table 1. Keratinophilic fungi isolated from Animal and Bird habitats of Jaipur District.

Parameter	Animal habitat		Bird habitat		Total species	
Total no. of soil samples	37		10			
Culture +ve soil samples	31		10			
Species	No.	%	No.	%	No.	%
<i>Trichophyton simii</i>	3	6.98	-	-	3	4.00
<i>T. terrestre</i>	7	16.28	2	6.25	9	12.00
<i>T. mentagrophytes</i>	3	6.98	4	12.5	7	9.30
<i>T. verrucosum</i>	-	-	1	3.12	1	1.33
<i>Chrysosporium tropicum</i>	10	23.25	9	28.12	19	25.33
<i>C. indicum</i>	1	2.32	3	9.37	4	5.33
<i>Ctenomyces serratus</i>	1	2.32	-	-	1	1.33
<i>Microsporium gypseum</i>	-	-	1	3.12	1	1.33
<i>M. audouinii</i>	-	-	1	3.12	1	1.33
<i>Cephalophora irregularis</i>	-	-	4	12.50	4	5.33
<i>Histoplasma capsulatum</i>	2	4.65	-	-	2	2.67
<i>Cladosporium</i> sp.	-	-	1	3.12	1	1.33
<i>Aspergillus</i> sp.	4	9.30	-	-	4	5.33
<i>Fusarium</i> sp.	1	2.32	1	3.12	2	2.67
<i>Exserophilum</i> sp.	1	2.32	-	-	1	1.33
<i>Botrytis</i> sp.	1	2.32	-	-	1	1.33
<i>Gymnoascus</i> sp.	-	-	1	3.12	1	1.33
<i>Actinomyces</i> sp.	4	9.30	1	3.12	5	6.67
<i>Nocardia</i> sp.	5	11.62	-	-	5	6.67
<i>Trichophyton</i> sp.	-	-	3	9.37	3	4.00
Total	43		32		75	

Jaipur has a dry climate in general. Its temperature exceeds even 46°C in summer and has high humidity (97%) during monsoon season. All these condition favour the incidence of keratinophilic fungi in Jaipur.

During present investigation *Chrysosporium tropicum* was found to be the most predominant keratinophilic fungi in both habitat soil. This fungus was isolated singly or in the form of mix growth of 2-3 fungi. (i) *Chrysosporium tropicum* + *T. terrestris*, (ii) *C. tropicum* + *Cephalophora irregularis* and (iii) *C. tropicum* + *C. irregularis* + *T. terrestris* were the most commonly isolated fungal mixture. *C. tropicum* is a fast growing fungus easily grow in all habitats sites. During the studies of 17 animal herds in Kuwait Al-Musallam (1990) reported *C. tropicum* as a most predominant species. Ghosh and Biswas (1995) also reported *C. tropicum* as the most common fungi followed by *C. indicum* during the survey of Bharatpur bird sanctuary soil in Rajasthan. Similar result was also obtained by various researchers (Deshmukh, 2004; Moharram and Abdel-Gawad, 1989; Sharma and Williamson, 1984).

During the present investigation it was also observed that soil pH plays an important role in the occurrence of a particular fungi or organism. Soil pH range of animal

habitat was found to be 7 -10.5. While in bird habitat it was slightly acidic to slightly alkaline (6.5 – 8.0 pH range). In bird habitat samples most of the fungi were isolated from neutral site while in animal habitat soils most of the fungi were reported at 8.5 to 9.5 pH range soil. One isolate of *T. terrestris* and one of *Aspergillus* sp. were isolated from higher pH range 10.5. Our present finding are in agreement with several other workers (Bhadoria and Sharma, 2001; Kaul and Sambali, 1998; Sharma and Sharma, 2010; Sharma and Williamson, 1984). *Nocardia* sp. and *Actinomyces* sp. were frequently observed in animal habitat soil samples due to high pH range.

Microsporium gypseum and *Microsporium audouinii* were isolated only from bird habitat site. Moharrum et al. (1989) reported very rare incidence of *M. gypseum* in rabbit claws in Egypt. Similarly Marsella and Mercanili (1986) isolated *Microsporium* sp. in four samples out of a total of 161 soil samples from different sites of Abruzzo national park, Italy.

T. simii and *Histoplasma capsulatum* were reported only from animal habitats Denton and Disalvo (1979) found *H. capsulatum* to be widely distributed and associated with bird droppings, chicken manure and bat guano. *T.*

Table 2. Hydrogen ion concentration (pH) and fungi isolated from soil samples.

S/N	Species	pH of soil samples								
		6.5	7.0	7.5	8	8.5	9	9.5	10	10.5
1.	<i>Trichophyton simii</i>	-	-	-	1	2	-	-	-	-
2.	<i>T. terrestre</i>	-	1	1	1	2	1	2	-	1
3.	<i>T. mentagrophytes</i>	-	3	-	3	-	-	1	-	-
4.	<i>T. verrucosum</i>	-	1	-	-	-	-	-	-	-
5.	<i>Chrysosporium tropicum</i>	1	7	1	2	3	3	2	-	-
6.	<i>C. indicum</i>	-	2	1	-	-	-	1	-	-
7.	<i>Ctenomyces serratus</i>	-	-	-	-	-	-	-	1	-
8.	<i>Microsporium gypseum</i>	-	1	-	-	-	-	-	-	-
9.	<i>M. audouinii</i>	-	1	-	-	-	-	-	-	-
10.	<i>Cephalophora irregularis</i>	1	3	-	-	-	-	-	-	-
11.	<i>Histoplasma capsulatum</i>	-	-	1	-	-	1	-	-	-
12.	<i>Cladosporium</i> sp.	-	1	-	-	-	-	-	-	-
13.	<i>Asperillus</i> sp.	-	-	-	-	-	1	1	1	1
14.	<i>Fusarium</i> sp.	-	1	-	-	-	-	1	-	-
15.	<i>Exserophilum</i> sp.	-	-	-	-	1	-	-	1	2
16.	<i>Botrytis</i> sp.	-	-	-	-	-	-	-	-	1
17.	<i>Gymnoascus</i> sp.	-	1	-	-	-	-	-	-	-
18.	<i>Nocardia</i> sp.	-	-	-	1	-	1	-	1	2
19.	<i>Actinomyces</i> sp.	-	1	-	-	-	1	-	3	-
20.	<i>Trichophyton</i> sp.	-	2	-	-	-	-	1	-	-

verrucosum was isolated first time in Jaipur from bird habitat soil sample. *Exserophilum* was a new reported as a keratinophilic fungi in Jaipur isolated from animal habitat site.

Aspergillus sp. and *Fusarium* sp. were frequently observed in present survey. Similar report was given by various workers (Abdel-Hafez and El-Sharouny, 1989; Ghosh and Biswass, 1995; Moharram and Abdel-Gawad, 1989). During this study higher incidence of keratinophilic fungi was observed in bird habitats as compare to animal habitats. Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophiles and dermatophytes. Environmental condition like heat, humidity and soil pH along with personal hygiene are involved in flourishing of fungal infections.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Botany, University of Rajasthan for providing laboratory facilities during research work.

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