Isolation of Keratinophilic Fungi from Soil Samples of Forests and Farm Yards

H Moallaei1, *F Zaini1, M Pihet2, M Mahmoudi3, J Hashemi1

1Dept. of Medical Parasitology and Mycology, School of Public Health & Institute of Public Health Research (SPH-IPHR), Tehran University of Medical Sciences, Iran
2Groupe d'Etude des Interactions Hôte-Parasite, Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire, 4 rue larrey, 49933 Angers cedex 9, France.
3Dept. of Epidemiology and Biostatistic, (SPH-IPHR), Tehran University of Medical Sciences, Iran

(Received 25 Apr 2006; accepted 25 July 2006)

Abstract
Soil is well known to support the transient or ongoing existence of keratinophilic fungi and potential sources of infection for humans and animals. Fifty soil samples were collected from various areas of forests and farmyards at Golestan Province in the north part of Iran to determine the prevalence of keratinophilic fungi and dominant species. A total of 357 fungal colonies including 13 genera with 11 species were isolated as follows: Anixiopsis stercoraria (16.24%), Arthroderma cuniculi (12.04%), Reniospora flavissima (9.24%), Fusarium oxysporum (9.24%), Aspergillus flavus (8.68%), Chrysosporium keratinophilum (8.40%), Trichophyton vanbreuseghemii (7.84%), and other fungi (37.56%). McNemar’s test showed that non-keratinolytic fungi were dominant in this investigation (P< 0.05). Anixiopsis stercoraria (16.24%) was the most prevalent and dominant keratinophilic fungus (P< 0.05). It can be concluded that soils from forest and farmyards of Golestan Province are rich in keratinophilic fungi including dermatophytes.

Keywords: Fungal flora, Forest, Farm yard, Keratinophilic, Fungi, Iran.

Introduction
The soils represent the main reservoir of fungi. Some soil fungi are potential pathogen to both human and animals. Soils that are rich in keratinous materials are the most conductive for the growth and occurrence of keratinophilic fungi. The potentially pathogenic keratinophilic fungi and allied geophilic-dermatophytic species are widespread worldwide. The forest, farmyard, park soils, as well as sediments of the rivers and oceans contained humus and organic material are the best candidate for growth of keratinolytic and saprophytic fungi (1).

Previous studies on the epidemiology of human dermatophytosis in Iran showed that it was prevalent in north and northeast of Iran especially in rural areas (2, 3). However, the soils of forest and farmyard in Golestan Province have never been investigated for keratinophilic fungi. Therefore, hygienic and ecological interests have led us to study the keratinophilic mycoflora of farm yards and forests, where farmers, tourists, and animals spend a large proportion of their time and may be exposed to pathogenic fungi. This would help us to know the distribution and occurrence of dermatophytes and other keratinophilic fungi and risk of human dermatophytosis in those regions, which could have a role in degradation of keratinous material as an industrial point of view.

Materials and Methods
Fifty soil samples were collected randomly from forests and farmyards at various sites at Golestan
Province in north part of Iran, which have not been exposed to sunshine and had enough humidity. These localities include Bandar-e gaz and Bandar-e torkman on southeastern coast of Caspian Sea, extending from Gorgan to Gonbad-e kavous on eastern region and Galikesh eastern land frontier with Northen-Khorasan Province (Fig. 1, 2). The samples were collected from the layer with depth not exceeding 3-5cm. The numbers of fungi in soils samples were assumed to had a Poisson distribution. The dominant species in studied area was determined by McNemara’s test with the level of significance at 5%.

**Results**

Totally 357 colonies from soil samples collected from five forests in Golestan Province. Eleven species in 13 genera and one non-sporolating fungus were recovered. Of those only seven species (55.46%) belonged to keratinolytic fungi. The results of the isolation in all sites are presented in Table 1.

Dermatophytes and closely related fungi were represented by seven species and comprised 32 (8.96%) of all found fungal isolates. Regarding the dermatophytes *Trichophyton vanbreuseghemii* was most common species (Fig. 3) followed by pathogenic *Microsporum gypseum* (Table I.). *M. gypseum* was only observed in forest at Gorgan but *T. vanbreuseghemii* was recovered from Galikesh and Gorgan. Among the closely related non-dermatophyte keratinophilic species *Anixiopsis stercoraria* was the dominant ($P<0.05$) and most frequently isolated species (21.84%), followed by *Arthroderma cuniculi* (9.24%), *Chrysosporium keratinophilum* (8.40%), *Myceliophthora vellera* (4.48%), *Reniospora flavissima* (3.64%). The commonest observed other species of fungi were (in decreasing rank); *Penicillium* sp. $>$ *Fusarium oxysporum* $>$ *Aspergillus flavus* $>$ non-sporolating fungi $>$ *Paecilomyces lilacinus* and *Geotricum candidum* $>$ *Acromonium* sp.

The data in Table II revealed that the highest number of colonies per soil unit belonged to *Anixiopsis stercoraria* in Galikesh followed by *Arthroderma cuniculi* in Bandar-e gaz. Overall, in different sites, Galikesh had the highest frequency of keratinophilic fungi followed by Gorgan. On the others, Gonbad-e kavus was appeared to be the lowest in the total count of keratinophilic fungi (Table 2).
**Fig. 1:** The location of the Golestan Province in Iran

**Fig. 2:** Sites of collection of soil samples in five forests and farmyards at Golestan Province, Iran

**Fig. 3:** Left: *Trichophyton vanbreuseghemii* on malt agar at 28°C after 10 days. Right: Microscopic feature of *Trichophyton vanbreuseghemii* (x400).
Table 1: Distribution of fungi isolated from soil samples of forests and farm yards in different sites at Golestan Province

<table>
<thead>
<tr>
<th>Species</th>
<th>Bandar-e Gaz</th>
<th>Bandar-e Torkman</th>
<th>Galikesh</th>
<th>Gonbad-e Kavus</th>
<th>Gorgan</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Acromonium sp.</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>30</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Anixiopsis stercoraria *</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>30</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Arthroderma cuniculi *</td>
<td>30</td>
<td>49</td>
<td>13</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chrysosporium keratinophilum *</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>12</td>
<td>20</td>
<td>13</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Geotricum candidum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Microsporum gypseum *</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myceliophthora vellera *</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-sporulating fungi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paecilomyces lilacinus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillium sp</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reniospora flavissima *</td>
<td>13</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichophyton vanbreuseghemii*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

| Total                                    | 61  | 100 | 66  | 100 | 70  | 100 | 78  | 100 | 82  | 100  | 357  | 100  |

*. Keratinolytic fungi

Table 2: Distribution of keratinophilic fungi in soil samples from forests and farm yards in different sites at Golestan Province

<table>
<thead>
<tr>
<th>sites</th>
<th>Keratinolytic fungi</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Bandar-e Gaz</td>
<td>43</td>
<td>20.28</td>
</tr>
<tr>
<td>Bandar-e Torkman</td>
<td>33</td>
<td>15.56</td>
</tr>
<tr>
<td>Galikesh</td>
<td>63</td>
<td>29.71</td>
</tr>
<tr>
<td>Gonbad-e Kavus</td>
<td>23</td>
<td>10.84</td>
</tr>
<tr>
<td>Gorgan</td>
<td>50</td>
<td>23.58</td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td>100</td>
</tr>
</tbody>
</table>
Discussion
Keratinophilic fungi are important ecologically and recently have attracted the attention throughout the world. They play a significant role in the natural degradation of keratinized residues (7-9), have many properties in common with dermatophytes and some can probably cause human and animal infections (10-16). Keratinophilic fungi are presented in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. Reports on the presence of these fungi in different soil habitats from different countries, e. g., Egypt, Australia, Palestine, Spain, India, Kuwait, Ukraine and Malaysia, have indicated that this group of fungi are distributed worldwide (1, 17).

Several investigations have been done in various part of Iran during last two decades and showed that a rich variety of keratinophilic fungal flora exists in the soils of studied area (18-25). However, there was no evidence of any study on mycoflora of Golestan Province with particular interest on forest soil. Therefore, the present investigation carried out for detection of keratinophilic fungi in soil of five different forests and farm yards. Use of these forests and farmyards by people and presence of animals and birds may introduce keratinous dander. These keratinous wastes may serve as substrata for keratinophilic fungi in soils of forest.

Two major techniques have been used for the qualitative and quantitative isolation of these fungi from soil: surface soil dilution plating (SSDP) and hair baiting technique (HBT). HBT which is the most common and reliable method used in present investigation and yielded two groups of keratinophilic fungi (11 species and one non-sporulating fungus) from soil of forests and farm yards in Golestan. The first set comprised dermatophytes including Microsporum gypseum and Trichophyton vanbreuseghemii. The later fungus rarely causes ectothrix hair infection and distributed commonly in Australia (26). It is interesting to note that this species was isolated for the first time from Galikesh and Gorgan and had never been found in other studied soil samples in Iran (18-25).

*M. gypseum* is a common geophilic dermatophyte widely distributed in soil globally (1). It causes ringworms of scalp and glabrous skin in human and animal (1).

*M. gypseum* previously was found in soil samples from Kerman (20), Isfahan (21), Gazvin (22) but Tehran (23), Ahvaz (24) and Ghochan (25). In the present study, it was isolated only from forest at Gurgan but comparatively with less percentage of occurrences than *T. vanbreuseghemii*. Dominik et al. (27) suggested that keratinophiles which are known as pathogens may be of medical interest. Some of the species isolated in this study are reported to be either well known agents of mycosis (*M. gypseum*) or have been recovered from human and animal lesions such as *Geotricum candidum*, *Aspergillus flavus*, *Fusarium oxysporum*, *Chrysosporium Anamorph of Arthroderma cuniculi* and *Paecilomyces lilacinus* (10-14).

*Aspergillus flavus* was the second dominant species in soils of Gorgan (19.5%) and Gonbad-e Kavus (19%) areas. This species has been cited as one of the fungi, which are present in atmosphera (28), and soil of various areas of world (29-32) as well as Iran (18-25).

*Aspergillus flavus* is also reported to be the commonest causative agent of sinusitis in Iran (33). On the other hand this species is a potentially mycotoxin producer (34).

The genus *Penicillium* was isolated from samples of Bandar-e Gaz, Bandar-e Torkman and Gorgan. The data are coincident with those reported by several authors who mention the constant presence of *Penicillium* in mycoflora from different area in the world (29, 31) and in Iran (18-25). *Paecilomyces lilacinus* can induce keratitis (1, 5). Species of *Geotricum* were reported from human dermal lesions (15), bronchial, oral, and vaginal infections (34, 35). On the bases of keratinolysis test according to the reports of Sharma (7) seven species (55.46%) were identified as keratinolytic with three in the genus.
Chrysosporium (C. keratinophilum, Chrysosporium Anamorph of Arthroderma cuniculi, Chrysosporium Anamorph of Reniospora flavissima) and remaining species were non-keratinolytic fungi. The most active keratinolytic fungi are dermatophytes and their correlates especially Microsporum, Trichophyton, Chrysosporium, Myceliophthora and Reniospora species, though forms of attack have equally been reported for species of Paecilomyces and Penicillium (7, 8). Cano et al. (16) have showed that three species of Aphanoascus (A. keratinophilus, A. fulvescence and A. verrucosus) could develop keratinolytic activity. Anixiopsis stercoraria (anamorphe of Aphanoascus fulvescens) a keratinophilic and keratinolytic species was frequently isolated in the present study and from soil all over the world (16, 36). It has been reported as responsible of human dermatomycosis (37) and granulomata in peritoneum and liver in experimental animals during last years (16, 38). Myceliophthora vellera isolated from the soil in many parts of Europe, Asia, America, and South Pacific Islands (17) where the temperature ranges from 10 °C to 30 °C. However, species with strong keratinolytic activity (M. gypseum, T. vanbreuseghemii) were generally found to have low population level in soil of forests. On the other hand, some of the species that showed weak or moderate keratinolytic activity (Geotricum candidum, Paecilomyces lilacinus) were found to be among the most dominant components of keratinophilic fungal communities of these habitats.

It must be concluded that the selection of certain keratinolytic isolates could become useful in managing heavily polluted habitats. Finally to our knowledge this is the first report concerning on isolation of Arthroderma cuniculi, Myceliophthora vellera, Reniospora flavissima, T. vanbreuseghemii as keratinolytic fungi from soil samples in studied areas in Iran.

Acknowledgements
The research has been supported by the (SPH-IPHR), Tehran University of Medical Science grant No. 240.7807.

The authors wish to thank Professor Chabbas Cheaf of the Laboratories of Centre Hospitalier Universitaire, Angers, France, for helping on with identification of some keratinophilic fungi. In addition, we thank all staffs of the Parasitology and Mycology Laboratory in CHU of Angers, France.

References
3. Nasery Bande Gharaey A (1992). The survey and study of cutaneous-superficial infections in patients referred to dermatology clinics in Mashhad, Iran. MSc thesis of Medical Mycology. Faculty of Medicine, University of Tarbiat Modares.


