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

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Abstract

Soil is well known to support the transient or ongoing existence of keratophilic 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 follow: *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 keratinophilc fungus ($P< 0.05$). It can be concluded that soils from forest and farmyards of Golestan Province are rich in keratiophilic 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

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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.

Soil samples were transferred immediately to the laboratory in sterile, tightly closed polythene bags and stored at -20 °C for one month to eliminate possible acarians. After freezing, the procedure of Vanbreuseghem (4) for selective isolation of keratinophilic fungi from soil was applied; using sterile healthy children hair fragments as described earlier (5). The baited soils were moistened with sterile distilled water, and incubated at 28 °C for one month. Ten Petri dishes were used for every soil sampling area. At weekly intervals all species colonizing the hair baits were transferred onto Yeast Extract Peptone Dextrose Agar (YPDA) with 0.1% chloramohenicol plates and also YPDA-0.1% chloramohenicol medium supplemented with 0.1% cyclohexamide for identification.

Identification of fungi Cultures were regularly examined during a maximum period of four weeks. Strains belonging to the Onygenales were identified by their morphological and physiological characteristics according to Kane et al. procedure (6). All filamentous isolates were subcultured on YPDA plates, oat-bran Borelli or Malt agar when necessary, and identified according to standard descriptions by performing the following tests: susceptibility to cyclohexamide, production of sexual states on YPDA-0.1% chloramohenicol, hair perforation, urease production on Christensen medium, thermosensibility at 37°C and slide culture for microscopy.

Statistical method The count of each species was expressed as the number of cases of isolation in every site i.e. each species was counted as one in every soil sample even if recorded several times.

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 fungi were recovered. Of those only seven species (25.46%) 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).

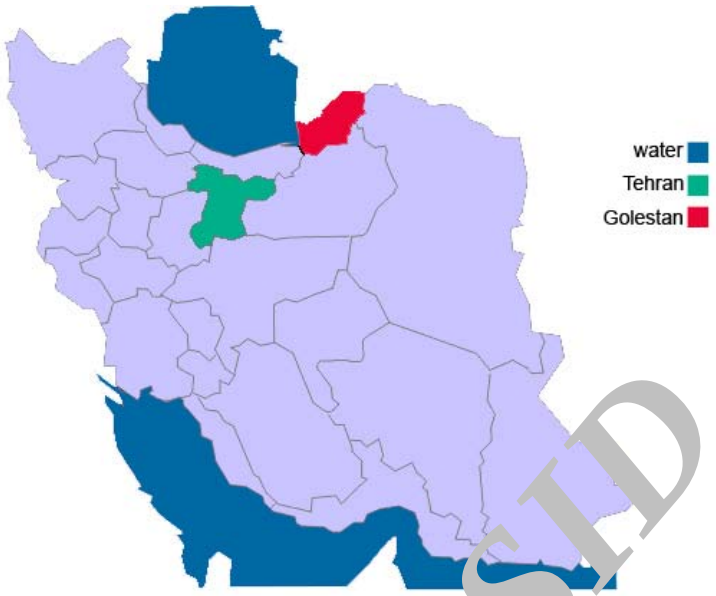

Fig. 1: The location of the Golestan Province in Iran

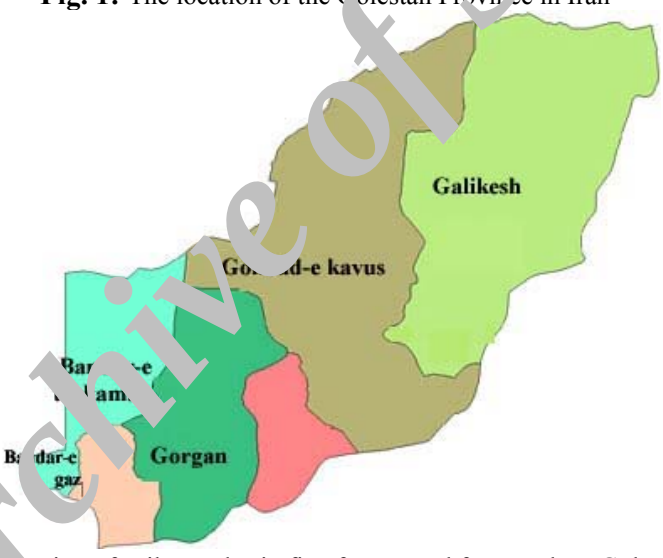

Fig. 2: ... of collection of soil samples in five forests and farmyards at Golestan Province, Iran

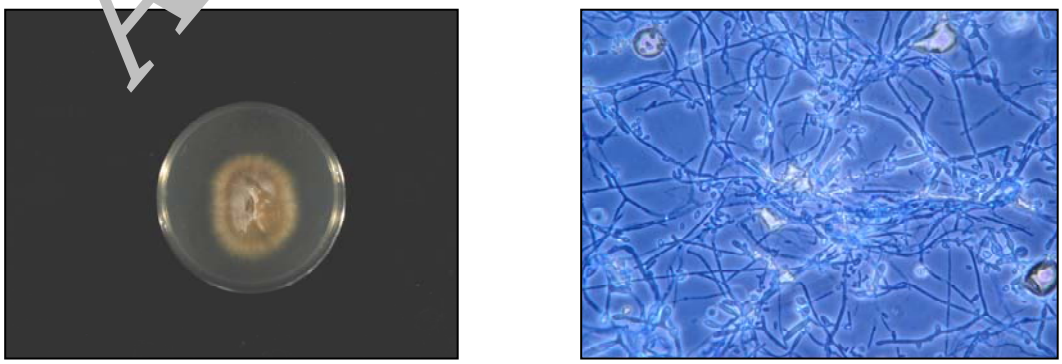

Fig. 3: Left: *Trichophyton vanbreuseghemii* on malt agar at 28ºC after 10 days.
Right: Microscopic feature of *Trichophyton vanbreuseghemii* (x400).

Table 1: Distribution of fungi isolated from soil samples of forests and farm yards in different sites at Golestan Province

Species	Bandar-e Gaz		Bandar -e Torkman		Galikesh		Gonbad-e Kavus		Gorgan		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
Acromonium sp.	0	0	20	30	7	10	0	0	0	0	27	7.56
Anixiopsis stercoraria *	0	0	20	30	35	50	15	19	8	9.75	78	21.84
Arthroderma cuniculi *	30	49	13	20	0	0	0	0	0	0	43	12.04
Aspergillus flavus	0	0	0	0	0	0	15	[illegible]	16	19.51	31	8.68
Chrysosporium keratinophilum *	0	0	0	0	14	20	0	0	16	19.51	30	8.40
Fusarium oxysporum	12	20	13	20	0	0	8	[illegible]	0	0	33	9.24
Geotricum candidum	0	0	0	0	0	0	8	10	0	0	8	2.24
Microsprum gypseum *	0	0	0	0	0	0	0	0	4	4.87	4	1.12
Myceliophthora vellera *	0	0	0	0	0	[illegible]	8	10	8	9.75	16	4.48
Non-sporlating fungi	0	0	0	0	0	[illegible]	8	10	16	19.51	24	6.72
Paecilomyces lilacinus	0	0	0	0	0	0	8	10	0	0	8	2.24
Penicillium sp	6	10	0	0	0	0	8	10	0	0	14	3.92
Reniospora flavissima *	13	21	0	0	0	0	0	0	0	0	13	3.64
*Trichophyton vanbreuseghemii**	0	0	0	0	14	20	0	0	14	17.01	28	7.84
Total	61	10	6[illegible]	100	70	100	78	100	82	100	357	100

*. Keratinolytic fungi

Table 2: Distribution of ke[illegible]tinophilic fungi in soil samples from forests and farm yards in different sites at Golestan Province

sites	Keratinolytic fungi		Others	
	n	%	n	%
Bandar-e Gaz	43	20.28	18	12.41
Bandar-e Torkman	33	15.56	33	22.75
Galikesh	63	29.71	7	4.82
Gonbad-e Kavus	23	10.84	55	37.93
Gorgan	50	23.58	32	22.06
Total	212	100	145	100

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 particularly 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 wastes under. 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-sporolating 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 of Gurgan but comparatively with less percentage of occurrences than *T. vanbreuseghemii*. Dominik et al. (27) suggested that keratinophilic 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 forest. 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, Mycliophthora vellera, Reinospora flavisima, T. vanbreuseghemii* as keratinolytic fungi from soil samples in studied areas in Iran.

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