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Fungi associated with sheep hairs in Saudi Arabia

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The frequency of occurrence of fungi in 25 hair samples of nine kinds of sheep, collected from different localities in Saudi Arabia, was estimated using three isolation methods at 28 °C. Forty-five species and one variety representing 23 genera were isolated and the most common genera were *Chrysosporium, Alternaria, Aspergillus* and *Penicillium*. The most prevalent species of the above genera were *C. indicum, C. tropicum, Alternaria alternata, Aspergillus flavus, A. fumigatus. Penicillium chrysogenum* and *P. oxalicum*. Other fungi were also isolated with variable frequencies.

Key words: Keratinophilic, non-keratinophilic fungi, sheep hairs.

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Ve 25 vzorcích srsti sbíraných v různých lokalitách Saudské Arábie a pocházejících z devíti plemen ovcí byla stanovena frekvence výskytu hub pomocí tří metod izolace při 28 °C. Z izolovaných 45 druhů a variet (23 rodů) byly nejčastějšími rody *Chrysosporium, Alternaria, Aspergillus* a *Penicillium*. Převládajícími druhy z jmenovaných rodů byly *Chrysosporium indicum, Ch. tropicum, Alternaria alternata, Aspergillus flavus, A. fumigatus, Penicillium chrysogenum* a *P. oxalicum*. Další houby byly izolovány s různou frakvencí.

Several studies have been made of the mycoflora associated with the hair of different kinds of animals (Kuttin and Beemer 1975, Aho 1983, Bagy and Abdel-Hafez 1985, Bagy 1986, Abdel-Hafez 1987, Ali-Shtayeh et al. 1988a,b, 1989, Chabasse 1988, Kubo et al. 1990, El-Said and Abdel Sater 1994 and others).

In Saudi Arabia, information on the existence of keratinophilic and non-keratinophilic fungi are not available. Thus the present work aims to make an extensive survey of the fungi colonizing the sheep hairs in Saudi Arabia.

MATERIALS AND METHODS

Twenty-five healthy hair samples, from nine kinds of sheep (Saudian 4; Somalian 2; Sudanian 3; Syrian 3; Turkish 3; Italian 3; Iranian 3 and Indian 4) were collected from different localities in Saudi Arabia. The samples were placed in clean plastic bags and transferred to the laboratory. For the isolation of the mycoflora inhabiting the hairs the following three isolation methods were used.

1. Soil-plating technique

The soil-plating technique was used as described by Vanbreuseghem (1952). The soil was double-sterilized by autoclaving at 121 °C for 15 min. The hair bundles (5 from each sample) were scattered on the surface of moistened sterile soil (20–25 % moisture content) in sterile plates (2 plates for each sample). The plates were incubated at room temperature (25 °) for 10–12 weeks and the soil in the plates were remoistened with sterile distilled water whenever necessary. The moulds which appeared on the baits were transferred to the surface of Sabouraud's dextrose agar medium (Moss and McQuown 1969) containing 0.5 g/l cycloheximide and 40 g/ml chloramphenicol. The plates were incubated at 28 °C for 14 days and the developing fungi were isolated, identified and calculated for 10 hair fragments for each sample.

2. Hair-plating directly on the medium

Five bundles from each sample were scattered on the surface of Sabouraud's dextrose agar medium. Three plates were used for each sample. The plates were incubated at 28 °C for 2–3 weeks and the developing fungi were counted, identified and calculated as previously mentioned.

3. The dilution-plate method

For the isolation of fungi inhabiting hairs, the dilution-plate method as described by Johnson and Curl (1972) was used. Five grams of each sheep hair sample are put in a 250 ml Eyrlenmeyer flask. A sufficient quantity of sterile distilled water was added to obtain the desired dilution. The flask was shaken for 15 minutes. One ml of the suspension was transferred aseptically into each of 3 Petri-dishes, and 20 ml of Sabouraud's dextrose agar medium cooled to exactly the solidifying temperature were added to each dish. The dishes were rotated by hand in broad swirling motion. Three plates were used for each sample and the plates were incubated at 28 °C for 2–3 weeks. The developing fungal colonies were counted, identified and calculated per g of hair.

RESULTS AND DISCUSSION

The results in Table 1 indicate that 45 species and one variety belonging to 23 genera were collectively isolated using different techniques, from which 18 species and variety (14 genera) were obtained using soil plating technique, 29 and 1 (14) using hair plating and 31 (15) using dilution-plate method. The most common genera isolated were: *Chrysosporium, Alternaria, Aspergillus* and *Penicillium.* Similar observations were obtained from goats and sheep in many parts of the world (Otčenášek and Dvořák 1962, Aho 1983, Bagy and Abdel-Hafez 1985, Abdel-Hafez 1987, Ali-Shtayeh et al. 1988a,b, El-Said and Abdel-Sater 1994).

Chrysosporium was the most frequent genus and emerged in 68 %, 52 % and 56 % of the samples comprising 48.3 %, 10.0 % and 8.2 % of the total count, using soil-plating, hair-plating and dilution-plate techniques, respectively. This genus was also isolated from goat and sheep hairs in the Gaza Strip as reported by Abdel-Hafez (1987). He observed that *Chrysosporium* was represented in 97.3 % of goat or sheep hair samples matching 36.1 % of total isolates. Also, El-Said and Abdel-Sater (1994) indicated that *Chrysosporium* was the most prevalent fungi on the hair of goats and sheep emerging in 92 % and 96 % of the samples comprising 91.2 % and 87.8 % of total isolates respectively. In our experiments, it was represented by 6 species of which *C. indicum* and *C. tropicum* were the most common species in the three methods used. They occurred in 16 %, 32 % and 40 %, and 36 %, 20 % and 44 % of the samples constituting 10.3 %, 4.7 % and 2.9 %, and 26.4 %, 2.2 % and 4.8 % of all fungal isolates using the three methods, respectively. The remaining 4 species were less common (Table 1). This is in agreement with the results obtained from goat and sheep hair from El-Bahrin (El-Said and Abdel-Sater 1994), who found that *C. indicum* and *C. tropicum* were the most prevalent keratinophilic fungi. The above species and others were also isolated, but with different frequencies, from some animal hairs, cloven hooves of sheep, claws of buffaloes and cows, horse hooves, poultry feathers and soil baited with human or animal hairs in Egypt (Maghazy 1983, Bagy and Abdel-Hafez 1985, Abdel-Gawad 1984, 1990, Moharram and Abdel-Gawad 1989, Abdel-Hafez et al. 1990, Abdel-Hafez 1991) as well as in other parts of the world (Rees 1968, Gugnani et al. 1975, Pugh and Evans 1970, Hubálek et al. 1973, Takatori et al. 1980, Ali-Shtayeh et al. 1988a, b, Chabasse et al. 1989, Filipello Marchisio 1986 and several others).

Further, several saprophytic and cycloheximide resistant fungi were also encountered on sheep and the most predominant species were members of *Alternaria, Aspergillus* and *Penicillium.*

Alternaria (2 species) was found in 28 %, 96 % and 92 % of the samples constituting 12.7 %, 28.5 % and 29.8 % of the total count for the three isolation methods, respectively. It was represented by two species, of which *A. alternata* was prevalent. Similarly, Ali-Shtayeh et al. (1988a) isolated *A. alternata* from the hair of goats in the West Bank of Jordan. Also, this genus was also the most common fungi on hairs of Table 1 goats and sheep (El-Said and Abdel-Sater 1994), skin of dogs and cats (Bone and Jackson 1971), cloven-hooves and horns of goats and sheep (Abdel-Hafez *et al.* 1990), hairs of goats, cows, donkeys and cats (Ali-Shtayeh *et al.* 1988a), and camel and goat hairs (Bagy and Abdel-Hafez 1985).

Aspergillus was the third common genus emerging in 28 %, 80 % and 76 % of the samples contributing to 9.2 %, 19.1 % and 19.0 % of the total number of fungi for the three isolation methods, respectively (Table 1). It was represented by 7 species and one variety of which *A. flavus* and *A. fumigatus* were prevalent. They

Table 1 Fungi isolated from sheep hair (out of 25 samples) using soil-plating (A); hair-plating (B) and dilution-plate method (C) techniques at 28 °C.

Genera & species	A		B		C	
	TC	NCI & OR	TC	NCI & OR	TC	NCI & OR
Chrysosporium	42	17H	32	13H	257	14H
Ch. carmichaelii V. Oorschot	—	—	1	1R	—	—
Ch. georgii (Var. et Ajello) V. Oorschot	4	2R	—	—	—	—
Ch. indicum (Randhawa et Sandhu) Gary	9	4L	15	8M	90	10M
Ch. keratinophilum (D. Frey) Carmichael	6	2R	7	4L	17	2R
Ch. pannicola (Corda) V. Oorschot	—	—	2	1R	—	—
Ch. tropicum Carmichael	23	9M	7	5L	150	11M
Acremonium strictum W. Gams	7	7M	1	1R	109	12M
Acrophialophora fusispora (Saksena) Samson	1	1R	—	—	—	—
Alternaria	11	7M	91	24H	937	23H
A. alternata (Fr.) Keissler	11	7M	90	24H	917	23H
A. tenuissima (Kunze) Wilt.	—	—	1	1R	20	2R
Aspergillus	8	7M	61	20H	596	19H
A. alutaceus Berk. et Curtis	—	—	2	2R	20	1R
A. flavus Link	6	6L	24	11M	87	9M
A. flavus var. *columnaris* Raper et Fennell	2	2R	3	2R	—	—
A. fumigatus Fresenius	—	—	17	11M	443	17H
A. niger v. Tieghem	—	—	7	5L	3	1R
A. oryzae (Ahlb.) Cohn	—	—	—	—	3	1R
A. sydowii (Bain. et Sart.) Thom et Church	—	—	1	1R	40	1R
A. terreus Thom	—	—	7	4L	—	—
Botryotrichum piluliferum Sacc. et Marchal	—	—	1	1R	—	—
Candida albicans (Robin) Berkhout	—	—	—	—	7	1R
Chaetomium globosum Kunze	3	3R	6	5L	17	3R
Emericella nidulans (Eidam) Vuill.	1	1R	6	6L	—	—
Gibberella fujikuroi (Sawada) Ito	—	—	1	1R	—	—
Gymnoascus reesii Baran	—	—	1	1R	—	—
Mucor	2	2R	5	4L	—	—
M. circinelloides v. Tieghem	—	—	3	2R	—	—
M. hiemalis Wehmer	2	2R	—	—	—	—
M. racemosus Fresenius	—	—	2	2R	—	—

Table 1 Fungi isolated from sheep hair (out of 25 samples) using soil-plating (A); hair-plating (B) and dilution-plate method (C) techniques at 28 °C. (Continued)

Genera & species	A		B		C	
	TC	NCI & OR	TC	NCI & OR	TC	NCI & OR
Myrothecium verrucaria (Alb. et Schw.) Dit.	1	1R	—	—	7	1R
Paecilomyces	2	2R	—	—	16	3R
P. lilacinus (Thom) Samson	2	2R	—	—	13	3R
P. variodtii Bainier	—	—	—	—	3	1R
Penicillium	1	1R	90	21H	1009	25H
P. aurantiogriseum Dierckx	—	—	4	3R	3	1R
P. chrysogenum Thom	1	1R	12	9M	245	10M
P. citrinum Thom	—	—	—	—	40	4L
P. corylophilum Dierckx	—	—	1	1R	20	3R
P. duclauxii Delacroix	—	—	—	—	37	8M
P. oxalicum Currie et Thom	—	—	73	20H	661	20H
P. variabile Sopp	—	—	—	—	3	1R
Phoma glomerata (Corda) Wollenw. & Hochapfel	—	—	—	—	3	2R
Rhizoctonia solani Kühn	—	—	—	—	5	1R
Rhizopus stolonifer (Ehrenb.) Lind	—	—	3	2R	22	5L
Scopulariopsis	—	—	10	6L	135	11M
S. brevicaulis (Sacc.) Bainier	—	—	10	6L	125	11M
S. brumptii Salvanet-Duval	—	—	—	—	10	1R
Syncephalastrum racemosum (Cohm.) Schroeter	1	1R	11	4L	5	1R
Thermoascus aurantiacus Miehe	6	4L	—	—	—	—
Trichothecium roseum (Pers.) Link	—	—	—	—	5	1R
Ulocladium botrytis Preuss	1	1R	—	—	—	—
Total isolates		87		319		3140
Number of genera = 23		14		14		15
Number of species = 45 + 1 var.		18+1		29+1		31

TC = total count, NCI = number of cases of isolation; OR = occurrence remarks; H = high occurrence, 13-25 (out of 25) cases; M = moderate occurrence, 7-12 cases; L = low occurrence, 4-6 cases; R = rare occurrence, 1-3 cases.

were found in 24–68 % of the samples matching 2.8–14.5 % of the total number of fungi for the three isolation methods, respectively. The remaining *Aspergillus*

species were less common (Table 1). Members of *Aspergillus* were also among the most common fungi on the hair of different animals as reported by several workers.

Penicillium was the most frequent fungus found using the dilution-plate method (100 % of the samples and 23 % of total count), and hair-dilution method (84 % and 28.2 %), but rare in the soil-plating method (4 % and 1 %). It was represented by 7 species, of which *P. oxalicum* was the most frequent, and was encountered each in 80 % of the samples representing 22.9 % and 21.1 % of the total count recovered by the hair-plating and dilution-plate methods, respectively. *P. chrysogenum* emerged in 4 %, 36 % and 40 % of the samples comprising 1.1 %, 3.8 % and 7.8 % of the total number of fungi in the three isolation methods, respectively. The remaining *Penicillium* species isolated had a moderate, low or rare frequency of occurrence (Table 1). In this respect, El-Said and Abdel-Sater (1994) reported that *Penicillium* was the second most predominant fungi occurring in 80 % of the samples contributing to 12.1 % and 10.3 % of the total number of moulds isolated from goat and sheep hairs, respectively. This genus was also isolated from hairs of goats, cows, donkeys and cats (Ali-Shtayeh et al. 1988a,b) and from camel and goat hairs (Bagy and Abdel-Hafez 1985).

Acremonium (1 species) and *Scopulariopsis* (2 species) showed moderate frequencies of occurrence in most isolation methods. These genera were isolated in rare or low occurrence from sheep hair in El-Bahrin (El-Said and Abdel-Sater 1994).

The remaining fungal species were isolated in rare or low frequencies of occurrence (Table 1). Most of these fungi were previously isolated from the hairs of different animals (Aho 1983, Bagy and Abdel-Hafez 1985, Marsella et al. 1985, Bagy 1986, Abdel-Hafez 1987, Abdel-Gawad 1990, Abdel-Hafez et al. 1990, El-Said and Abdel-Sater 1994).

In conclusion, an analysis of the mycoflora of sheep hairs indicated that there are several keratinophilic and saprophytic fungi inhabiting hairs of these animals. A smaller total of isolates and a narrower spectrum of species were collected from soil-plating technique than the other two isolation methods. This method enumerated only fungal species which were able to grow on keratin (moist sheep hair) as their sole source of carbon. Therefore, some species were encountered only using the soil-plating method and not isolated from the others such as: *Chrysosporium georgii, Acrophialophora fusispora, Thermoascus aurantiacus*. On the other hand, the other two methods provided and ideal chance for fast growing fungi inhabiting sheep hair to appear. For example, *Chrysosporium carmichaelii, C. pannicola, Aspergillus terreus, Botryotrichum piluliferum, Gibberella fujikuroi, Gymnoascus reesii, Mucor circinelloides, M. racemosus* by hair-plating; and *Aspergillus oryzae, Candida albicans, Paecilomyces variotii, Penicillium citrinum, P. duclauxii, P. variabile, Phoma glomerata, Rhizoctonia solani, Scopulariopsis brumptii, Trichothecium roseum* by the dilution-plate method.

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