

KERATINOPHILIC FUNGI ASSOCIATED WITH HORSE HOOFS IN EGYPT

Khyria M. ABDEL-GAWAD

Botany Department, Faculty of Science,
Assiut University, Assiut, Egypt.

ABSTRACT - The frequency of occurrence of keratinophilic fungi in 26 samples of hoofs (26 horses) collected from different localities at Assiut governorate were determined by using the soil plate technique. Twenty species which belong to 14 genera were collected: *Trichophyton equinum* was the most prevalent, *Chrysosporium tropicum* was isolated in moderate frequency, but in low frequencies keratinophilic and several species of non-keratinophilic fungi were isolated. At 45°C, on YPSs medium, 16 species belonging to 15 genera of thermophilic and thermotolerant fungi were isolated from 60 hoof samples: *Aspergillus* was the genus most commonly recovered. Among the true thermophilic fungal species, *Talaromyces thermophilus*, *Malbranchea sulfurea* and *Thermomyces lanuginosus* occurred in low incidence. Several species of thermophilic and thermotolerant fungi were of rare occurrence.

RÉSUMÉ - 20 espèces (14 genres) de champignons kératinophiles ont été isolées sur 26 sabots de cheval provenant de différentes localités (Gouvernorat d'Assiut). *Trichophyton equinum* est l'espèce la plus fréquente, suivie par *Chrysosporium tropicum*. A 45°C, sur milieu YPSs, 16 espèces (15 genres) de champignons thermophiles et thermotolérants ont été isolées à partir de 60 échantillons de sabots de cheval: *Aspergillus* est le genre le plus fréquent. Parmi les espèces thermophiles vraies, *Talaromyces thermophilus*, *Malbranchea sulfurea* et *Thermomyces lanuginosus* ont une faible fréquence.

KEY WORDS : keratinophilic fungi, thermophilic fungi, thermotolerant fungi, horse hoofs.

INTRODUCTION

The occurrence of dermatophytes and other keratinophilic fungi from animal hoofs has not been examined extensively although they are known to occur from the hair or skin of mammals (Ajello, 1959; Otcenasek & Dvorak, 1962; Rees, 1967; Scott & al., 1980; Aho, 1983; Marsella & al., 1985; Kuttin & al., 1986) and in Egypt (Bagy & Abdel-Hafez, 1985; Bagy, 1986). Others

studies have been performed on ringworm and horses infected by dermatophytes (de Vroey & al., 1983; Aho & Soveri, 1984; Aho & Skutnabb, 1984; Connole & Pascoe, 1984; Weiss & al., 1984; Elmula & Idris, 1985; Vrazal & al., 1985). Hence the aim of this investigation is to study the incidence of these fungi on horse hoofs.

MATERIALS AND METHODS

Twenty-six hoof samples from 26 horses were collected from different localities at Assiut governorate. These samples were placed in clean plastic bags and transferred immediately to the laboratory. The hoof samples were dissected into small pieces and 5 pieces from each sample were placed in 5 sterilized Petri dishes with double sterilized soil (autoclaving at 121°C for 30 min.) moistened with sterile distilled water, remoistened whenever necessary, and incubated at 26°C for up to 8 weeks. The moulds which appeared on the baits were transferred to the surface of Sabouraud's dextrose agar medium (Moss & McQuown, 1969) supplemented with 40 µg/ml streptomycin, 20 units of Penicillin/ml (SSA) and 0.05% cycloheximide (Actidione). The plates were incubated at 28°C for 2 weeks and the growing fungi were examined, isolated and identified.

To examine thermophilic fungi, 60 hoof samples were used with the same method of soil plate technique, but plates were incubated at 45°C and medium YPSs was used for isolation (g/l: water tap 1/4, distilled 3/4; yeast extract, 4.0; K₂ HPO₄, 1.0; MgSO₄.7 H₂O, 0.5; soluble starch, 12.0; agar, 20.0. Rose bengal (66 ppm) was added as a bacteriostatic agent (Smith & Dawson, 1944). Eight plates were used for each sample and the plates were examined after 7 days of incubation at 45°C. The developing fungi were identified and counted.

RESULTS AND DISCUSSION

Twenty species belonging to 14 genera were collected from horse hoofs (Tab. 1). *Trichophyton equinum* was the most common species on the horse hoofs (57.7% of the samples). *Trichophyton equinum* was previously reported as a cause of tinea corporis in man and a cause of ringworm in animals, particularly in horses. Colony appeared to be white and fluffy but later velvety with central folding and cream to tan in colour. Reverse is yellow to reddish brown. Macroaleuriospores are rare and they are slightly clavate, smooth and thin-walled, 3-4 celled. Microaleuriospores are abundant, slightly clavate to elongate, or nearly spherical, single-celled and either sessile or borne on short, delicate conidiophores. Chlamydo-spores are abundant in old cultures. It was also isolated from horses with ringworm by Vrazal & al. (1985) and studied on dermatophytoses in horses by Garcia & al. (1981), Aho & Soveri (1984) and Zakopal (1985).

Table 1: Numbers of cases of isolation (NCI out of 26 samples) and percentage counts (%) of various genera and species recovered from horse hoofs on Sabouraud's medium at 25°C.

Tableau 1: Genres et espèces de champignons isolés à partir de 26 échantillons de sabots de cheval, sur milieu Sabouraud à 25°C.

Genera and species	NCI	%	O.R.
<i>Trichophyton equinum</i> (Matruchot & Dassonville) Goedelst	15	57.7	H
<i>Chrysosporium tropicum</i> Carmichael	11	42.3	M
<i>C. keratinophilum</i> (Frey) Carmichael	4	15.4	L
<i>C. georgii</i> (Varsavsky & Ajello) Van Oorshot	1	3.9	R
<i>C. merdarium</i> (Link ex Grev.) Carmichael	1	3.9	R
<i>C. pannicola</i> (Corda) Van Oorschot et Stalpers	1	3.9	R
<i>Myceliophthora vellerea</i> (Sacc. & Speg.)	2	7.7	R
<i>Geotrichum candidum</i> Link ex Lemm	1	3.9	R
<i>Aspergillus flavus</i> Link ex Fries	5	19.2	L
<i>A. terreus</i> Thom	2	7.7	R
<i>A. sydowi</i> (Bainier et Sartory) Thom et Church	1	3.9	R
<i>Acremonium</i> sp.	4	15.4	L
<i>Fusarium solani</i> (Mart.) Sacc.	2	7.7	R
<i>Trichothecium roseum</i> Link	2	7.7	R
<i>Alternaria alternata</i> (Fr.) Keissler	1	3.9	R
<i>Cladosporium herbarium</i> (Pers.) Link ex Fr.	1	3.9	R
<i>Mucor</i> sp.	1	3.9	R
<i>Penicillium chrysogenum</i> Thom	1	3.9	R
<i>Paecilomyces lilacinus</i> (Thom) Samson	1	3.9	R
<i>Scopulariopsis brevicaulis</i> (Saccardo) Bainier	1	3.9	R
Yeast-like	3	11.5	L

O.R. - occurrence remarks: H = high occurrence, between 13-26; M = moderate occurrence, between 6-12; L = low occurrence, between 3-5; R = rare occurrence, between 1-2.

Chrysosporium tropicum was the second most frequent fungal species (42.3% of the samples). It was also isolated by Bagy & Abdel-Hafez (1985) from camel and goat hairs and from dog, donkey and cow hairs (Bagy, 1986). It was also isolated from mammals by Rees (1967), Moraes & al. (1967) and Gugnani & al. (1975).

A. flavus was the third dominant species (19.2% of the samples). It was also isolated by Bagy & Abdel-Hafez (1985) and Bagy (1986). *Chrysosporium keratinophilum* and *Acremonium* sp. were the fourth common species (15.4% of the samples). *C. keratinophilum* was the most common species on the hairs of goat and camel (Bagy & Abdel-Hafez, 1985) and was also found on dog, donkey and cow hairs (Bagy, 1986). It was isolated from mammals by Otcenasek & Dvorak (1962), Hoffman & al. (1970) and Gugnani & al. (1975). *Acremonium* sp. was also isolated by Filipello Marchisio (1986) from children's sandpits.

Among the group of *Chrysosporium*-like fungi the species isolated from the horse hoofs are the same mostly recovered from soils by Guarro & al. (1981) and Marsella & Mercantini (1986) and from Egyptian soils baited with different keratinaceous materials. Abdel-Fattah & al. (1982) showed that *C. indicum*, *C. tropicum* and *C. keratinophilum* were common in Egyptian soil baited with human hair, animal hair or pigeon feathers. Also, Maghazy (1983) isolated *C. tropicum*, *C. keratinophilum*, *C. indicum*, *C. pannicola* and *C. queenslandicum* from Egyptian soils baited with human hair, cow and buffalo hair and sheep wool. Also they were isolated from feathers of birds by Otcenasek & al. (1967), Sur & Ghosh (1979), and in Egypt, Bagy (1982) isolated *Chrysosporium* species from chicken's and wild sparrow's feathers and their nests.

Myceliophthora vellerea (*Chrysosporium asperatum*) isolated from horse hoofs, was commonly recovered in soil by Caretta & al. (1977). *Geotrichum candidum* was also isolated by Zaror (1979) which cause human nails infection. Yeasts, also isolated by Rippon (1982), and diverse filamentous fungi are referred collectively as onychomycosis.

Most of the other fungi recovered from the horse hoofs are common soil fungi in Egypt, frequently isolated by Moubasher and his collaborators on Czapek's agar.

These keratinophilic fungi which concentrated in the superficial layers of soil may become hoofs saprophytic or infections. The dispersal of these fungi in the air and incidence on animal skin or hair may cause infection; for example *Trichophyton equinum* which cause ringworm of horses was isolated by several authors; it was also isolated from the horse hoofs in this investigation.

At 45°C, 16 species belonging to 15 genera of thermophilic and thermotolerant fungi were isolated from the hoof samples (Tab. 2).

Aspergillus was the genus most commonly recovered (83% of total samples). Of the three *Aspergillus* species, *A. fumigatus* was of high incidence (72%) while *A. nidulans* was of low incidence (20% of the samples).

Among the true thermophilic fungal species, *Talaromyces thermophilus*, *Malbranchea sulfurea* and *Thermomyces lanuginosus* occurred in low incidence (21, 20 and 11% of total samples respectively).

Hyphae of *Malbranchea sulfurea* were colorless at first, later yellow, 1.5-4.5µm in diameter, the branches arising at right angles to the supporting hyphae, at first nonseptate, later becoming regularly septate and forming blocklike cells from the apex toward the base with thick-walled spores separated by thin-walled, sterile cells which soon die and collapse; spores pale yellow or colorless subcylindrical, often slightly curved as irregular, 3-7 x 3-4.5µm.

Table 2: Incidence (out of 60 samples) and % incidence of thermophilic and thermotolerant fungi recovered from horse hoofs on YPSs Agar medium at 45°C.

Tableau 2: Champignons thermophiles et thermotolérants isolés à partir de sabots de cheval sur milieu agar-YPSs, à 45°C.

Genera and species	Incidence	%	O.R.
<i>Aspergillus</i>	50	83	H
<i>A. fumigatus</i> Fres.	43	72	H
<i>A. nidulans</i> (Eidam) Wint.	12	20	L
<i>A. terreus</i> Thom	4	7	R
<i>Talaromyces thermophilus</i> Stolk	13	21	L
<i>Malbranchea sulfurea</i> (Miche) Sigler & Carm.	12	20	L
<i>Thermomyces lanuginosus</i> Tsi Kitnsky	7	11	L
<i>Chaetomium thermophilus</i> La Touche	6	10	R
<i>Mucor</i> sp.	5	8	R
<i>Acremonium alabamensis</i> Morgan-Jones	3	5	R
<i>Myriococcum albomyces</i> Cooney & Emerson	3	5	R
<i>Paecilomyces inflatus</i> (Burnside) Carmichael	2	3	R
<i>Corynascus sepedonium</i> Emmons	1	2	R
<i>Hunicola stellata</i> Bunce	1	2	R
<i>Rhizomucor pusillus</i> Lindpt (Schipper)	1	2	R
<i>Rhizopus stolonifer</i> (Ehrenb. ex Fries) Lind.	1	2	R
<i>Thermoascus thermophilus</i> (Sopp) Von Arx	1	2	R
<i>Torula thermophila</i> Cooney & Emerson	1	2	R

Occurrence remarks: H = high occurrence, between 30-60; M = moderate occurrence, between 15-29; L = low occurrence, between 7-14; R = rare occurrence, between 1-6.

Hyphae of *Myriococcum albomyces* were colorless, branched, decumbent to erect, forming a loose mucedinous subiculum in culture; prostrate hyphae constricted at the septa, forming branched, chainlike series of cells that break apart easily, ascocarps superficial, 100-250µm diameter, globose, dark brown, nonostiolate, wall glabrous, asci pyriform produced in one or more tufts from a group of central cells.

Paecilomyces inflatus has colonies floccose-funiculose, yellow and center faintly pink; reverse white becoming buff or faintly yellow. Conidiophores are usually lacking and phialides borne irregularly on aerial hyphae; phialides have a septum near the base and are usually inflated in the basal portion appearing flask-like in the upper portion to narrow apical tube, conidia sometimes develop irregularly, becoming lopsided or squared, distinctly lemon-shaped with slightly truncate base and apex with rounded point.

Hyphae of *Torula thermophila* were colorless, septate, 2-5µm broad; spores dark brown, smooth walled, translucent, generally globose, to oval, basipetally produced in chains on hyphal branches or developed intercalarily, colonies white at first, turning jet black. The rest of thermophilic and thermotolerant fungi were rare.

Most probably, the hoofs are infested with propagules of these fungi (spores or mycelial fragments) from the soil, the different types of feedstuffs distributed to the horses, the air or the air-dust mycoflora. These suggestions are based on the fact that all of the fungi recovered from the horse hoofs were previously recovered from Egyptian soils (Moubasher & al., 1981 a-b; Moharram, 1984), wheat and broad-straw, composts (Moubasher & al., 1982) and other sources.

REFERENCES

- ABDEL-FATTAH H.M., MOUBASHER A.H. and MAGHAZY S.M., 1982 - Keratinolytic fungi in Egyptian soils. *Mycopathologia* 79: 49-53.
- AHO R., 1983 - Saprophytic fungi isolated from the hair of domestic and laboratory animals with suspected dermatophytosis. *Mycopathologia* 83: 65-73.
- AHO R. and SKUINABB K., 1984 - *Microsporium equinum*- uusin hevosen sieni-ihottuman aiheuttaja Suomessa. [= Equine dermatophytosis caused by *Microsporium equinum*]. *Sumen Eläinlääkärilehti* 90: 200-201, 203-204.
- AHO R. and SOVERI T., 1984 - *Trichophyton equinum* aiheuttama sieni-ihottuma hevosella. [= Equine dermatophytosis caused by *Trichophyton equinum*]. *Sumen Eläinlääkärilehti* 90: 192-193, 195-197.
- AJELLO L., 1959 - A new *Microsporium* and its occurrence in soil and on animals. *Mycologia* 51: 69-76.
- BAGY M.M.K., 1982 - Some ecological and physiological studies on keratinolytic fungi associated with birds in Egypt. Ph. D. Thesis, Bot. Dept., Fac. Sci., Assiut Univ., Egypt.
- BAGY M.M.K. and ABDEL-HAFEZ A.I.I., 1985 - Mycoflora of camel and goat hairs from Al-Arish, Egypt. *Mycopathologia* 92: 125-128.
- BAGY M.M.K., 1986 - Fungi on the hair of large mammals in Egypt. *Mycopathologia* 93: 73-75.
- CARETTA G., DEL FRATE G., PIONTELLI E. e TODARO F., 1977 - Distribuzione di funghi cheratinofili nel suolo del vulcano Enta (Sicilia). *Rivista Parasitol.* 38: 115-127.
- CONNOLLE M.D. and PASCOE R.R., 1984 - Recognition of *Trichophyton equinum* var. *equinum* infection of horses. *Austral. Veterin. J.* 61: 94.
- DE VROEY C., WUYTACK-RAES C. and FOSSOUL F., 1983 - Isolation of saprophytic *Microsporium praecox* Rivalier from sites associated with horses. *Sabbouraudia* 21: 255-257.
- ELMULA A.F. and IDRIS D.F.A.A., 1985 - Ringworm in a horse caused by *Trichophyton verrucosum*. *Bull. Animal Health & Produc. Africa* 33: 17-18.
- FILIPPELLO MARCHISIO V., 1986 - Keratinolytic and keratinophilic fungi of children's sandpits in the city of Turin. *Mycopathologia* 94: 163-172.
- GARCIA C., PLANAS A., CORDOVES CESPEDES C.O., DELGADO V. and CASANAS P., 1981 - [Preliminary report on the incidence of dermatophytes in

- domestic animals]. Acad. Ci. Cuba Informe Científico-Técnico No. 157: 1-4, in Span. with Span & Engl. summ. (from Biological Abstract: 75, 19290).
- GUARRO J., PUNSOLA L. and CALVO M.A., 1981 - Keratinophilic fungi from soil of Tarragona, Catalunya. *Mycopathologia* 76: 69-71.
- GUGNANI H.C., WATTAL B.L. and SANDHU R.S., 1975 - Dermatophytes and other keratinophilic fungi recovered from small mammals in India. *Mykosen* 18: 529-538.
- HOFFMANN R., KOLIPP D. and KOCH H.A., 1970 - Die Bedeutung von Mäusen und anderen Kleinsäugetern für die Vorbereitung von Dermatophyten und anderen Keratinophilen Pilzen. *Mykosen* 13: 353-387.
- KUTTIN E.S., ALHANATY E., FELDMAN M., CHAIMOVITS M. and MÜLLER J., 1986 - Dermatophytosis of camels. *J. Med. Veterin. Mycol.* 24: 341-344.
- MAGHAZY S.M., 1983 - Further studies on keratinolytic fungi in Egyptian soils. Ph. D. Thesis, Bot. Dept., Fac. Sci., Miniya Univ., Egypt.
- MARSELLA R., MERCANTINI R., SPINELLI P. and VOLTERRA I., 1985 - Occurrence of keratinophilic fungi in animals of zoological park of Rome. *Mykosen* 28: 507-512.
- MARSELLA R. and MERCANTINI R., 1986 - Keratinophilic fungi isolated from soil of the Abruzzo National Park, Italy. *Mycopathologia* 94: 97-107.
- MOHARRAM A.M., 1984 - Studies on thermophilic and thermotolerant fungi in Egyptian soils. Ph. D. Thesis, Bot. Dept., Fac. Sci., Assiut Univ., Egypt.
- MORAES M., BORELLI D. and FEO M., 1967 - *Microsporium amazonicum* nova species. *Med. Cutan.* 2: 281-286.
- MOSS E.S. and MCQUOWN A.L., 1969 - *Atlas of Medical Mycology*, 3rd ed. Baltimore, Williams & Wilkins Comp.
- MOUBASHER A.H., ABDEL-FATTAH H.M., ABDEL-HAFEZ S.I.I. and MOHARRAM A.M., 1981a - Studies on Egyptian Thermophilic soil fungi. I. Taxonomical and Environmental Studies. *Bull. Fac. Sci., Assiut Univ.* 10: 13-28.
- MOUBASHER A.H., ABDEL-FATTAH H.M., ABDEL-HAFEZ S.I.I. and MOHARRAM A.M., 1981b - Studies on Egyptian thermophilic soil fungi II. Seasonal fluctuations of thermophilic soil fungi. *Bull. Fac. Sci., Assiut Univ.* 10: 29-40.
- MOUBASHER A.H., ABDEL-FATTAH H.M., ABDEL-HAFEZ S.I.I. and MOHARRAM A.M., 1982 - Fungi of wheat and broad-bean straw composts. II. Thermophilic fungi. *Mycopathologia* 78: 169-176.
- OTCENASEK M. and DVORAK J., 1962 - The isolation of *Trichophyton terrestre* and other keratinophilic fungi from small mammals of south eastern Moravia. *Sabouraudia* 2: 111-113.
- OTCENASEK M., HUDEC K., HUBALEK Z. and DVORAK J., 1967 - Keratinophilic fungi from the nests of birds in Czechoslovakia. *Sabouraudia* 5: 350-354.
- REES R.G., 1967 - Keratinophilic fungi from Queensland. I. Isolation from animal hair and scales. *Sabouraudia* 5: 165-172.

- RIPPON J.W., 1982 - *Medical mycology*. Philadelphia, W.B. Saunders Comp.
- SCOTT D.W., KIRK R.W. and BENTINCK-SMITH J., 1980 - Dermatophytosis due to *Trichophyton terrestre* infection in a dog and cat. *J. Amer. Animal Hospital Assoc.* 16: 53-59.
- SMITH N.R. and DAWSON V.T., 1944 - The bacteriostatic action of rose bengal in media used for the plate count of soil fungi. *Soil Sci.* 58: 467-471.
- SUR B. and GHOSH G.R., 1979 - Keratinophilic fungi from Orissa. I. Isolations from soils. *Sabouraudia* 18: 269-274.
- VRAZAL V., RYBNÍKĀR A., CHUMELA J. and LYSÁK J., 1985 - *Trichophyton equinum* - hlavní původce dermatofytóz ukořů. [= *Trichophyton equinum* - the main cause of ringworm in horses]. *Veterinárství* 35: 119-120.
- WEISS R., FÖLDY I. and CHRISTOPH H., 1984 - *Trichophyton verrucosum* infektion bei pferden. [= *Trichophyton verrucosum* infection in horses]. *Tierärztliche praxis* 12: 49-53.
- ZAKOPAL J., 1985 - Zvyšit pozornost dermatofytózám ■ koni. [= Better attention to dermatophytoses in horses]. *Veterinárství* 35: 216-217.
- ZAROR L., 1979 - Onicomycosis en Valdivia. [= Onychomycosis in Valdivia]. *Revista Med. Chile* 107: 558-559.