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REGULAR ARTICLE

KERATINOPHILIC FUNGI IN SOILS STRESSED BY OCCURRENCE OF ANIMALS

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ABSTRACT

The aim of this study was to isolate and identify keratinophilic fungi from soils stressed by occurrence of animals, both pets and farm animals. Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance. This article draws the attention towards the incidence of fungal opportunistic pathogen (*Pseudallescheria boydii*) in soil sample, stressed by occurrence dog. This substrate should be considered as a potential source of the opportunists. *Trichophyton ajelloi* was representative and encountered in all the samples investigated. A human pathogen, a geophilic dermatophyte *Microsporum gypseum* was isolated from all 6 soil samples, stressed by occurrence of a dog. Another soil samples genus *Chrysosporium* has also occurred, namely *Chrysosporium keratinophilum* and *Chrysosporium queenslandicum*. Preliminary results showed that keratinophilic fungi were richly represented in the soils in Slovakia and should pay attention to their occurrence especially in the human environment.

Keywords: animals, *Chrysosporium*, keratinophilic fungi, *Microsporum*, *Pseudallescheria boydii*, *Trichophyton*

INTRODUCTION

Keratinophilic fungi are an ecologically important group of fungi that cycle one of the most abundant and highly stable animal proteins on earth **(Sharma and Rajak, 2003).** The biological function of keratinolytic fungi in the soil is the degradation of keratinized materials such as hides, furs, claws, nails and horns of dead animals. In point of refractyl keratin substances decay (specialized animal fibre), these fungi are important in soil ecosystem, that is why they are important from the point of element cycle on one hand, but their appearance in the man´s nature obtains even hygienical – epidemologic meaning on the other hand, because many of them are potential pathogen of warm – blooded animals. The species of this group have been divided into three categories according to their natural habitats: anthropophilic, when humans are the natural hosts; zoophilic, when a variety of animals represent the natural hosts; geophilic, when soil is the natural habitat **(Chabasse and Contet-Andonneau, 1994; De Hoog *et al.*, 2000)**. Keratinophilic fungi include both saprophytic species and dermatophytes. The occurrence of dermatophytes in soil was reported for the first time by **Vanbreuseghem (1952),** who used the hairbaiting technique.

Keratinophilic fungi are important ecologically and recently have attracted attention throughout the world **(Sharma and Rajak, 2003; Marchisio, 2000)**. In Slovakia, however, the occurrence and distribution of these fungi has been studied mainly in cultivated agricultural soils **(Chmel and Vláčilíková, 1977)**, forest soils, in rodent burrows **(Volleková, 1965, 1983, 1985, 1992)** or on pastures **(Javoreková *et al.* 2012)** and in the human environment (**Labuda *et al.*, 2008; Labuda, 2007; Labuda and Kačinová, 2007).** Domestic animals, especially dogs and cats, serve as reservoirs of *Microsporum* spp. and *Trichophyton* spp., and their infections are considered to have zoonotic importance **(Romano, 1999; Cabañes, 2000; Iorio *et al.*, 2007; Simpanya, 2000)**. Dermatophytes are also isolated from asymptomatic cats that act as carriers; in light of that fact cats without lesions should be considered in the epidemiology and risk of infection for in-contact humans **(Patel *et al.*, 2005; Pier *et al.*, 1994; Romano *et al.*, 1997).** The studies on the isolation of dermatophytes from dogs and cats have shown that the prevalence of infections is approximately 4 – 20% in dogs, and higher than 20% in cats **(Lewis *et al.*, 1991; Cabañes *et al.*, 1997; Brilhante *et al.*, 2003; Cafarchia *et al.*, 2004).** Dermatophytes are cited among the most frequent causes of dermatological problems in domestic animals. The superficial mycoses caused by dermatophytes are called dermatophytosis, and they are commonly referred to as ringworm or tinea. Dermatophytes are classified in three genera, *Epidermophyton, Microsporum* and

Trichophyton, which include about 40 accepted species. However, only a few species belonging to the genera *Microsporum* and *Trichophyton* are usually the cause of dermatophytosis in domestic animals **(Cabañes, 2000)**. The transmission of dermatophytes to humans from dogs and cats usually occurs through direct contact or indirectly through fungus-bearing hair and scales from infected animals **(Careta *et al.*, 1989).** Considering the close contact between pets and their owners, especially between children and cats and dogs, these animals that are often asymptomatic carriers of dermatophytes can be important sources of infection **(Ozgur, 2001)**.

The objective of our study was isolation and identification of keratinophilic fungi from soils stressed by occurrence of animals, both in pets and in farm animals.

MATERIAL AND METHODS

Isolation of keratinophilic fungi from soil

A total of 31 samples were taken from different location in Slovakia (Table 1) during a period of March – August 2012. Keratinophilic fungi were isolated by the hair-baiting method **(Vanbreuseghem, 1952)**. For the isolation of keratinophilic fungi, only the superficial layer 5 cm of the humus horizon was used. Soil samples were poured into Petri dish (up to 5 per sample), i.e. 1 sample = 5 subsamples. Based on soil moisture, we applied 10 mL cykloheximid 500 mg/L + 50 mg/L chloramphenicol solutions. As bait were applied 5 fragments of sterilized horsehair on a Petri dish. Cultivation was carried out at 25°C for 2 – 3 months (every week check if any growth does occur on the fragments).

The pH of each soil sample (25 g) was measured using a pH meter (Sentron), after dilution in sterile distilled water 125 ml with 10 minutes of agitation.

Isolation of the fungi from colonized hair fragments

PDA (Potato Dextrose Agar) was prepared and 100 µL of the antibiotic solution (chlortetracycline/chloramphenicol) was applied on the surface and evenly spread. Solution (chlortetracycline/chloramphenicol) was prepared: 100 mg/L + 100 mg/L osmotic watter; sterilization at 120°C for 15 min. The mycelium was transferred from a fragment colonized hair into the PDA plate with antibiotic solution. We did three / four lines on plate (scars) to be

sure that the contaminating fungi will be well separated from the keratinophillic fungi. The cultivation temperature for PDA plates was 25°C, darkness, 4 – 6 days, until colonies appeared. Cultures were transferred to 2% SGA (Sabouraud Glucose Agar) for identification. These plates were incubated at 25°C for 7 days and then were used for identification.

Identification of keratinophilic and keratinolytic fungi

The identification of the resulting keratinophilic and keratinolytic fungi was based on their phenotypic characteristics according to **Domsch *et al*. (1980), Van Oorschot (1980),** and **De Hoog *et al*. (2000)**.

Table 1 Overview of studied soil samples

Soil samples	Locality/district	Occurence animals	pH soil samples
1	Nitra/Nitra	goat	7.32
2	Nitra/Nitra	cat	7.53
3	Žarnovica/Žarnovica	wild animal	6.67
4	Voznica/Žarnovica	wild animal	7.00
5	Nitra/Nitra	ducks	7.80
6	Vinodol/Nitra	chicken	6.65
7	Dubnica nad Váhom/Ilava	horse	8.36
8	Nové Zámky/Nové Zámky	dog	6.55
9	Nové Zámky/Nové Zámky	pigeon	6.37
10	Nové Zámky/Nové Zámky	chicken	6.39
11	Žemberovce/Levice	ducks	7.01
12	Doľany/Pezinok	chicken	8.58
13	Nitra/Nitra	wild animal	7.22
14	Moravany nad Váhom/Piešťany	pigeon	7.37
15	Nová Lehota/Nové Mesto nad Váhom	camel	7.89
16	Michalovce/Michalovce	chicken	7.69
17	Michalovce/Michalovce	chicken	7.05
18	Michalovce/Michalovce	chicken	7.70
19	Nové Sady/Nitra	chicken	8.39
20	Nové Sady/Nitra	rabbit	7.91
21	Veľké Zálužie/Nitra	sheep	8.14
22	Púchov/Púchov	dog	8.47
23	Suchá nad Parnou/Trnava	chicken	8.06
24	Rybníčky/Trnava	wild animal	7.76
25	Detva/Detva	wild animal	4.44
26	Nitra/Nitra	dog	7.35
27	Krškany/Levice	dog	8.28
28	Lehota/Nitra	dog	7,73
29	Trnava/Trnava	chicken	6.41
30	Trnava/Trnava	dog	7.96
31	Nitra/Nitra	horse	9,05

RESULTS AND DISCUSSION

All the collected soil samples from the observed localities (Table 1) were found to be positive for keratinophilic fungi. A total diversity of keratinophilic fungi recovered in this study accounted for 237 isolates, 9 species belonging to 7 different genera, including 5 known to be keratinolytic.

Keratinolytic fungi are associated with human and/or animal activities. Physico-chemical factors (including climatic conditions) exert a considerable impact on the compositions of keratinolytic fungal communities in keratin-rich environments. Among the factors, pH, temperature, organic carbon and nitrogen content, C:N-ratio, salts, humidity, faecal bacteria and oxygen availability appear to be the most important **(Ulfig, 2000)**. The influence of pH on the development of keratinophilic fungi in the soil is very discussed. The pH analysis from soil samples showed that keratinophilic fungi developed in a large pH margin: both acid and alkaline (4.44 – 9.05), and 77% of these samples were in alkaline pH (Table 1). **Da Silvia Pontes and Oliveira (2008)** also recorded that the keratinophilic fungi develop much better in alkaline pH.

Trichophyton ajelloi was representative and encountered in all the samples investigated (Table 2, 3). From the total of 237 isolates of keratinophilic fungi represents *T. ajelloi* 119, which represent 50.21%. *Trichophyton ajelloi* is a geophilic fungus with a worldwide distribution which may occur as a saprophytic contaminant on humans and animals, but infections in man and animals are not often reported. Only a few species belong to the genera *Microsporum* and *Trichophyton* are usually the cause of dermatophytosis in these animals. In very few cases, anthropophilic and geophilic species have been mentioned as a cause of dermatophytosis in animals. Dermatophytic species *T. ajelloi* was cited as etiological agents of dermatophytosis and was isolated from the fur of healthy cats and dogs (**Solans, 1988)**.

A human pathogen, a geophilic dermatophyte *Microsporum gypseum* was isolated from all 6 soil samples, stressed by occurence dog. *Microsporum gypseum* is geophilic fungus, and its natural habitats are soils, especially rich garden soils, where it decomposes keratinous debris **(Foil, 1993)**. *Microsporum gypseum* was isolated from samples stressed by occurrence of a dog in the number of 29 isolates (Table 2). It is known to cause infections in many species of animals as well as in humans. It is quite often isolated from dogs, accounting for between 10 and 25% of the dermatophyte infections in this species **(Lewis *et al.*, 1991)**.

Table 2 Keratinophilic fungi isolated from soils with occurrence domestic animals: cat and dog

Keratinophilic strains	Domestic animals		Total (n)
	cat (n)	dog (n)	
Metacordyceps chlamydosporia	0	3	3
*Microsporum gypseum**	0	29	29
*Myceliophthora vellerea**	2	1	3
*Pseudallescheria boydii***	0	2	2
Purpureocillium lilacinum	0	3	3
*Trichophyton ajelloi**	11	12	23

*keratinolytic, ** BSL – 2 (BioSafety Level), n – number of isolates

Table 2 draws attention to incidence of fungal opportunistic pathogen (*Pseudallescheria boydii*) in soil sample, stressed by occurrence of a dog (locality Nitra, sample 26). This substrate should be considered as a potential source of the opportunists. **Richard *et al.* (1994)** reported that in rural areas up to 80% and in an urban environment up to 20% of fungal infections of human glabrous skin may be due to close contact with pet animals. *Pseudallescheria boydii* occur in the respiratory tract where it may cause allergic reactions, sinusitis or pneumonia. The species is frequently involved in arthritis and otitis. In addition, cutaneous and ophthalmic cases have been reported. The species is an emerging opportunist in immunocompromised hosts, e.g. in leukemic, transplant (**De Hoog *et al.*, 2000**). An animal case was described by **Käufer and Weber (1977)**. Cutaneous infections have been reviewed by **Miyamoto *et al.* (1998)**.

Chrysosporium keratinophilum was detected from soil samples with occurrence of chicken, duck and *Chrysosporium queenslandicum* was detected from soil sample with occurrence of horse (Table 3). Most *Chrysosporium* species are keratinophilic fungi, living on remains of hairs and feathers in soil (**De Hoog *et al.*, 2000**). *Chrysosporium keratinophilum* and *Chrysosporium queenslandicum* are geophilic keratinolytic species (BSL – 1). *Chrysosporium keratinophilum* was repeatedly isolated from onychomycoses and superficial infections and *Chrysosporium queenslandicum* was isolated from skin and nail infections **(Reboux *et al.*, 2005)**.

Table 3 Keratinophilic fungi isolated from soils stressed by occurrence other observed animals

Keratinophilic strains	studied animals									Total (n)
	wild animal (n)	chicken (n)	rabbit (n)	pigeon (n)	goat (n)	horse (n)	duck (n)	sheep (n)	camel (n)	
Arthroderma uncinatum		3			2	1				6
*Chrysosporium keratinophilum**		6					3			9
*Chrysosporium queenslandicum**						3				3
Chrysosporium sp.	2	1		1		2				6
Metacordyceps chlamydosporia						3	10			13
*Microsporum gypseum**	3	5			3	1		6	3	21
*Myceliophthora vellerea**	6			2						8
Purpureocillium lilacinum	2	2	1		2			2		9
*Trichophyton ajelloi**	4	29	4	2	15	16	13	2	11	96
Trichophyton terrestre		3								3

*keratinolytic, n – number of isolates

CONCLUSION

This research reports the prevalence and distribution of keratinophilic fungi in soils stressed by occurrence other observed animals, both in pets and in farm animals. From these preliminary outcomes and from those older reported from our area, it is obvious that it will be necessary to continue in such a kind of study especially when the clinically important fungi are encountered in soil samples.

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