SEASONAL BEHAVIOUR OF KERATINOPHILIC FUNGI ISOLATED FROM PARKS SOIL IN MILAN

(Comportamiento estacional de hongos queratinofílicos aislados en suelos de parques en Milán)

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ABSTRACT

Soil samples collected from 10 parks in Milan were examined for the presence of keratinophilic fungi by the Orr's hair baiting technique. The organisms isolated and their frequency were as follows: ***Arthroderma uncinatum*** *and its anamorph* ***Keratinomyces ajelloi*** *were dominant and recovered from 22.5% - 43.7% and 40% - 50% of the soil samples collected in summer and winter respectively.* ***Myceliophthora vellerea*** *was isolated in 31.2% of the samples, particularly in winter,* ***Microsporum gypseum*** *in winter (20%) and* ***Trichophyton terrestre*** *(10%) in summer.* ***Chrysosporium keratinophilum*** *(16.2% in summer and 18.7% in winter)* ***C. indicum*** *(15% s- 23.7% w),* ***C. pannicola*** *(5% s - 37% w).* ***Aphanoascus fulvescens*** *was isolated in summer (23.7%). Among the cycloheximide-resistant keratinophilic fungi* ***Alternaria alternata*** *(27.5% s – 33.7% w),* ***Paecilomyces lilacinus*** *(26.2% s - 18.7% w) and* ***Acremonium strictum*** *(8.7% s - 20% w) also were observed. The distribution of the different species is discussed.*

RESUMEN

Se examinaron muestras de suelo procedentes de 10 parques de Milán para determinar la presencia de hongos queratinofilicos utilizando la técnica de Orr. Los organismos aislados y su frecuencia fueron: ***Arthroderma uncinatum*** *y su anamorfo* ***Keratinomyces ajelloi*** *fueron dominantes y se recuperaron desde un 22,5%-43,7% y 40%-50% de las muestras de suelo colectadas en verano e invierno respectivamente.****Myceliophthora vellerea*** *fue detectada en el 31,2 % de las muestras, particularmente en invierno,* ***Microsporum gypseum*** *en invierno (20%) y* ***Trichophyton terrestre*** *(10%) en verano.* ***Chrysosporium keratinophilum*** *(16.2% en verano y 18.7% en invierno)* ***C. indicum*** *(15% v - 23.7% i),* ***C. pannicola*** *(5% v - 37% i).* ***Aphanoascus fulvescens*** *fue aislado en verano (23.7%). Entre los hongos queratinofílicos resistentes a la cicloheximida, se presentó* ***Alternaria alternata*** *(27.5% v – 33.7% i),* ***Paecilomyces lilacinus*** *(26.2% v - 18.7% i) y* ***Acremonium strictum*** *(8.7% v- 20% i). Se discute la distribución de las diferentes especies.*

INTRODUCTION

Studies on the incidence of keratinophilic fungi in the Italian soils have been reported from agricultural and public gardens and parks in many countries (1-4). These fungi, according to Garrett's terminology, are a substrate group and the final colonisers in all soils (5). They have the ability to digest keratin *in vitro* (6-9) in their saprophytic state and some may invade tissues *in vivo* and provoke well-defined infections in animals and humans (10).

Several species displayed distinct limitations in their distribution; some species are ubiquitous, others peculiar to the region and others rare (11,12).

The present survey was undertaken to acquire information on the natural occurrence and seasonal distribution of keratinophilic fungi in park area and ex zoological garden soils from the metropolitan area in Milan.

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MATERIALS AND METHODS

Eighty soil samples were taken from 10 randomly selected sites (8 samples for each park) in Milan's metropolitan area. Fungi were isolated by the hair baiting technique as recorded by Vanbreuseghem (13) and modified by Orr (14). The soil was collected in late summer in the year

2001 and in winter sampling in the year 2002. Each sample was composed of soil collected in a $5m^2$ area, just below (2-3 cm) the ground surface after the removal of superficial vegetative material and other debris from the surface of the soil. A sterile glass Petri dish (2 x 150 mm) was half-filled with 40 g of soil sample and sterile human hair, horsehair and pieces of feathers (approximately 2-3 cm long), were scattered on the surface of the soil samples. The soil was then moistened with about 15 ml of sterile water containing 0.05 mg/ml chloramphenicol and cycloheximide at a 1.5 g/L concentration and incubated at room temperature. The Petri dishes were checked regularly (destilled water being added as required) over a period of 8 weeks for any growth of keratinophiles. When mycelial growth was visible on the hair under a stereoscopic microscope a part of this growth was transferred onto potato dextrose agar (PDA) and the isolates were identified.

Identifications were made directly from the growth on the baiting plates especially when the teleomorphic isolates were obtained. Fungi were identified by their macro and micromorphological characters in lacto-phenol, cotton blue or lacto-acid fuchsin 1‰.

In every soil sample each fungal species was counted only once even if it was present on all the keratinous substrate.

RESULTS

The keratinophilic fungi isolated from soil collected in the metropolitan area of Milan in summer sampling and winter sampling are listed in tables 1 and 2. As shown in tab. 1 (summer) 186 isolates of 18 species of fungi and 2 varieties classified in 8 different genera, were identified. In winter 173 isolates of 14 species of fungi and 2 varieties classified in 8 genera were identified.

The anamorphic and teleomorphic forms of some of the fungi were reported separately because the two states of these fungi did not always occur together. ***Arthroderma uncinatum*** and its anamorphic state, ***Keratinomyces ajelloi***, were predominant and both were isolated from 22.5%-43.7% and from 40%-50% of the soil samples collected in summer and in winter respectively. ***Ctenomyces serratus*** was rarely isolated but its anamorph ***Myceliophthora vellerea*** **(=*Chrysosporium asperatum*)** was frequently isolated from soil collected, particularly in winter (31.2%).

Among the geo-keratinophilic fungi, anamorphs ***Microsporum gypseum*** complex was isolated from soil collected in winter (20%) and ***Trichophyton terrestre*** (10%) from soil sampled in summer. Among the zoophilic ***Microsporum*** species, ***M. canis*** was the only zoophilic dermatophyte isolated from a soil sample at Solari's park. Species of ***Chrysosporium*** found in the order of their relative frequency were: ***C. keratinophilum*** (16.2% in summer and 18.7% in winter), ***C. indicum*** (15% and 23.7%), ***C. pannicola*** (5% and 3.7%), ***C. merdarium*** (only in winter 5%) and occasionally ***C. tropicum*** and ***C. carmichaelii***. In numerous plate the teleomorph ***Aphanoascus fulvescens*** developed in summer (23.7%). In some plates the teleomorphs ***A. clatrathus, A. reticulisporus, A. terreus*** and ***A. verrucosus*** were observed.

Among non Onygenales, the cycloheximide-resistant keratinophilic fungi the most frequently recorded species present in the majority of the soil samples collected in summer and winter were respectively ***Alternaria alternata*** (27.5% - 33.7%), ***Paecilomyces lilacinus*** (26.2% - 18.7%), ***Acremonium strictum*** (8.7% - 20%).

The fungi only occasionally recorded were ***Phialophora cyclaminis*** (6.2%), ***Clonostachys rosea*** (7.5%), ***Sepedonium chrysospermum*** (7.5%) and ***Pochonia chlamydosporia*** *(=Verticillium chlamydosporium)* (7.5%).

DISCUSSION

A distinctive feature of this survey is the dominance in the fungal spectrum of ***A. uncinatum*** and its anamorph ***K. ajelloi*** in all garden soils collected in the metropolitan area of Milan . Being a widespread soil inhabitant, this species has been recovered from soil in Belgium (15) in Italy (2-4,16), New Zealand (17) in soil from the metropolitan area of Detroit (18). Domsch *et al*. (19) list birds' nests, hair and skin scraping as sources. Rippon (20) reports it from cattle, dogs and squirrels but it is a rare pathogen of humans; human cutaneous cases are rare (21). In culture, the formation of sexual structures is inhibited at temperatures above 25°C and there is no growth at 37°C. According to Marples, the high incidence of ***K. ajelloi*** in New Zealand is related for to the populations of sheep; it seems possible that the constant increments of wool, which the soil receives, serve as an enrichment to the substrate for ***K. ajelloi***. The association of this species with strongly acid soils is suggestive for Marples (17) and its widespread dominance may be related to the pH of the substrate, to the temperature and the proportion of direct sunlight on the habitat. This author finds it curious that ***K. ajelloi*** is absent from the soil samples of the Pacific Islands. The rarity of domestic mammals in the Pacific Island could contribute to the scarcity of ***K. ajelloi*** in these soils.

Among geophilic dermatophytes whose pathogenicity is universally recognised we have isolated ***M. gypseum***, ***M. cookei*** and ***M. canis***. The anamorph ***Microsporum gypseum*** complex was isolated in eight of the ten parks in Milan. This was the most frequently found species from the soil of public gardens and parks in Rome (3) from sand boxes in Turin (4) and from park soil in Pavia (1,2). ***M. gypseum*** has been well documented as a pathogen in man and animals where the source of most infections is

Table 1 Member of Onygenales isolated from soils in the area of Milan in summer.

Localities sampled n° of samples examined : 80	A 8	B 8	C 8	D 8	E 8	F 8	G 8	H 8	I 8	L 8	Total isolates	Percentage on:	
Fungal taxa:												**samples**	**isolates**
Aphanoascus clatrathus Cano & Guarro							1				1	1.25	0.54
Aphanoascus fulvescens (Cooke) Apinis	2	1	4	3	3		1	1	1	3	19	23.75	10.21
Aphanoascus reticulisporus		1		1		1		1	1	1	6	7.5	3.23
Aphanoascus terreus (Randhawa & Sandhu) Apinis								1			1	1.25	0.54
Aphanoascus verrucosus Cano & Guarro		1						1			2	2.5	1.07
Arthroderma quadrifidum Dawson & Gentles			1								1	1.25	0.54
Arthroderma uncinatum Dawson & Gentles	3		2	3		3	1	2	1	3	18	22.5	9.68
Chrysosporium carmichaelii van Oorschot	1										1	1.25	0.54
Chrysosporium indicum (Randhawa & Sandhu) Garg	1				1	2	2	2	2	2	12	15	6.45
Chrysosporium keratinophilum (Frey) Carmichael	1			2	2	2	2	2	1	1	13	16.25	6.99
Chrysosporium pannicola (Corda) van Oorschot & Stalpers			3						1		4	5	2.15
Chrysosporium sp.	2	2	1	2	3		2	2	2	3	19	23.75	10.21
Chrysosporium tropicum Carmichael		2									2	2.5	1.07
Ctenomyces serratus Eidam			2								2	2.5	1.07
Geomyces pannorum (Link) Sigler & Carmichael var. *asperulatus* (Sigler & Carmichael) van Oorschot				1		1	1	1			4	5	2.15
Geomyces pannorum (Link) Sigler & Carmichael var. *pannorum*									1		1	1.25	0.54
Microsporum cookei Ajello	3	1									4	5	2.15
Microsporum gypseum (Bodin) Guiart & Grigorakis	3			2	2	2	3	2	2		16	20	8.6
Myceliophthora vellerea (Sacc. & Spegazzini) van Oorschot	2	2	4	1	1	3		2	2		17	21.25	9.14
Keratinomyces ajelloi Vanbreu.	4	1	3	4	4	4	4	3	4	4	35	43.75	18.82
Trichophyton terrestre Durie & Frey	2			1			3	1	1		8	10	4.3
TOTAL											**186**		

A. Porta Venezia park (ex ZOO), **B.** Piazza Sempione park, **C.** Giardini park, **D.** Indipendenza park, **E:** Insubria square park, **F.** Martini square park , **G.** Marinai d'Italia square park , **H.** Solari square park , **I.** Cantore square park, **L:** Aquileia square park.

the soil or exposure to soil-borne propagules of this fungus (22). ***M. cookei*** has occasionally been isolated in Milan and occur in the soil samples of the ex-zoological park at Porta Venezia. This widely distributed species has been isolated from soil, and hair of wild animals.

Two strains of ***M. canis*** were isolated from the park soil of Solari in Milan during the winter. This zoophilic species has exceptionally been isolated from soil by Vanbrueghem's hair baiting technique (22-25). It infects a wide variety of lower animal especially cats, and humans readily

Tab. 2. Member of Onygenales isolated from soils in the area of Milan in winter.

Localities sampled n° of samples examined : 80	A 8	B 8	C 8	D 8	E 8	F 8	G 8	H 8	I 8	L 8	Total isolates	Percentage on:	
Species isolated :												**samples**	**isolates**
Aphanoascus fulvescens (Cooke) Apinis	2					1					3	3.75	1.78
Arthroderma uncinatum Dawson & Gentles	4	4	4	4	3	3	1	4	3	2	32	40	18.93
Chrysosporium indicum (Randhawa & Sandhu) Garg	1	2	3		3	2	1	3	2	2	19	23.75	11.24
Chrysosporium keratinophilum (Frey) Carmichael	1	2		1	1	3		4	2	1	15	18.75	8.88
Chrysosporium merdarium (Link : Grev.) Carmichael			1	1		2					4	5	2.37
Chrysosporium pannicola (Corda) Oorschot & Stalpers						2			1		3	3.75	1.78
Chrysosporium tropicum Carmichael										1	1	1.25	0.59
Ctenomyces serratus Eidam									1		1	1.25	0.59
Geomyces pannorum (Link) Sigler & Carmichael var. *asperulatus* (Sigler & Carmichael) van Oorschot	1					1					2	2.5	1.88
Geomyces pannorum (Link) Sigler & Carmichael var. *pannorum*									1		1	1.25	0.94
Microsporum canis Bodin								2			2	2.5	1.18
Microsporum cookei Ajello	2							1		2	5	6.25	2.96
Microsporum gypseum (Bodin) Guiart & Grigorakis	3	1		3	2	2	2	2	1		16	20	9.47
Myceliophthora vellerea (Sacc. & Spegazzini) van Oorschot	4	3	3	2	2	4		4	2	1	25	31.25	14.79
Keratinomyces ajelloi Vanbreu.	4	4	4	4	4	4	4	4	4	4	40	50	23.67
Trichophyton terrestre Durie & Frey	2			1							3	3.75	1.78
TOTAL											**169**		

get infections through direct contact with infected animals. ***M. canis*** was isolated from soil sample collected in the garden of San Gallicano hospital in Rome (3).

The geophilic species, which may be considered as true inhabitants, it is interesting to note the highest occurrence of ***Myceliophthora vellerea*** in all soil park; the teleomorph ***Ctenomyces serratus*** was only rarely isolated. The occurrence of the anamorphic state rather than the teleomorphic state is probably a reflection of the heterothallic nature of the species. This species is world-wide and has a strong predilection for feathers but it has also been found on dung and in soil and it is considered a soil saprophytic fungus.

Special attention should be paid to the species closely related to dermatophytes, particularly ***Aphanoascus, Chrysosporium, Geomyces*** and ***Trichophyton***, which were rather common in the garden and park soils. Their potential as human or animal pathogens has been recognised.

T. terrestre is able to infect domestic pets (26) or humans (27). ***A. fulvescens*** and some ***Chrysosporium*** such as ***C. keratinophilum, C. tropicum*** and ***C. pannicola*** are the most frequent fungi isolated from domestic or wild mammals or birds (28). Van Oorschot (29) suggested that ***C. keratinophilum*** represented the anamorph of ***A. fulvescens,*** but for Cano and Guarro (30) the teleomorph of ***C. keratinophilum*** discovered by keratin-baiting of soil in Spain has been described as ***Aphanoascus keratinophilus,*** forming ascomata on cultura only on oatmeal medium. Among the non dermatophytic filamentous keratinophilic fungi recorded in this survey, ***Alternaria alternata, Paecilomyces lilacinus, Acremonium strictum*** and ***Verticillium chlamydosporium*** were frequently found in

park soil. They are common saprophytes in soil and plant debris. Cutaneous infection due to ***Alternaria*** species have been reported from several parts of the world, as the species of the genera ***Acremonium*** and ***Paecilomyces*** in human cases of cutaneous and pulmonary infections (31).

Results obtained in this survey for keratinophilic fungi in the soil from the gardens and parks of Milan, show that some genera and species are always present and that the less frequent species survive in the soil. Some seasonal variation in the presence of keratinophilic fungi in soil has been detected.

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