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Prevalence of keratinophilic fungi in soils of Bonaire (Dutch Caribbean), West Indies

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ABSTRACT

This report represents the first study of keratinophilic fungi present in soils of Bonaire in the Dutch Caribbean. Out of the 76 soil samples examined from different habitats, 39 (51.3%) were positive for the presence of keratinophilic fungi, yielding 43 isolates. The demonstration of a high frequency (31.6%) of occurrence of *Microsporum gypseum* complex represented by 16 isolates of *M. gypseum* and 8 of *M. fulvum*, two well-known species of geophilic dermatophytes in the soils of Bonaire is an important finding of public health significance. Other keratinophilic fungi were represented by *Chrysosporium* spp. (*C. indicum*-9, *C. keratinophilum*-5, *Chrysosporium* sp- 2 isolates) and *Sepedonium* sp-3 isolates.

Key words: Keratinophilic fungi, *Microsporum gypseum* complex, *Chrysosporium* sp, *Sepedonium* sp, soil, Bonaire

INTRODUCTION

Keratinophilic fungi are involved in the breakdown of keratinaceous substrates and are present in various environments worldwide. These belong to hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a variety of non-dermatophytic keratinophilic fungi (Gugnani, 2000). Most dermatophytic are anthropophilic or zoophilic in their natural habitat, while some of them like *Microsporum gypseum* and *Trichophyton terrestre* occur in soil as saprophytes and are termed geophilic (Summerbell, 2000; Padhye & Summebell, 2010). Non-dermatophytic keratinophilic fungi¸ represented predominantly by species of *Chrysosporium* and other genera, occur as saprobes in soil; some of them are potential pathogens for humans and animals (Gugnani, 2000; DeHoog et al., 2000).

Studies carried out in several countries in different parts of the world have demonstrated the occurrence of a variety of keratinophilic fungi, such as *Chrsyosporium* species and dermatophytes including *Microsporum gypseum*, *Trichophyton terrestre* and *T. ajelloi* in soil (Al-Doory, 1966; Otcenasek *et al*., 1968; Volz, 1971; Deshmukh, 1999; DeHoog *et al*., 2000; Summerbell, 2000; Padhye & Summerbell, 2010; Gugnani *et al*., 2007, 2012). Other dermatophytes known to occur infrequently or sporadically in soil in some countries are *M. cookei*, *M. vanbreuseghem*i and *T. gloriae* (DeHoog *et al*., 2000; Padhye & Summerbell, 2010). *Trichophyton simii*, *T. mentagrophytes*, *M. nanum* and *M. persicolor*, well-known zoophilic dermatophytes have also been frequently recovered from soil in some countries (Gugnani *et al*., 1967, 2007; Da Silva Pontes & Oliveira 2008; Sharma *et al*., 2008; Volz, 1971). Information on the occurrence of keratinophilic fungi in West Indies is very scanty and is limited to only three reports, one each from Abaco Island in Bahamas (Volz, 1971), Cuba (Otcenasek *et al*., 1968), and St. Kitts and Nevis (Gugnani *et al*., 2012). In view of the paucity of information on prevalence of keratinophilic fungi in West Indies, particularly in the Dutch Caribbean, it was considered of interest to investigate the occurrence of keratinophilic fungi in different kinds of soils in Bonaire.

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MATERIALS AND METHODS

Area of study

The island of Bonaire is part of the Leeward Islands in the Caribbean, situated in the southern Caribbean (12°10'N, 68°15'W) approximately 100 km north of Venezuela. Bonaire is approximately 40 km long by 11 km at its widest point. It has a land area of 288 sq Km (111 sq miles). The climate of Bonaire is semi-arid with savannah type of vegetation, consisting of dry woodland, cactus scrubs, and several species of cactus, dry grasses and typical divi-divi trees. The soil is generally sandy loam, or loam, sometimes clayey loam, and saline in many places. Temperatures are reasonably constant all year round varying from 23-32 degrees Celsius. Because of the influence of the Caribbean Sea and a north east trade wind there is little variation in temperatures. Rainfall is

scanty with an annual precipitation of about 500 mm, with an average humidity of 76%.

Collection of soil samples

A total of 76 soil samples was collected from several natural habitats in sterile zip polythene bags from a depth of 3-5 cm, with the help of a stainless steel spatula disinfected with 70% isopropyl alcohol each time before and after use. Fifty-two samples were collected during October to December 2012, while the remaining 24 were collected between May and June 2013. The samples were promptly transported to the laboratory after collection, and processed within 16-24 hours.

Processing of soil samples and identification of isolates

The well-known hair-baiting technique of Vanbreuseghem (1952) was employed for isolation of keratiophilic fungi from soil. For this, pieces of mixed adult human (male) and child (male and female) hair 0.5- 1.5 cm long, sterilized by autoclaving were spread on the soil samples (about 25g each) in disposable sterile Petri dishes (9 x 1.5 cm). Small quantities of sterile distilled water (10-15 ml) were poured on the hair-baited plates. All samples were processed individually and each soil sample was hair-baited in duplicate. The plates were incubated at room temperature. Sterile water was added periodically to provide moisture needed for fungal growth. Fungal growths appearing on hair baits after 2-4 weeks of incubation were microscopically examined and transferred to plates of Mycobiotic agar (Oxoid). The prepared Mycobiotic agar contained 0.05 mg/ml of chloramphenicol, and 0.4 mg/ml of cycloheximide to cut down contamination due to bacteria and saprophytic molds respectively. The cultures were microscopically examined to check for purity and sub-cultured to get pure cultures of the isolates. Identification of the isolates was accomplished by studying in detail the colonial and microscopic characters of the isolates and comparing with descriptions of suspected fungi in standard books and manuals (van Oorschot, 1980; DeHoog *et al.*, 2000, Ellis *et al.* 2007; Padhye & Summerbell, 2010).

RESULTS

The distribution of keratinophilic fungi recovered from different types of soils in several habitats in Bonaire is shown in Table 1. Out of 76 samples of soil examined, 39 (51.3%) were positive for kertinophilic fungi, with mixed growth of two species in 4 of the samples, thus yielding a total of 43 isolates. *Microsporum gypseum* was the most frequently recovered species, being present in 21.1% of the soil samples examined (Table 1). The isolates of *M. gypseum* originated from a variety of soil types, namely, garden, flower-beds, poultry farm, under trees, and forest. The closely related species, *M. fulvum* in the *M. gypseum* complex was present in 10.5% of the soil samples. Occurrence of *M. fulvum* was restricted to a relatively

Table 1. Distribution of keratinophilic fungi in different types of soils in Bonaire.

Habitat	Soil Type	No. of Samples Examined*	No. of isolates of different species of keratinophilic fungi *M. gypseum*	*M. fulvum*	*C. indicum*	*C. keratin.*	*Chrys.* sp.	*Sepedonium* sp	Total
Under trees	Loam	11	2	2	1	1	-	-	6
Forest	Loam with humus	10	2	2	3	-	1	1	9
Flower beds	Sandy loam	8	3	**2**	1	1	-	-	7
Grassland	Clayey loam with gravel	10	1	-	1	2	-	-	4
Garden	Clayey loam with gravel	10	4	2	-	1	-	-	7
Poultry farm	Loam with farm wastes	11	3	-	1	-	1	-	5
Debris in tree hollows	Woody debris with humus	8	1	-	2	1	-	2	5
Sandy beach	Sandy Loam	8	-	-	-	-	-	-	-
Total		76	16 (21.1%	8 (10.5%)	9 (11.8%)	5 (6.6%)	2 (3.9%)	3 (3.9%)	43

*No of samples positive for keratinophilic fungi = 39 (51.3%), 4 samples yielding mixed growth of two species
M-Microsporum, *C.*, *Chrys* –*Chrysosporium*, *kerat.-keratinophilum*
M. gypseum complex (*M. gypseum*+*M. fulvum*) = 24 (31.6%)

lesser number of soil types. Over all, the *M. gypseum* complex (*M. gypseum* + *M. fulvum*) was present in 24 (31.6%) of the soil samples examined. In the soil samples yielding M. *gypseum*/*M. fulvum*, growth of the isolate on the hair-bait was abundant. The differentiation of *M. gypseum* and *M. fulvum* was based on phenotypic characteristics. The isolates of *M. gypseum* formed buff to cinnamon-colored colonies with yellow-brown pigment on reverse. Microscopic examination demonstrated characteristic thin, rough-walled macroconidia with slightly rounded terminal ends, and truncated proximal ends **(Fig. 1)**. The isolates of *M. fulvum* formed buff to pinkish-buff colored colonies with reverse colorless to yellow-brown. Microscopically numerous, thin, rough-walled, and relatively longish and bullet shaped macroconida were observed **(Fig. 2)**, a feature typical of the species. Abundant pyriform to clavate microcondia were also seen in both species of the complex.

The other keratinophilic fungi recovered from soils were *Chrysosporium indicum* (9 isolates), *C. keratinophilum* (5 isolates), *Chrysosporium* sp. (2 isolates), and *Sepedonium* sp. (3 isolates). *Chrysosporium* species formed moderately fast growing, cream-coloured colonies, dense or powdery at the center. Conidia were hyaline, smooth-walled sessile or on short protrusions or short branches. *Chrysosporium indicum* isolates were characterized by sub-hyaline, smooth, thin-walled, obovoid, ellipsoidal conidia, frequently elongate, less than

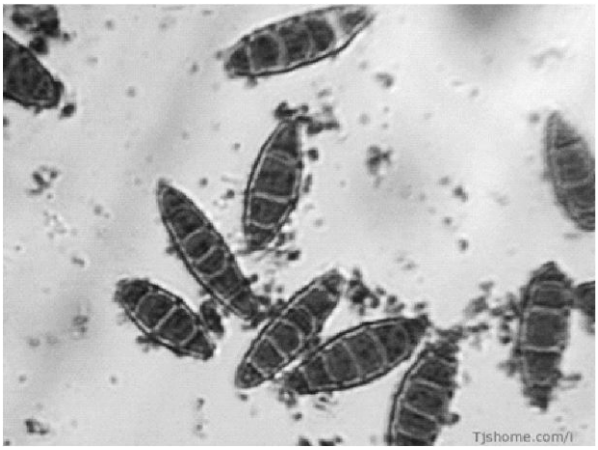

Fig. 1. Macroconidia of a soil isolate of *Microsporum gypseum* with slightly rounded terminal ends, and truncated proximal ends. Lactophenol blue mount, x 375

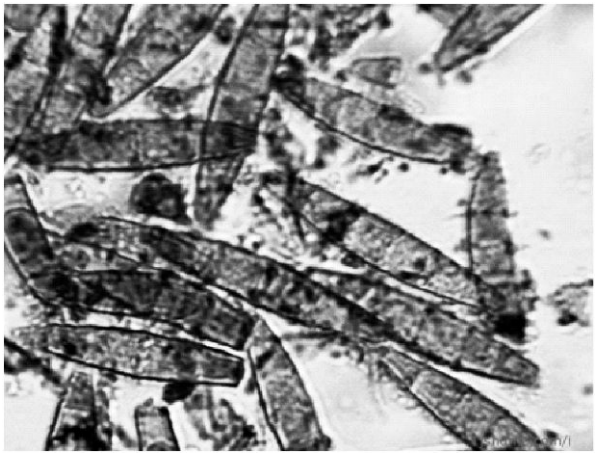

Fig. 2. Macroconidia of a soil isolate of *Microsporum fulvum*, long and bullet shaped. Lactophenol blue mount, x 425

3 µm broad with a concave upper surface; intercalary complex, conidia were also observed. The conidia of *C. keratinophilum* isolates were subhyaline, thick-walled obovoid or clavate with conspicuous basal scars, smooth-walled or slightly rough-walled. *Sepedonim* sp. formed yellowish orange coloured colonies; micrsocpically characteristic globose spiny macroconidia were observed. Several saprophytic molds, specifically species of *Aspergillus*, *Penicillium*, *Scopulariopsis* and *Paecilomyces*, grew sparsely on the hair-baits in some of the soil samples positive for *Chrysosporium* or *M. gypseum*; no attempt was made to specifically identify them. In two of the soil samples, one each from grassland and garden, *Fusarium* grew abundantly as the sole fungus on.. These two isolates were identified as *F. solani*.

DISCUSSION

The present investigation dealing with keratinophilic fungi of soils of Bonaire is the first study of its kind from the Dutch Caribbean. The results of the study provide basic original information on the prevalence of geophilic, keratinophilic fungi in Bonaire. The demonstration of a high frequency of occurrence of *Microsporum gypseum* complex (*M. gypseum* and *M. fulvum*, well-known geophilic dermatophytes) in soils of Bonaire (31.6%) is an important finding of public health significance. Occurrence of *M. gypseum* complex in soil has been reported with varying frequency in different countries. For instance, it was recovered from 15.7% of soils of St. Kitts and 40% of soils of Nevis (Gugnani *et al*., 2012), 21.8% of soils in India (Deshmukh, 1999) and from 20.8% of soils in Brazil (Da Silva Pontes & Oliveira, 2008). Regarding the relative distribution of two species of the *M. gypseum* complex, *M. gyseum* was much more frequent than *M. fulvum*, in contrast to nearly equal prevalence of the two species in soils of St. kitts and Nevis (Gugnani et al., 2012). It is also interesting to note that isolates of *M. gypseum* were recovered from a variety of soil habitats (Table 1) including one isolate from the woody debris of the hollow of a living tree, an unusual habitat for this fungus.

Human infections due *M. gypseum* and *M. fulvum* are generally infrequent or rare, probably due to low pathogenic potential of these two dermatophytes (Padhye & Summerbell, 2010). A thorough search of the literature did not reveal any information on the occurrence of human or animal infections due to dermatophytes in the Dutch Caribbean. Clinically suspected cases of dermatophytosis though not reported, possibly occur but no mycological investigations have been done. There are a few reports of dermatophytic infection from other Caribbean countries. In one study of etiological agent of tinea capitis in Jamaica during 1998-2002 (East Innis *et al*., 2006), *M. gypseum* was represented by only one isolate. In a similar study in Haiti, only one of the 55 isolates of dermatophytes was identified as *M. gypseum* (Racourt *et al.*, 2009). Also three cases of tinea capitis caused by *M. gypseum* were recorded in a study from Trinidad (Moore & Suite, 1993).

The first report of *Chrysosporium indicum* in the Caribbean was from Cuba (Otcenasek et al., 1968). In our study, *C. indicum* was the second most common keratinophilic fungus with an incidence of 13.5%, as also in a study of keratinophilic fungi of St. Kitts and Nevis with an incidence of 13.9% and 12.7% respectively in the two islands (Gugnani *et al*., 2012). It may be mentioned here that *C. indicum* has been recorded as the most abundant keratinophilic species in some soil surveys in India (Gugnani 1970; Deshmukh, 1999; Deshmukh, 2003) and in Egypt (Abdel-Fatah et al., 1982). In some other studies, *C. tropicum* has been recorded as the most common geophilic keratinophilic species (Ramesh & Hilda, 1998; Jain & Sharma, 2012). *Fusarium solani*, a well-known plant pathogen, and associated with a variety of human diseases like keratitis, onychomycosis,

eumycetoma, skin lesions and disseminated infection (Kuruvilla & Dias, 2012) has been occasionally recovered as a keratinophilic fungus from soil (Kaul & Sumbali, 2000), as is the case with two samples in our study. As expected, the soil samples from a sandy beach were negative for keratinophilic fungi.

In conclusion it may be mentioned that our study on prevalence of keratinophilic fungi in soils of several natural habitats in Bonaire is the first of its kind from the Dutch Caribbean and contributes to knowledge of ecology of this group of fungi. Prior to this, there have been no such studies from Bonaire, or any other country in the Dutch Caribbean., viz. Sint Eustatius, Saba, Aruba, Curacao, and Sint Maartan. The frequent occurrence of *M. gypseum* and *M. fulvum* in soils of Bonaire demonstrated in this study is a finding of public health importance. Comprehensive mycological investigations of skin and scalp lesions suggestive of mycotic infection in different population groups in Bonaire may lead to detection of cases of infections due to these geophilic dermatophytes, and other dermatophytic species.

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