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A PRELIMINARY STUDY ON THE OCCURRENCE OF KERATINOPHILIC FUNGI IN SOILS OF JAMAICA

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SUMMARY

This report represents the first study of keratinophilic fungi present in soils of Jamaica. Out of the 40 soil samples examined from different habitats, 30 (75%) were positive for the presence of keratinophilic fungi, yielding 36 isolates of keratinophilic fungi. *Microsporum gypseum* complex (represented by 16 isolates of *M. gypseum*, and four of *M. fulvum*) was most frequent, being present in 50% of the samples. A very high occurrence of this dermatophyte in Jamaican soil is of public health significance. The remaining isolates of keratinophilic fungi were represented by *Chrysosporium* spp (mainly *C. indicum* and *C. tropicum*) and *Sepedonium* sp.

KEYWORDS: Keratinophilic fungi; *Microsporum gypseum; M. fulvum; Chrysosporium* spp; Soil; Jamaica.

INTRODUCTION

Keratinophilic fungi are a group of fungi that colonize various keratinous substrates degrading them to components of low molecular weight. These include a variety of filamentous fungi comprising mainly hyphomycetes and several other taxonomic groups[3]. Hyphomycetes include dermatophytes and a variety of non-dermatophytic keratinophilic fungi[3,10]. Most species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur in soil as saprophytes and are termed geophilic, for example, *Microsporum gypseum* and *Trichophyton terrestre*[3,15,19]. Non-dermatophytic keratihnophilic fungi¸ including species of *Chrysosporium* and other genera of fungi, are known to occur as saprobes in soil; some of them are potential pathogens of humans and animals[3,10]. Studies on keratinophilic fungi of soils of varying habitats in different countries have demonstrated frequent occurrence of *Chrysosporium* spp and dermatophytes like *Microsporum gypseum* complex (*M. gypseum* and *M. fulvum*), *Trichophyton terrestre*, and *Trichophyton ajelloi*[1-5,10-13,19]. Other dermatophytes known to occur infrequently or sporadically in soil in some countries are *M. cookei*, *M. vanbreuseghemi*¸ and *T. gloriae*[3]. *Trichophyton simii*, *T. mentagrophytes*, *M. nanum* and *M. persicolor*, well-known zoophilic dermatophytes, have also been frequently recovered from soil in some countries[3,9,11,15,18,19,22].

Information on the prevalence of keratinophilic fungi in the West Indies is scanty and limited to only a few reports, one each from Abaco Island in Bahamas[22], Cuba[16], and St. Kitts and Nevis[12]. There is no report on isolation of keratinophilc fungi including dermatophytes from Jamaican soil. Thus it was considered of interest to investigate the occurrence of keratinophilic fungi in soils from different kinds of habitats in Jamaica.

MATERIAL AND METHODS

Area of study: Jamaica lies between the latitudes 170 and 190 N, and longitudes 760 and 7W. Jamaica is a limestone plateau, with an average elevation of about 460 m (1500 ft). The interior of the island is mountainous and peaks of over 2100 m (7000 ft) are found in the Blue Mountains, which dominate the eastern part of the island. The coastal plains are largely alluvial and the largest plains areas lie along the south coast. The climate in Jamaica is largely tropical with hot humid weather, although higher inland regions are relatively temperate. Jamaica can be divided into 14 parishes. Samples were collected from localities in some of the parishes, viz. May Pen in the parish Clarendon, New Green, Northern Caribbean University (NCU) in Mandeville in the Manchester parish in the Middlesex county, from Montago Bay in the Saint James Parish, and Beach soil in Saint Elizabeth parish in the Cornwall County, and Kingston Harbor in the parish Kingston Surrey county. These areas are warm tropical with a relative humidity of approximately 70-80%. The temperature in the greater part of the year is 22-30 ºC. Jamaica receives annual rainfall of 811 mm.

Collection of soil samples: Soil samples were collected from a depth of approximately 4-6 inches in zip polythene bags during December 2011 to February 2012, with the help of a stainless steel spatula, 6" inch long and 2" wide disinfected with 70% isopropyl alcohol each time before and after use. The polythene bags were sterilized with 70% isopropyl alcohol and left to dry for 3-4 minutes. Following this, the inside of each bag was rubbed with a sterile swab, which was then rolled on a plate of Sabouraud dextrose agar (SDA) (HiMedia Laboratories, Mumbai, India). The SDA plates were incubated at room temperature for 4-5 days. No growth of molds occurred; however, in some of the plates single bacterial colonies were observed. Thus the sterility of the zip polythene bags was

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Table1
Species of keratinophilic fungi recovered from soils of different habitats in Jamaica

Locality/ies	Habitat	No. samp. exam.	No. positive for different species						
			M. gypseum	*M. fulvum*	*C. indicum*	*C. tropicum*	*C. keratin*	*Chrys*. sp	*Seped*. sp
New Green Manchester Cedar grove, Hat field	Aviary-pigeons	3	2		2	2			
Maypen, Osbane stone	Poultry farm	2	-	-	1	-	-	-	-
Hat field, New Green Manchester, Georges Dulke, Dembigh, Clarendon	Animal habitat (Cow, goat, pig, dog, horse)	15	7	4	3	1	1	2	1
Mandville, Dorus	Garden soil	3	2	-	-	-	-	-	1
Ensula, St. Elizabeth, Wontago Bay	Beach soil	3	1	-	-	-	-	-	-
Maypen	Sugar-cane field	2	-	-	-	-	-	-	-
Maypen	Banana plantation	2	1	-	-	-	-	-	-
NCU, Bonitocres, Ingle side	Bamboo Trees	4	1		1	1			
Kingston	Harbor	2	-	-	-	-	-	-	-
NCU, Manchester	Potato field	2	1						
NCU, Manchester	Clay	2	1						
	Total	40	16 (40%)	4 (10%)	7 (17.5%)	4 (10%)	1 (2.5%)	2(5%)	2 (5%)

Samp. - samples; exam. - examined; *M*. - *Microsporum; C* - *Chrysosporium*; *Chrys*. - *Chrysopsorium*; *Seped*. - *Sepedonium*

ensured for the purpose of this study. The collected soil samples were shortly transported to St. Kitts. For determining the approximate pH of the soil samples, a small quantity of the sample was suspended in distilled water five times its volume, its pH was determined with the help of pH indicator strips, and then confirmed by testing with a pH meter. The pH of the samples varied from 5.5 to 6.2. The types of soil samples collected are shown in the table 1.

Processing of soil samples and identification of isolates: Samples were processed in the Microbiology laboratory of Windsor University School of Medicine, Cayon, St. Kitts. The well-known hair-baiting technique of VANBREUSEGHEM[21] was employed. For this, pieces of human hair 0.6 to 1.6 cm in length (sterilized by autoclaving), were spread on the soil samples in sterile Petri dishes. Small quantities of sterile distilled water (10-15 mL) were poured on the hair-baited plates. The plates were incubated at room temperature on the laboratory bench under normal light. 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 cultured on slopes of Mycobiotic agar (Neogen Corporation, Lansing, Michigan, USA) to get pure cultures. Identification of the isolates was accomplished by studying the colonial and microscopical characters of the isolates in detail and comparing them with descriptions of suspected fungi in standard books and manuals[3,7,15,20].

RESULTS

The distribution of keratinophilic fungi recovered from soil samples collected from different habitats is shown in the Table 1. Thirty of the 40 samples were positive for keratinophilic fungi with mixed growth in six of the samples, thus yielding a total of 36 isolates. *Microsporum gypseum* complex was the most frequently isolated keratinophilic fungus being present in 50% of the samples. The majority of the isolates of this species complex originated from soil samples collected from habitats of cows, goats, pigs, dogs and horses. Out of the 20 isolates of the *M. gypseum* complex, 16 of them were identified as *M. gypseum*, and the remaining four as *M. fulvum*. 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 the reverse. Microscopic examination of lactophenol blue mounds of growth demonstrated characteristic thin, rough-walled macroconidia with slightly rounded terminal ends, and truncated proximal ends (Fig. 1), characteristic of *M. gypseum.*[3,7,15]. The isolates of *M. fulvum* formed buff to pinkish-buff colored colonies with the reverse colorless to yellow-brown. Microscopically numerous, thin, rough-walled, and relatively longish and bullet shaped macroconidia (Fig. 2), characteristic of the species[3,7,15] were observed in lactophenol blue mounds of the growth. Abundant pyriform to clavate microconidia were also seen in both species of the complex.

Chrysosporium was represented by 14 isolates, seven identified as *C. indicum*, four as *C. tropicum*, one as *C. keratinophilum*, and two as unidentified *Chrysosporium* sp. Two isolates were identified as *Sepedonium* sp. *Chrysosporium* species formed moderately fast growing, cream-colored colonies, dense or powdery at the center. Conidia were hyaline, smooth-walled sessile or on short protrusions or short branches. Species differentiation was done according to characteristics of conidia[3,20]. C. *indicum* isolates were characterized by sub-hyaline, smooth, thin-walled, obovoid, ellipsoidal conidia, frequently elongated,

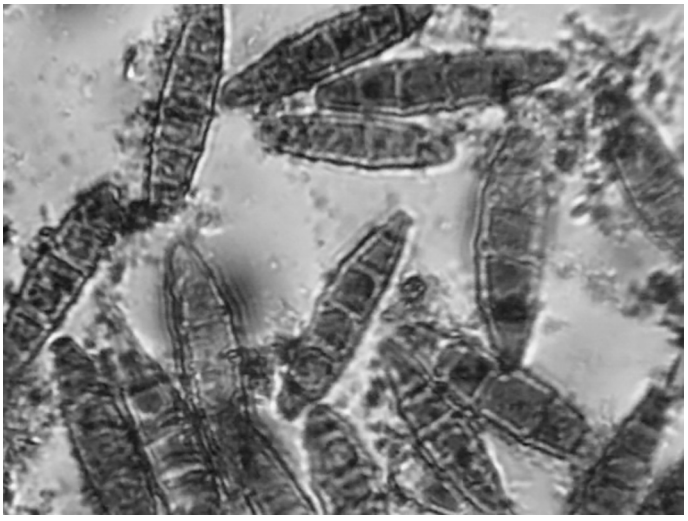

Fig. 1 - Macroconidia of a soil isolate of *M. gypseum* with slightly rounded terminal ends, and truncated proximal ends. Lactophenol blue mound, x 475

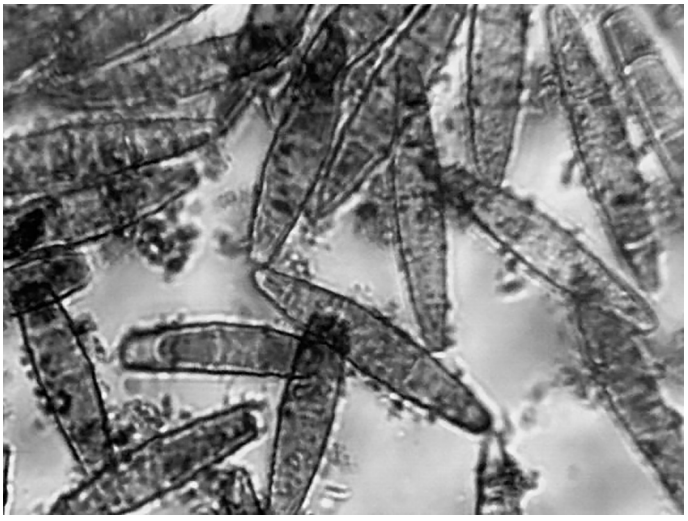

Fig. 2 - Macroconidia of a soil isolate of *M. fulvum*, longish and bullet shaped. Lactophenol blue mound, x 475

less than 3 µm broad with a concave upper surface; intercalary, conidia were also observed. *C. tropicum* isolates formed pyriform, smooth-walled conidia of 6-8 µm. The conidia of the isolate of *C. keratinophilum* were subhyaline, thick-walled obovoid with conspicuous basal scars, smooth-walled or slightly rough-walled. *Sepedonim* sp. formed yellowish orange colored colonies; microscopically characteristic globose spiny macroconidia were observed.

DISCUSSION

Investigators from several countries have reported on the occurrence of a variety of keratinophilic fungi including dermatophytes in soils of varying habitats. The predominant keratinophilic fungi reported in most studies include *Chrysosporium* spp (mainly *C. indicum*, *C. tropicum* and *C. keratinophilum*) and the dermatophyte *M. gypseum*[2-5,10-13,16,22]. There have been only a few studies on the geophilic keratinophilic fungi from the Caribbean region. One study dealt with the recovery of a few isolates of *M. gypseum*, *M. nanum*, *Trichophyton mentagrophytes*, *T. terrestre*, and *C. indicum* from soil in Abaco Island in the Commonwealth of the Bahamas[22], and another reported the isolation of *Chrysosporium indicum* and *C. evolceanui* from the soil of Cuba[16]. The third one was a comprehensive investigation on the distribution of *M. gypseum* and other keratinophilic fungi in soils of varying habitats in St. Kitts and Nevis[12]. The present study is the first of its kind from Jamaica. A very high incidence of *M. gypseum* complex demonstrated in the soils of Jamaica (50%) is a noteworthy finding of public health significance. A similar high incidence (40%) of *M. gypseum* complex has been recorded in the soils of Nevis[12]. *M. gypseum* has also been found to occur frequently in soils of Brazil, 20.8% in one survey[2], and 19.2% in another very recent study of 692 samples from different geographic regions of the country[8]. The distribution of geophilic dermatophytes is influenced by soil pH and climatic factors. In a study from Kenya, the majority of the isolates of dermatophytes including *M. gypseum* were recovered from soils with acidic pH[14]. In another study from Brazil[2], the majority of the isolates of *M. gypseum* were recovered from soils with almost neutral pH i.e. 7.0-7.09. A high prevalence of *M. gypseum* complex in soils of Nevis and Jamaica can be attributed mainly to tropical humid climate, a temperature of 22-30 ºC for most of the year and the acidic pH of the soils in these islands.

The frequent occurrence of *Chrysosporium* as a geophilic keratinophilic fungus in Jamaican soil is in agreement with that recorded in surveys of keratinophilic fungi of soils in several other countries[4,5,10-13]. Our knowledge of ecology and epidemiology of dermatophytes and the factors influencing their transmission has helped us understand better the natural history of dermatophytoses. Human infections due to the geophilic dermatophyte, *M. gypseum* complex are infrequently or rarely known, particularly in the Caribbean region. In one study of the etiological agent of tinea capitis in Jamaica during 1998-2002, *M. gypseum* was represented by only one isolate[6]. Also in a similar study in Haiti, only one of the 55 isolates of dermatophytes was identified as *M. gypseum*[17]. Comprehensive studies may reveal many more cases of skin and scalp infections due to *M. gypseum*.

RESUMO

Estudo preliminar sobre a ocorrência de fungos queratinofílicos em solos da Jamaica

Esta comunicação representa o primeiro estudo sobre fungos queratinofílicos presentes em solos da Jamaica. De 40 amostras de solo examinadas de diferentes localidades, 30 (75%) foram positivas para a presença de fungos queratinofílicos permitindo 36 isolamentos dos mesmos. O complexo *Microsporum gypseum (*representados por 16 isolamentos de *M. gypseum* e quatro de *M. fulvum)* foi o mais frequente, estando presente em 50% das amostras. A muito alta ocorrência deste dermatófito no solo da Jamaica é significante para a saúde pública. Os isolados remanescentes de fungos queratinofílicos foram representados pelo *Chrysosporium* spp (principalmente *C. indicum* e *C. tropicum*) e *Sepedonium* sp.

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