Original Article

Prevalence of keratinophilic fungi in soils of St. Kitts and Nevis

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Abstract

Introduction: Information on the prevalence of keratinophilic fungi in West Indies is scanty. Occurrence of keratinophilic fungi in soils of St. Kitts and Nevis has not been investigated previously.

Methodology: The prevalence of keratinophilic fungi was investigated in 108 samples of soils of varying habitats from St. Kitts and 55 such samples from Nevis by hair-baiting technique. Fungal growths appearing on the hair baits after four to eight weeks of incubation at room temperature were microscopically examined and cultured on mycological media. Cultures were identified on the basis of colonial and microscopic features.

Results: Forty-nine (45%) of the samples from St. Kitts and 38 (69%) from Nevis were positive for keratinophilic fungi. *Microsporum gypseum* complex, a well-known geophilic dermatophyte, was the most frequently recovered species being present in 15.7% of soils of St. Kitts and 40% of soils of Nevis. The next commonest species recovered was *Chrysosporium indicum*, represented by 15 (13.9%) isolates from St. Kitts and seven (12.7%) isolates from Nevis. Other infrequently isolated keratinophilic species included *Chrysosporium tropicum*, *Chrysosporium keratinophilum*, and unidentified *Chrysosporium* species.

Conclusions: This study is the first of its kind in the islands of St. Kitts and Nevis. A high incidence of *M. gypseum* complex in the soil of these islands is a noteworthy finding of public health significance.

Key words: *Microsporum gypseum*; *Chrysosporium* spp; soil; St. Kitts; Nevis

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Introduction

Keratinophilic fungi are involved in the breakdown of keratinaceous substrates and are present in the environment worldwide. These belong to hyphomycetes and several other taxonomic groups. Hyphomycetes include dermatophytes and a variety of non-dermatohytic keratinophilic fungi [1]. 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* [1]. 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 for humans and animals [1-2]. Surveys conducted 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 mentagrophytes*, *T. terrestre* and *T. simii*, in soils of varying habitats [1-7].

Information on the prevalence of keratinophilic fungi in West Indies is scanty and is limited to only two reports, one from Abaco Island in Bahamas [8], and the other from Cuba [9]. There is no report of isolation of keratinophilc fungi including dermatophytes from soil in the twin islands of St. Kitts and Nevis, although cases of dermatophytosis in humans and animals are clinically suspected. Thus it was considered of interest to investigate the occurrence of keratinophilic fungi in soils from different kinds of habitats in St. Kitts and Nevis.

Methodology

Location, land area and climate of the islands

West Indies is a large group of islands that separate the Caribbean Sea from the Atlantic Ocean. These comprise three main island groups, specifically Bahamas (north), Greater Antilles (central), and Lesser Antilles (southeast). The Lesser Antilles are comprised of two subgroups, the Leeward Islands and Windward islands. The islands of St Kitts and Nevis, a part of the Leeward Islands, are volcanic in origin

Table 1. Prevalence of different species of keratinophilic fungi in soil in St. Kitts

Type of soil	No. of samples examined	No. of isolates of different species recovered				
		M. gypseum complex	***C. indicum***	***C. tropicum***	***C. kertinophilum***	***Chrysosporium* spp.**
Beaches along Atlantic Ocean and the Caribbean Ocean	17	2	2	1	0	0
Black rock soil	10	1	0	0	2	3
Cultivated fields	14	3	3	0	1	1
Grasslands and playground	15	2	2	1	0	2
Gardens and public grounds	9	0	4	1	1	2
Garden plant soil	8	2	1	1	0	0
Flower beds	7	1	1	1	O	1
Animal habitats (dog, cat)	12	3	1	O	0	2
Soil under and near trees	10	3	1	1	0	0
Roadside soil	6	0	0	0	0	0
Total	108	17(15.7)	15(13.9)	6(5.6%)	4(3.70%	11(10.2%)

and are located in the northern part of the Lesser Antilles chain of islands in the Eastern Caribbean. St Kitts is located at 17°15' North latitude and 62°45' West longitude while Nevis is located at 17° North latitude and 61°30' longitude. St Kitts has an area of about 69 square miles while the area of Nevis is 36 square miles. Both islands have a tropical climate with temperatures ranging from 17°C to 33°C. The temperature averages about 26°C and average humidity is 71.5%. Cooling breezes from the northeast trade winds blow throughout most of the year. The average seawater temperature is 27°C. Average annual rainfall is 55 inches in St Kitts and 48 inches in Nevis.

Collection and processing of soil samples

Soil samples were collected in sterile, tightly closed polythene bags. The samples were processed in the laboratory within a day or stored 4°C until processed within two or three days. The hair-baiting technique of Vanbreuseghem [10] was employed for selective isolation of keratinophilic fungi. Approximately 50 g of each sample was transferred to a sterile Petri plate, and pieces of sterilized adult or child hair were spread over the soil in the plate. The hair-baited soils in the plates were moistened with sterile distilled water and incubated at room temperature (22°C to 30°C) for four to eight weeks. The soil samples were periodically moistened with small quantities of sterile distilled water.

Isolation and identification of keratinophilic fungi

The soil samples were regularly checked for fungal growths on the hair baits. Portions of the growth were microscopically examined for the presence of keratinophilic fungi, and loopfuls were transferred to slopes/plates of Sabouraud dextrose agar (HiMedia Laboratories Mumbai, India)

supplemented with 0.05 mg/L chloramphenicol (CDH, New Delhi, India) and 0.5 mg/L cycloheximide (Sigma, St Louis, MO, USA), and Mycobiotic agar (Neogen Corporation, Lansing, Michigan, USA). The latter medium also contained chloramphenicol and cycloheximide at the stated concentrations in the dehydrated product. The inoculated slopes/plates were incubated at room temperature for five to ten days following which the cultures were microscopically examined to check for purity and sub-cultured to get pure cultures. The purified fungal isolates were identified up to species level as far as possible by a detailed study of their colonial characters and microscopic morphology in lacto-phenol blue (LB) mounts, and comparison of their characteristics with the descriptions of the species in the standard books and manuals [2,11,12].

Results

The data on species of keratinophilic fungi isolated from soils of St. Kitts and Nevis are presented in Tables 1 and 2. Out of a total of 108 samples of soils of different types examined from St. Kitts, 49 (45%) were positive for keratinophilic fungi yielding 53 isolates (Table 1) with mixed growth of two species being obtained from three of the samples. Out of 55 samples of different types examined from Nevis, 38 (69.0%) samples were positive for growth of keratinophilic fungi with mixed growth in three samples, thus yielding 41 isolates (Table 2). *Microsporum gypseum* complex, a well-known geophilic dermatophyte, was the most frequently recovered species being present in 15.7% of the soils of St. Kitts and 40% of soils of Nevis. It grew luxuriantly on hair bait in many of the soil samples. The other species recovered included *Chrysosporium indicum*, represented by 15 (13.9%) isolates from St. Kitts and 7 (12.7%) isolates from Nevis. In addition, a few isolates of *C. tropicum*, *C. keratinophilum* and several unidentified isolates of *Chrysosporium* spp. were recovered from soils in both islands (Tables 1 and 2). Several saprophytic molds, specifically species of *Aspergillus*, *Penicillium* and *Fusarium*, also grew on the hair-baits in some of the soil samples. No attempt was made to specifically identify these molds, as they are generally not considered kertinophilic species; thus their frequency of isolation is not recorded in the tables. However, three of the *Fusarium* isolates, which grew luxuriantly on hair-baits, were identified as *F. solani*. Most of the isolates of *M. gypseum* in St. Kitts originated from soil samples from animal (dog, cat) habitats, cultivated fields, and from the base of garden plants and trees. Soil samples yielding *M. gypseum* in Nevis were mostly from rainforest, vegetable garden, and goat habitats. The incidence of *M. gypseum* was much higher in Nevis soil samples than in soil samples from St. Kitts.

As shown in Table 1, the soil samples from the sandy beaches were mostly negative for keratinophilic fungi. The two isolates of *M. gypseum* complex from beach soil in St. Kitts were collected from the sites in Shipwreck Beach, one of the beaches frequently visited by natives as well tourists in the island. Of the 17 isolates of *M. gypseum* complex from St. Kitts, 8 were *M. gypseum* and 9 were *M. fulvum*. Likewise, of the 22 isolates of the complex from Nevis, 13 were *M. gypseum* and nine were *M. fulvum*. The isolates of *M. gypseum* formed buff to cinnamon-coloured colonies with yellow-brown pigment on reverse. Microscopic examination demonstrated characteristic thin, rough-walled macroconidia with slightly rounded terminal rounded ends, and truncated proximal proximal ends. Clavate microconidia were also abundant. The isolates of *M. fulvum* formed buff to pinkish-buff coloured colonies with reverse colourless to yellow-brown. Microscopically numerous, thin, rough-walled, and relatively longish and bullet shaped macroconida were observed, a feature typical of the species. Abundant pyriform to clavate microcondia were also seen. *Chrysosporium* species formed moderately fast growing, cream-coloured colonies, dense or powdery at the centre. Conidia were hyaline, smooth-walled sessile or on short protrusions or short branches. *Chrysosporium indicum* isolates were characterized by subhyaline, smooth, thin-walled, obovoid, ellipsoidal conidia, frequently elongate with a concave upper surface, Intercalary conidia were also observed. *C. tropicum* isolates showed characteristic subhyaline, smooth to slightly thick-walled obovoid to clavate conidia with a truncate base. The conidia of isolates of *C. keratinophilum* showed subhyaline, thick-walled obovoid or clavate with conspicuous basal scars.

Table 2. Prevalence of different species of keratinophilic fungi in soil in Nevis

Type of soil	No. of samples examined	No. of isolates of different species recovered:				
		M. gypseum complex	***C. indicum***	***C. tropicum***	***C. kertinophilum***	***Chrysosporium* spp.**
Under trees in rainforest	10	5	1	1	0	2
Near spring in rainforest	9	1	2	0	1	1
Under trees of curry leaves, golden apple, pumpkin and palm	16	9	1	0	0	2
Grassland	7	1	1	0	0	1
Humus	3	0	0	1	0	1
Goat habitat	4	4	0	0	0	0
Flower beds	6	2	2	1	0	0
Total	55	22(40%)	7(12.7%)	3(5.5%)	1(1.8%)	7(12.7%)

Discussion

Investigators from several countries have reported on the occurrence of a variety of keratiophilic 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. keratiophilum*) and the dermatophyte *M. gypseum* [2,11]. Other dermatophyte species known to occur sporadically in soil are *M. cookei*, *M. vanbreuseghem*i¸ *T. terresrte* and *T. gloriae* [2,5,11]. *T. simii*, *T. mentagrophytes*, *M. nanum* and *M. persicolor*, well-known zoophilic dermatophytes, have also been frequently recovered from soil in some countries [4,10,15]. To our knowledge, there have been only two publications on the keratnophilic fungi from Caribbean soil. One deals with the recovery of a few isolates of *M. gypseum* and *C. indicum* from Abaco Island in the Commonwealth of the Bahamas [8], and the other concerns the isolation of *Chrysosporium indicum* and *C. evoluceanui* from the soil of Cuba [9]. The present investigation of a large number of soil samples from St. Kitts and Nevis for the presence of keratinophilic fungi represents the first study of its kind in the Lesser Antilles in the West Indies. A high incidence of *M. gypseum* complex (*M. gypseum* and *M. fulvum*) in soil in the islands, *i.e.* 15.7% in St. Kitts and 40% in Nevis, is a noteworthy finding of public health significance. Mycological studies of cases of dermatophytosis in St. Kitts and Nevis have not been performed; thus information on the occurrence of human infections due to these or other dermatophyte species in the islands is lacking. However, it may be mentioned here that two fungal isolates from ringworm lesions in dogs from the veterinary clinic in Ross were identified as *M. gypseum*. Thus cases of human infection due to *M. gypseum* and *M. fulvum*, though not detected so far, possibly occur in St. Kitts and Nevis. *M. gypseum* complex has been reported with varying frequency in different countries. This dermatophyte was recovered from 31% of samples of soil and bat guano in Panama and the Canal zone [15], 15.2% of soil samples in Thailand [16], 20.6% of soils in the glacier bank soils of the Kashmir Valley, India [17], from 14% of soils of school and college playgrounds in Jaipur, India [18], and 20.8% from urban soils of João, Pessoa, Brazil [19].

Our survey constitutes one of the few studies of geophilic keratinophilic fungi that have attempted to differentiate between *M. gypseum* and *M. fulvum* in the isolates of *M. gypseum* complex. Occurrence of *C. indicum* in the Caribbean has been earlier recorded from Cuba [9]. In our study, *C. indicum* was the second commonest keratinophilic fungus with an incidence of 12% in St. Kitts and 5.5% in Nevis. It may be mentioned here that *C. indicum* has been recorded as the most abundant keratinophilic species in some soil surveys in India [6,7]. Both *M. gypseum* and *C. indicum* have been recorded earlier from Bahamas [8]. *Fusarium solani* has been occasionally recovered as a keratinophilic fungus from soil [20], as was the case with three samples in the present study. A relatively much higher prevalence of

keratinophilic fungi in soil samples from Nevis (69%) than in those from St. Kitts (45%) may due to the fact that the samples from St. Kitts included several samples from sandy beaches, which are usually not a favourable habitat for the growth of keratinophilic fungi. There have not been any studies so far on the prevalence of geophilic keratinophilc fungi in the group of islands in the Lesser Antilles, including St. Kitts and Nevis.

There is also paucity of information on the occurrence of dermatophytes in the environment, and human and animal infections caused by them in St. Kitts and Nevis, and other countries in the East Caribbean. It is hoped that this study would stimulate further work in this area to understand the ecology of human infections caused by dermatophytes and related keratinophilic fungi in the region.

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References

1. Gugnani HC (2000) Non-dermatophytic filamentous keratinophilic fungi and their role in human infections. In Kushwaha RKS, Guarro J, editors. Biology of Dermatophytes and other Keratinophilic Fungi. Revista Iberoamericana de Micologia Apartado, Bilbao, Spain. 109-114.
2. DeHoog GS, Guarro J, Gene J, Figueras MJ (2000) Atlas of Clinical Fungi. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands/Universitat Rovirai Virgili. Spain.
3. Al-Doory Y (1966) The occurrence of keratinophilic fungi in Texas soil. Mycopathologia 33: 105-112.
4. Gugnani HC, Shrivastav JB, Gupta NP (1967) Occurrence of *Athroderma simii* in soil and on hair of small mammals. Sabouraudia 6: 77-80.
5. Summerbell RC (2000) Form and function in the evolution of dermatophytes. In Kushwaha RKS, Guarro J, Editors Biology of Dermatophytes and other Keratinophilic Fungi.Revista Iberoamericana de Micología Bilbao, Spain. 30-43.
6. Deshmukh SK (1999) Keratinophilic fungi isolated from soils of Mumbai, India. Mycopathologia; 146: 115-116.
7. Deshmukh SK, Verekar SA (2006) The occurrence of dermatophytes and other keratinophilic fungi from the soils of Himachal Pradesh (India). Czech Myco 58: 117-124.
8. Volz PA (1971) A preliminary study of the keratinophilic fungi from Abaco Island, The Bahamas. Mycopathologia 43: 3-4.
9. Otcenasek M, Hubalek Z, Devorak J, Kunert J (1968) *Chrysosporium indicum*-like and *Chrysosporium evoluceanui* from the soil of Cuba. Mykosen 11: 19-24.
10. Vanbreuseghem R (1952) Technique biologique pour I' isolement des dermatophytes du sol. Ann Soc Beige Med Trop 32: 173-178.
11. Padhye AA, Summerbell RC Dermatophytes (2010) In Merz WG, Hay J editors. Topley & Wilsons Microbiology and Microbial Infections Vol. 6 Medical Mycology, London: Arnold. 220-239.
12. Ellis D, Davis S, Alexiou H, Handke R, Bartley R (2007) Descriptions of Medical Fungi, Mycology Unit, Women's and Children's Hospital, and School of Molecular and Biomedical Science, University of Adelaide. University of Adelaide: Adlelaide: 198 p.
13. Gugnani HC, Paliwal-Joshi A, Rahman H, Padhye AA, Singh TS K, Das TK, Khanal B, Bajaj R, Rao S, Chukhani R (2007) Occurrence of pathogenic fungi in soil of burrows of rats and of other sites in bamboo plantations in India and Nepal. Mycoses 50: 507-511.
14. Sharma R, Wolfgang PB; Rajak RC, Yvonne, G (2008) Molecular detection of *Microsporum persicolor* in soil suggesting widespread dispersal in central India. Medical Mycology 46: 67-73.
15. Taylor R, Shacklette MH, Kelley HB (1962) Isolation of *Histoplasma capsulatum* and *Microsporum gypseum* from soil and bat guano in Panama and the Canal Zone. Am J Trop Med Hyg11: 790-795.
16. Taylor R (1962) Occurrence of *Microsporum gypseum* in Thailand soils. Mycologia 58: 648-649.
17. Deshmukh SK (2002) Incidence of dermatophytes and other keratinophilic fungi in the glacier bank soils of the Kashmir valley, India. Mycologia 16: 165-167.
18. Sharma M and Sharma M (2010) Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, India. Afr J Microbiol Res 24: 2647-2654.
19. Da Silva Pontes ZBV and Oliveira AC (2008) Dermatophytes from urban soils in João Pessoa, Paraíba, Brazil. Revista Argentina de Microbiología 40: 161-163.
20. Kaul S and Sumbali G (2000) Keratinophilic fungi from poultry farm soils of Jammu, India. Mycologist 42: 89-91.

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