Mycoscience 41: 259–262, 2000

Short Communication

Fungi colonizing hair-baits from three coastal beaches of Mexico

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Accepted for publication 22 March 2000

The abundance of hair-bait colonizing fungi was investigated on the beaches of Caracoles, Mocambo, and Icacos, located on the coasts of the Caribbean Sea, Gulf of Mexico and Pacific Ocean, respectively. On each beach a sample of sandy soil was collected. The samples were analyzed by two selective isolation methods for keratinophilic fungi resulting in a total of 544 occurrences. A total of 17 species was found, of which 4 were ascomycetes and 13 hyphomycetes. *Gymnascella dankaliensis* and *Aspergillus terreus* were the most abundant. *Arthroderma curreyi* and *Chrysosporium tropicum* were found in low percentages in this survey. From the three beaches sampled, Icacos beach, on the Pacific Ocean coast had the highest number of isolated species.

Key Words——Ascomycetes; hair-baiting method; Mexico; sand beaches.

The micromycetes that inhabit the sand of the seacoastal beaches are important ecologically because they contribute to the process of remineralization of the nutrients in this environment (Kohlmeyer and Kohlmeyer, 1979). The keratinophilic fungi are those that degrade substrates with keratin (Hawksworth et al., 1995). These fungi are principally deuteromycetes, and where the sexual states are known they usually belong to the Onygenales (Ascomycetes) (Currah, 1987). These fungi have been isolated from sandy beaches (Dabrowa et al., 1964; Kishimoto and Baker, 1969; Bergen and Wagner-Merner, 1977; Crespo et al., 1985), where they are generally saprobic, but species pathogenic or potentially pathogenic to man also have been found (Kishimoto and Baker, 1969). This study was undertaken to assess the prevalence of potential pathogens in selected beaches in Mexico. This paper represents a preliminary report on the abundance of hair-colonizing fungi found in three Mexican beaches of touristic importance.

Materials and Methods

Areas of study Three touristic beaches were studied based on the vast numbers of people that visit them every year; these were Caracoles, Cancun, State of Quintana Roo (21° 08′19″N, 86°45′W); Mocambo, State of Veracruz (19° 08′56″N, 96° 05′44″W), and Icacos, Acapulco Bay, State of Guerrero (16°50′N, 99°56′W) on the coasts of the Caribbean Sea, Gulf of Mexico and Pacific Ocean, respectively. These beaches were sampled from August 3–5, 1996, during low tide.

Collection of materials and processing of the samples In the mesobeach (a zone continually covered by water and exposed to the air in a rythmic and alternate manner) of each of the beaches 10 quadrants 1m^2 were randomly drawn and 5 samples of 200 g were collected with sterile plastic spoons from each one, at a depth 2–5 cm, and placed in sterile plastic bags. The samples were processed in the laboratory on the same day or were stored at 4°C until the following day. In the laboratory the samples were analyzed by two different methods. In the first method, incubation of hair-baits in a damp chamber (Orr, 1969), each sample of sand was subdivided into triplicate portions of aproximately 50 g each; these were placed in Petri dishes on two circular sterile filter paper discs (Whatman No. 1). Each sample was then baited with 25 sterile 5 cm long filaments of children's hair and moistened with 10 ml of a sterile distilled water solution containing antibiotics (SDWA) (cycloheximide 2 mg/ml, chloramphenicol 1 mg/ml, penicillin G 500 μg/ml, streptomycin 300 μg/ml). A positive control consisted of sterile sand moistened with 5 ml of a sterile water suspension (SDW) of conidia from *Microsporum gypseum* (100 conidia/ml) and a negative control with sterile sand moistened only with SDW. Each control was baited with 25 pieces of sterile hair. All the Petri dishes were incubated for 6 wk at 30°C and observed on a daily basis. The Petri dishes were periodically moistened with 10 ml of SDW to avoid dehydration. Fungal colonization of the hair bait was checked with a stereoscopic microscope. From each Petri dish 2 hair filaments colonized by fungi were taken and transfered to

a Petri dish with fresh culture medium. The culture media used were Sabouraud agar (SDA) (Difco) [36 g, (with the antibiotics chloramphenicol 1 mg/ml, penicillin 500 μg/ml, streptomycin 300 μg/ml, cycloheximide 2 mg/ml added after autoclaving), distilled water 1 L] (Stockdale, 1971), and dermatophyte test medium (DTM): mycobiotic agar (Difco) [36 g, phenol red (Sigma) 40 ml, hydrochloric acid (HCl) 0.8 N 6 ml, distilled water 1 L] (Rebel and Taplin, 1970). The plates were examined every day with a stereoscopic microscope and each one that developed a different colony was transfered to a slant of SDA without antibiotics for later identification. For the second method, direct inoculation of selective agar plates with sandy soil, the method of Stockdale (1971) was followed. Triplicate Petri plates were prepared with the same culture media used for the first method. The surface of the culture media was inoculated with sand from each sample and the plates were incubated for 6 wk at 30°C. A positive control was made with sterile sand moistened with 5 ml of SDW suspension of conidia from *M. gypseum* (100 conidia/ml) and a negative control with sterile sand. At the end of the incubation period, each different colony was transferred to a tube with SDA medium without antibiotics for later identification. In order to determine qualitatively *in vitro* the ability of the fungi to colonize the hair baits, 10 pieces of sterile children's hair 5 cm in length were placed on plates of Sabouraud agar and each plate was inoculated with one of the different fungi that was isolated by the previous methods. The colonization of the hairs was interpreted using the model proposed by Filipello-Marchisio (1986). Material for SEM was prepared following the procedure in Goh and Hanlin (1994). Cultures and slides of the isolated species are deposited in the collection of fungi of the Instituto de Biología, Universidad Nacional Autónoma de México (MEXU).

Analyses of the data In order to compare the relative abundance of the species and to distinguish the dominant fungi, the total abundance of each species is presented in descending order. The abundance of species is expressed as the number of individual isolations of a species and the percentage abundance as the number of isolations of a species divided by the total number of recovered isolations of the sample (Bills and Polishook, 1994).

Results and Discussion

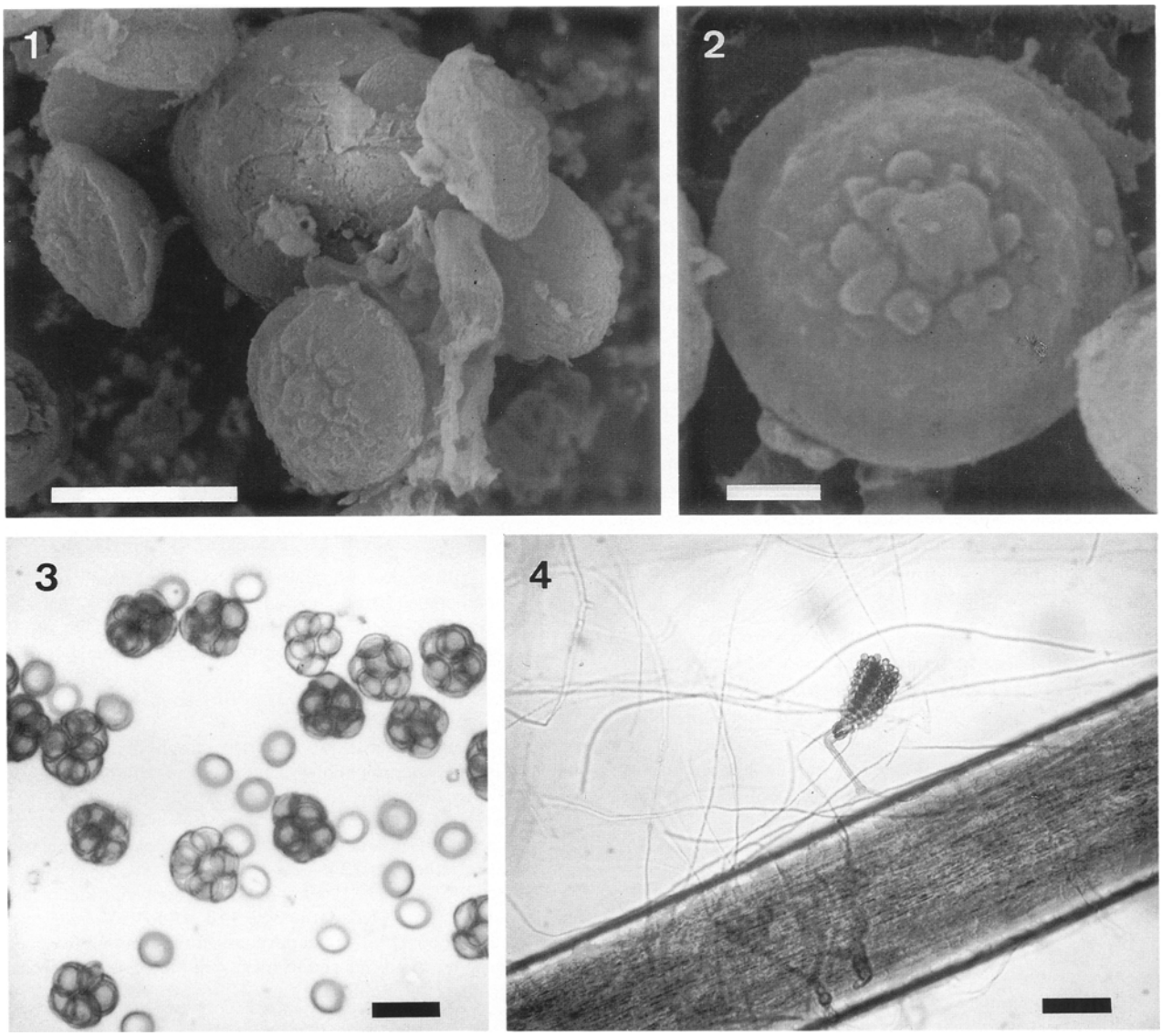
From the samples of sandy soil from the beaches of Caracoles, Mocambo, and Icacos a total of 544 occurrences was obtained (Table 1). A total of 17 fungi was found, of which 4 belonged to the ascomycetes and 13 to the hyphomycetes. The species *Gymnascella dankaliensis* (Castell.) Currah and *Aspergillus terreus* Thom were the most abundant of the three samples with an abundance percentage of 36.2% and 22.6% of the total of occurrences, respectively. The species that were isolated only once (0.2% of the total occurrences) were *Arthroderma curreyi* Berk., *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Fusarium solani* (Mart.) Sacc., and *Scopulariopsis parvula* F. J. Morton & G. Sm. The dominant fungi that were found in one or more of the

Table 1. Abundance of keratinophilic fungi isolated from Caracoles Beach, Cancun (CCQ), Mocambo Beach, Veracruz (MBV) and Icacos Beach, Acapulco (IAG).

Fungi	Samples			Total abundance	Percentage abundance	S	P
	CCQ	MBV	IAG				
Gymnascella dankaliensis	0	116	81	197	36.2	1	0
Aspergillus terreus	28	43	52	123	22.6	1	0
Gymnoascus sp.	0	46	31	77	14.1	1	0
Aspergillus fumigatus	0	18	34	52	9.5	1	0
Aspergillus flavo-furcatis	0	0	46	46	8.4	1	0
Aspergillus recurvatus	0	0	13	13	2.4	0	1
Scopulariopsis brumptii	0	9	0	9	1.6	1	0
Fusarium semitectum	0	8	0	8	1.5	1	0
Chrysosporium tropicum	6	0	0	6	1.1	0	1
Scopulariopsis carbonaria	0	0	2	2	0.4	1	0
Geotrichum candidum	2	0	0	2	0.4	1	0
Microascus cinereus	0	0	2	2	0.4	1	0
Acremonium murorum	0	0	2	2	0.4	1	0
Arthroderma curreyi	1	0	0	1	0.2	0	1
Fusarium solani	0	1	0	1	0.2	1	0
Scopulariopsis brevicaulis	1	0	0	1	0.2	1	0
Aspergillus auricomus	0	1	0	1	0.2	1	0
Scopulariopsis parvula	1	0	0	1	0.2	1	0
Totals	39	242	263	544	100		

S=surface hair erosion, P=radial hair penetration

samples, with an abundance above 5% of total occurrences, were *Gymnascella dankaliensis*, *Gymnoascus* sp., *Aspergillus terreus*, *A. fumigatus* Fresen., and *A. flavo-furcatis* Bat. & H. Maia. The samples of sand from the Icacos beach had the highest number of species, whereas the samples from the Caracoles beach had the lowest numbers. The results of the positive control showed abundant growth of *Microsporum gypseum* (E. Bodin) Guiart & Grigoraki, in the hair fragments placed on the sand. The aspergilli in the samples from the three beaches were predominant. The low number of species that was isolated in this study probably was due to the selectivity of the isolation methods employed and to the ecological characteristics of the habitat at the time of sampling. Various types of fungi are found mixed in the sand of beaches, which is why two selective methods were followed in this study in order to retrieve the keratinophilic fungi from the samples. In addition, the beaches were sampled during the period of the most tourist visits. The presence of keratinophilic fungi depends mainly on the enrichment of the soil due to human activities (Soo-Hoo, 1991). Also, these can tolerate up to 12% sodium chloride concentrations and their propagules can remain alive in sand for up to 6 mo (Kane and Fisher, 1975). However, only two keratinolytic species, *Arthroderma curreyi* and *Chrysosporium tropicum* J. W. Carmich. were isolated, with a low abundance value. Pugh (1962) found that *Arthroderma curreyi* was the most frequent species in the coastal beaches of England. The physicochemical nature of the organic residues, the



Figs. 1–3. *Gymnascella dankaliensis*. 1. Mature ascus with free ascospores. 2. Oblate ascospore with polar and equatorial thickenings and irregular exterior wall surface. 3. Asci containing ascospores and free ascospores.
Fig. 4. Conidiophore of *Aspergillus recurvatus* with conidia and boring hypha on hair filament.
Scales: 1, 5 μm; 2, 1 μm; 3–4, 10 μm

climatic conditions, the contamination and the management of the ecosystem affect the functioning and the structure of the communities of fungi (Dighton,1995). Notable in this study was the elevated number of occurrences that was obtained of *Gymnascella dankaliensis* in the beach at Mocambo and also at Icacos (Table 1; Figs. 1–3). This species is cosmopolitan and is principally coprophilous (Currah, 1985). In addition, many species in the family Gymnoascaceae (Onygenales) tolerate high concentrations of cycloheximide (Kuehn and Orr, 1962). The ecological factors that favor the dominance of *G. dankaliensis* in the beaches at Mocambo and Icacos are unknown. Another interesting isolate was *Aspergillus recurvatus* Raper & Fennell which forms a boring hypha on the surface of the hair (Fig. 4). This saprobic species was described first from a desert area of California from lizard dung (Raper and Fennell, 1977) and the sand beaches of Enewetak Atoll, Marshall Islands (Dunn and Baker, 1983). Also isolated was *Aspergillus fumigatus*, which is considered a potential pathogen because it can cause allergies and invasive infections (Kwon-Chung and Bennet, 1992). This species has been isolated from beaches in California, Florida and Hawaii (Kishimoto and Baker, 1969; Dabrowa et al., 1964; Bergen and Wagner-Merner, 1977). In this study various species of *Scopulariopsis* were isolated, including those producing the *Microascus* sexual state, such as *M. cinereus* (Émile-Weil & L. Gaudin) Curzi. Some species of *Scopulariopsis* have been isolated from human sources, mainly from skin or nails (Morton and Smith, 1963). All of the fungi isolated in this study colonized *in vitro* the hair baits, although only *Arthroderma curreyi* and *Chrysosporium tropicum* are potentially pathogenic to man (Simpanya and Baxter, 1996). On the basis of this preliminary study it does not appear that pathogenic species represent a significant threat to the humans that frequent these beaches. More research is necessary, however, on the keratinophilic fungi that live in the sands of Mexican coastal beaches in order to fully identity them and to understand their ecology.

Acknowledgements—We are grateful to Francisca Hern á ndez and Ruben López-Martínez, Laboratorio de Micología Médica, Facultad de Medicina, Universidad Nacional Autónoma de México, for valuable advice. This study was funded by Dirección General de Asuntos del Personal Académico (DGAPA Proyect IN-203 895). Some aspects of this study were also supported by CONACYT/NSF Mexico-US Cooperative Project INT-E120.0274.

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