THE KERATINOPHILIC FUNGI OF TAIWAN

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Abstract: Soil from the northern, eastern and southern areas of Taiwan were studied for pathogenic and nonpathogenic keratinophilic fungi. In addition, soil samples from the playground area of 250 primary schools throughout Taiwan were obtained for the study. Most of the isolated species were nonpathogens but additional pathogenic fungi not previously reported in nature from Taiwan were also isolated.

INTRODUCTION

A total of 567 soil samples collected throughout Taiwan from elementary schools, rural and metropolitan areas were screened for the purpose of identifying pathogenic and nonpathogenic keratinophilic fungi. Similar ecological investigations have been conducted in many areas of the world to identify the soil keratinophilic fungi and to isolate species present with potential pathogenic capabilities. These studies have directed attention to species distribution as well as the perfect and imperfect forms of the organism. *Microsporum gypseum* has received the greatest attention of the keratinophilic species. Some literature citings only discuss one species, primarily when first isolated from soil using the hair baiting technique.

A systematic survey of Taiwan keratinophilic fungi was made over a period of three months. Soil from northern, eastern and southern Taiwan and schools equally distributed throughout the country was collected from May through July, Soil serves as an excellent reservoir for the dermatophytic fung *Microsporum*, *Trichophyton* and *Epidermophyton* as well as several systemic species (Conant *et al.*, 1971), Genera such as *Chrysosporium* are keratinophilic but not pathogenic. Many Deuteromycetes are soil saprophytes and readily grow on organic material in the soil when suitable moisture and temperatures are provided. Direct soil contact and dust inhalation initiates many mycoses in man and animals, however, some keratinophilic fungal species have not been identified as soil inhabitants. Numerous mycoses are readily transmitted from and between humans and animals.

MATERIALS AND METHODS

Primary schools equally distributed throughout Taiwan were contacted for soil samples from school yard areas. A total of 250 schools kindly responded and each returned about 50 g soil samples in sterile self sealing plastic bags by mail to the laboratory. Soil was also collected from metropolitan areas including public parks

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parks as well as rural areas. Individual field collections from areas in Taiwan included 101 soil samples from the Taipei metropolitan area, 120 from the eastern coast of Taiwan, 44 from south Taiwan, and 52 from the National Taiwan University (NTU) campus. Soil collections in addition to the school yard soils were also packaged in 50 g quantities in sterile plastic bags and returned to the laboratory soon after collections were made. The soil was placed in sterile disposable plastic petri dishes and moistened with sterile distilled water. Sterile human hair was generously added to the surface of the soil cultures. After 2–4 weeks incubation at room temperature, hair directly supporting fungal growth was transferred with clear Scotch tape to microscope slides and mounted with lactophenol cotton blue. Keratinophilic isolates were identified to species and saprophytes to genus by spore forms and conidiophores (Gilman, 1957; Rebell & Taplin, 1970; Conant *et al.*, 1971; Ellis, 1971; Barnett & Hunter, 1972).

RESULTS

Of the 250 primary school soil samples, 195 schools had positive growth of fungi on the hair baited soil while 55 soil samples had no growth. The isolates and the school soil collections yielding the organisms were randomly distributed throughout Taiwan with no statistical significance of individual species to geographic collection sites.

Twelve keratinophilic species were isolated from the 250 soil collections of school yards, The number following each species refers to the number of schools with soil containing that particular species. The isolates included *Arthroderma multifidium* Dawso (5), *A. tuberculatum* Kuehn (9), *Chrysosporium asperatum* Carmichael (2), *C. evolceanui* (Randhawa & Sandhu) Garg (16), *C. indicum* (Randhawa & Sandhu) Garg (58) with *Aphanoascus terreus* (Randhawa & Sandhu) Apinis (3) as the perfect form of the species, *C. keratinophilum* (Frey) Carmichael (6), *C. pannorum* (Link) Hughes (1), *C. tropicum* Carmichael (17), *Microsporum fulvum* Uriburu (5), *M. gypseum* (Bodin) Guiart & Grigorakis (7), *Sporothrix schenckii* Hektoen & Perkins (58), *Trichophyton terrestre* Durie & Frey (4) with *Arthroderma quadrifidum* Dawson & Gentles (2) as the perfect form of the species. The dermatophytes that could cause skin, hair or nail infections were *M. fulvum* and *M. gypseum*. Sparce ectothrix growth on the hair and characteristic macroaleuriospores were present with the two *Microsporum* species. *Sporothrix schenckii* is considered a systemic species that has the ability to cause nodular lesions and indolent ulcers in lymph nodes, skin or subcutaneous tissues (Conant *et al.*, 1971). The other named species are nonpathogenic representatives of keratinophilic fungi.

Hair baited school yard soil also supported the growth of other fungi. The genera and the number of positive soil samples for each genus included *Allescheriella* P. Henn. (1), *Alternaria* Nees ex Wallr. (3) *Aspergillus* Mich, ex Fr. (2), *Bdellospora* Drechsler (1), *Botrytis* Pers. ex Fr. (2), *Cephalosporum* Nees ex Stilbum (2), *Cladosporium* Link ex Fr. (1), *Cunninghamella* Matr. (1), *Cirvularia* Boedijn (1), *Cylindrosporium* Grev. (1), *Fusarium* Link ex Fr. (2), *Haploglossa* Drescheler (10), *Helicosporium* Nees ex Fr. (1), *Helminthosporium* Link ex Fr. (1), *Hormodendrum* Bon. (2), *Monilia* Pers. ex Fr. (1), *Mucor* Mich. ex Fr. (1), *Paecilomyces* Bain. (4), *Penicillium* Link ex Fr. (13), *Rhizopus* Ehrenb. ex Corda (1), *Scopulariopsis* Bain (1), *Staphylotrichum* Meyer & Nicot (1), *Thielaviopsis* Went (1), *Trichoderma* Pers. ex Fr. (3), *Trichothecium* Link ex Fr. Species of the representative genera include saprophytes, plant pathogens, agents of human mycoses, and opportunistic fungi of animal and human diseases.

Soil collections separate from elementary school collections were made at the National Taiwan University, the City of Taipei, eastern Taiwan and southern Taiwan (Table (1). A total of 317 soil collections were made in the four areas. The number and distribution of collected soil samples examined for fungal growth included NTU (52), Taipei (101), E. Taiwan (120) and S. Taiwan (44). NTU and Taipei represented areas of high population densities while estern and southern areas of Taiwan included rural, coastal and small communities. No significant variation in species densities occurred between soil collection sites at National Taiwan University and other areas equally distributed throughout Taipei.

Table 1. Distribution of keratinophilic fungi in Taiwan soil

Species	Collection areas							
	NTU		Taipei		E. Taiwan		S. Taiwan	
	a	b	a	b	a	b	a	b
A. multifidium	0	0	0	0	1	0.8	0	0
C. asperatum	2	3.6	1	0.9	14	11.7	1	2.3
C. evolceanui	5	9.7	11	10.9	39	32.5	10	22.7
C. indicum	22	42.3	22	21.7	76	63.3	8	18.2
C. keratinophilum	1	1.9	6	5.9	22	18.3	16	36.4
M. fulvum	1	1.9	3	2.9	3	2.5	2	4.5
M. gypseum	5	9.7	9	8.9	19	15.8	2	4.5
T. ajelloi	0	0	1	0.9	0	0	0	0

Legend a. Number of soil samples supporting fungal growth for each species.
b. Percent incidence of positive soil samples. Data does not include soil collections from school yards

Variations in population density of keratinophilic species between eastern and southern areas of Taiwan were noted. *Microsporum gypseum* and *Chrysosporium* species were generally more frequently found in eastern Taiwan than in other collection areas. *Microsporum gypseum* was isolated on several occasions from all areas of Taiwan. The species most commonly isolated from the soil samples was *Chrysosporium indicum*. One separate occasion each, *A. multifidium* and *Trichophyton ajelloi* (Vanbreuseghem) Ajello were isolated. *Arthroderma tuberculatum*, *C. pannorum*, *C. tropicum*, *Sporothrix schenckii* and *Trichophyton terrestre* were only found in school yard soil samples. A high incidence of *S. schenckii* was noted in the soil samples.

DISCUSSION

The fungal microflora of soils is an area of study requiring more analyses. When a species becomes a animal or plant parasite or opportunistic organism, the disease and the organism is studied in detail while the existence of the species in an environment of no immediate pathogenic importance initiates few studies. In relatively recent years the soil fungal populations have been identified. The first isolation of keratinophilic fungi from soil habitats included soil from a shed which housed ringworm infected calves (Muende & Webb, 1937). Further identification of keratinophilic fungi in soil was established with the direct isolation of macroaleuriospores of *Microsporum gypseum* from soil by means of a membrane filter method (Gordon *et al.*, 1952). Independent investigations by Emmons (1951) and Van Brussel (1950, 1952) demonstrated that dermatophytes would grow on sterilized soil as a medium without added nutrients if suitable temperature and moisture were present.

The keratinophilic dermatophyte isolated in the early studies was *M. gypseum.* Search for keratinophilic fungi in soil has included the isolation of dermatophytes and the nonpathogens. In most all the fungal flora characterizations of soil baited with hair, *M. gypseum* was commonly found. The discovery of the perfect states of several Deuteromycetes species was also made with the baited soil in previous studies (Durie, 1962). Prior to interest in keratinophilic species, soil isolation of fungi responsible for systemic mycoses were made (Frey & Durie, 1955).

Cuticular mycoses have been identified in Taiwan with specific etiological agents. Species include *Trichophyton ferrugineum, T. violaceum, T. rubrum, T. mentagrophytes, Epidermophyton floccosum, Microsporum canis, M. gypseum* and *Pityrosporum orbiculare* (*Malassezia furfur*) (Jen, 1963). Systemic mycotic disease agents have included *Cryptococcus neoformans, Sporothrix* (*Sporotrichum*) *schenckii, Candida albicans, Aspergillus fumigatus, Fonsecaea* (*Hormodendrum*) *dermatitidis* and *F. pedrosoi* (Jen *et al.*, 1967, 1970). Lü (1964) previously isolated the dermatophyte *M. gypseum* from Taiwan soil along with the representative saprophtic species *Fusarium, Cephalosporium, Penicillium, Mucor, Monotospora, Chaetomium, Curvularia, Aspergillus, Helminthosporium, Trichoderma, Syncephalastrum.*

With the increase and prolonged usage of steroid drugs, many saprophytic deuteromycete fungal species have become opportunistic species causing mycotic diseases in man (Emmons *et al.*, 1970). *Trichophyton, Microsporum* and *Epidermophyton* species are causal agents of many dermatophytic diseases although *T. terrestre* is known only as a saprophyte (Rippon, 1974). The species can only grow on hair in vitro not in vivo (Rebell & Taplin, 1970).

Numerous ecological surveys have been conducted to identify the soil borne potentially pathogenic fungi, the keratinophilic species and the saprophytic species known as opportunists. The soil surveys have included both rural and densely populated areas (Otčenášek *et al.*, 1967). Studies have been conducted in Australia and New Guinea (Frey & Durie, 1955; Male, 1961; Ridley, 1961; Durie & Frey, 1962; Dunn & Morahan, 1964; Frey, 1965), The Bahamas (Volz, 1971), Belgium (Ajello *et al.*, 1965b), Brazil (Londero & Ramos, 1961; Londero, 1962; Rogers & Beneke, 1964); Bulgaria (Balabanov & Kasurov, 1962), Colombia (Rogers, 1971), the Congo (Vanbreuseghem, 1950), Costa Rica (Mata & Mata, 1959), Czeckslovakia (Kunert, 1965a, 1965b,), Easter Islands (Ajello & Alpert, 1972), Egypt and Ethiopia (Taylor *et al.*, 1964), England (Stockdale, 1958; Pugh & Mathison, 1962; Griffin, 1960a), Finland (Lundell *et al.*, 1960), Germany (Meinhof *et al.*, 1960; Böhme, 1965; Bühme, 1965, 1966; Köhler & Hoffman, 1965; Krentel, 1964; Schönborn, 1965; Böhme *et al.*, 1969), Hungary (Banhegyi, 1959), India (Puri, 1961; Sarker, 1962b; Randhawa & Sandhu, 1965; Garg, 1966a, 1966b; Padhye *et al.*, 1967), Italy (Ajello *et al.*, 1965a), Japan (Kaben, 1963), New Zealand (Marples, 1965), Norway (Lindquist, 1961), Panama (Ajello, 1954); Poland (Dominik & Majchrowicz, 1964, 1965, 1966), Russia

(Stepanitschewa, 1963; Pesterev, 1966; Polag & Szadeczky, 1966), Solomon Islands (Smith & Marples, 1964), Spain (Periro, 1962) and the United States (Dean & Haley, 1962; Dabrowa *et al.*, 1964; Orr *et al.*, 1972). The isolation of single keratinophilic species initiated several additional soil studies (Daniels, 1954; Evolceanu *et al.*, 1960, 1962; Ito *et al.*, 1961; Lundell *et al.*, 1961; Ajello & Cockshott, 1962; Heitmanek, 1962; Padhte & Thirumalachar, 1962; Sarker, 1962; Randhawa & Sandhu, 1963, 1964; Blaschke-Hellmessen, 1964; Flórián & Galgoczy, 1964; Refai & Rieth, 1964; Rioux *et al.*, 1965; Baxter, 1966; Evolceanu & Alteras, 1966; Garg, 1966b). The species receiving the greatest attention concerning isolation frequency, world distribution, life cycle and perfect stages as well as isolation in soil habitats is *M. gypseum* (Ajello, 1953a, 1953b; Gordon, 1953; Durie & Frey, 1955; Dey & Kakoti, 1955; Stockdale, 1958, 1961, 1964; Banhegyi, 1959; Doupagne, 1959; Mata & Mata, 1959; Randhawa, *et al.*, 1959; Griffin, 1960b, 1960c; Silva, 1960; Szathmary & Henfoy, 1960; Yarzabel *et al.*, 1960; Castro, 1961; Padhye, 1961; Sarker, 1962a; Kaben, 1963; Lü, 1964; Mohapatra & Gugnani, 1964; Weitzman, 1964: Varsavsky *et al.*, 1965). In Taiwan no medical records of *M. gypseum* in adults or children have been recorded in the literature. Of the kerationphilic dermatophytes isolated from Taiwan soils, *M. gypseum* was commonly found. The species is also in abundance in other areas of the world.

The soil serves as a potential reservoir for many fungal species, many previously isolated as causal agents of human mycoses. Species are not ecologically restricted to soil types or geographical areas. Soil has sufficient nutritional properties to support an almost ubiquitous flora, even allowing long dormancy periods until suitable moisture and temperature is present to support spore germination. Species recovered in Taiwan from soil samples capable of causing mycoses in humans include *M. fulvum*, *M. gypseum*, *T. ajelloi* and *S. schenckii* in addition to isolations of opportunistic fungal genera.

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