ISOLATION OF THE PATHOGENIC FUNGUS, MICROSPORUM GYPSEUM, FROM PHILIPPINE SOIL

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During the past few years, there has been a growing interest in the search for the natural habitat of the human pathogenic fungi. Current investigations are directed towards seeking an answer to the question of whether the pathogenic fungi are obligate parasites of man and lower animals or saprophytes that possess ability to infect susceptible individuals under certain conditions. A perusal of the literature on the subject, points to the soil as the natural habitat of many pathogenic fungi (1-12).

Interest has been focused on the search for pathogenic fungi in soil as a result of the pioneering studies of Emmons (1). He was the first to point to the role of the soil as a reservoir of pathogenic fungi including those causing systemic and superficial infections. Subsequent investigations done by other workers resulted in the isolation from soil of several pathogenic fungi including *Allescheria boydii, Sporotrichum schenckii, Candida albicans, Cryptococcus neoformans, Coccidioides immites, Histoplasma capsulatum, Microsporum gypseum* and *Trichophyton mentagrophytes.*

The saprophytic existence of the dermatophytes, the etiological agents of ringworm infections, was suggested by the ease with which these fungi were grown in soil under laboratory conditions. Early attempts, however, to isolate the dermatophytes directly from test soils ended in failure due to overgrowth of the culture tube by saprophytic molds. The introduction by Vanbreuseghem (12) of a selective procedure for isolating keratinophilic fungi has made possible the isolation

of the dermatophyte fungi from soil. By placing hair filaments on the surface of moistened soil in which *Trichophyton mentagrophytes, Trichophyton rubrum* and *Epidermophyton floccosum* had been grown, Vanbreuseghem observed that the bait became visible overgrown by mycelium which in some cases were seen to be penetrating the hair shafts by means of "perforating organs." Vanbreuseghem examined a number of soil samples from Belgium by this hair-baiting technic and isolated a keratinophilic fungus which is now known as *Keratinomyces ajelloi,* but none of the known dermatophyte fungi. His method, however, was successfully employed in the isolation from soil of *Microsporum gypseum* by Ajello (4, 5, 6), Gordon (8), Frey and Durie (9) and Rodriguez (10). This paper is a report on the isolation of *M. gypseum* from Philippine soil using the hair baiting technic.

MATERIALS AND METHODS

The soil samples examined in this study came from various parts of Manila, Makati town, Rizal, Quezon City and the campus of the College of Agriculture at Los Baños, Laguna. Soil samples were collected from the sides of streets, near the fence, near the house, under the house and in the woods. Specimens were collected directly in sterile Petri dishes by scooping up the top most layer of the earth with the bottom part of the Petri dish. The cover was replaced after enough soil was collected to half fill the container. The soil samples were processed the same day as collected.

In isolating the dermatophyte, the technic described by Vanbreuseghem (12) and later employed by Ajello (4) was employed. To the Petri dish half filled with soil sample, sufficient sterile distilled water was added to moisten the soil thoroughly. About 20 to 30 ml. of water was required depending upon the nature of the soil sample. Short strands of autoclaved human hair were placed on the surface of the moistened soil. The baited Petri dishes were then kept in a drawer at room temperature and observed over a period of 8 weeks. Hairs that became covered with mycelium were examined microscopically and cultured on a selective medium introduced by

Georg *et al.* (13) which contained 0.5 mg. cycloheximide (Actidione*), 20 units penicillin and 40 units of streptomycin per ml. of Sabouraud's dextrose agar.

Animal inoculation was performed to determine the pathogenicity of the isolated fungus. On a shaved area approximately 3 cm. by 4 cm. on the flank of a guinea pig, a heavy suspension of 10-day old highly sporulating culture of the fungus isolate was rubbed in with sandpaper. The sandpapering was done very lightly so as not to cause bleeding. The animal was kept in a separate cage for observation.

RESULTS

Of 104 soil samples examined, 23 (22.1%) yielded cultures of *M. gypseum*. The time of appearance of a visible growth of this fungus on the soil plates was very variable. In some plates growth was visible as early as the third week, while in others as late as the sixth week. The fungus made its first appearance as a fine creamy down covering the hair filaments (Fig. 1). The color usually turned to tan after several days. The growth of the fungus upon the hair is luxurious enough to be easily detected with the naked eye.

Microscopic examination of the hairs covered with mycelium showed abundant ellipsoid, rough, thin-walled macroconidia measuring 36—61 microns (ave. 51 microns) in length by 7.8—12.4 microns (ave. 9.9 microns) in width and containing from 4 to 7 cells (Fig. 2). Few single-celled, oval to clavate microconidia attached to the sides of hyphae were also observed. Many hair filaments were seen with wedge-shaped perforations caused by penetration of the hair with cone-shaped masses of mycelium (Fig. 3).

Pure culture of *M. gypseum* was obtained by inoculating the mycelium covered hair into Sabouraud's dextrose agar with cycloheximide, penicillin and streptomycin. Growth of the fungus on this medium was fairly rapid, its colony measuring about 2 cm. in diameter after one week incubation. The colony

* Cycloheximide used was generously supplied by Upjohn Company of Kalamazoo, Michigan.

was powdery and almost cinnamon brown in color, while the reverse side was pale orange yellow (Fig. 4). Microscopic examination of a young colony showed abundant ellipsoid macroconidia characteristic of this species and a few microconidia attached to the sides of hyphae (Fig. 5).

Of the 2 isolates inoculated into the skin of each of two guinea pigs, only one produced a skin lesion from which the growing fungus was demonstrated microscopically and recovered by culture. Inoculation of the fungus into the guinea pig was followed 2 days later by an acute dermatitis. This traumatic inflammatory reaction which subsided within a week, was replaced by a slight induration, crust formation and small, irregular, slightly erythematous areas. KOH mount of skin scrapings taken during the second week of infection, showed abundant branching hyphae in the scales. No infection of the hairs was noted. *M. gypseum* was cultured from the skin lesion using Sabouraud's dextrose agar with cycloheximide, penicillin, and streptomycin. A gradual clinical recovery was noted which was associated with the disappearance of the fungus from the skin scrapings. Gordon (8) has verified on a human subject the infectiveness of a culture of *M. gypseum* isolated from one soil sample.

DISCUSSION

A survey of published literature revealed that *M. gypseum* has been successfully isolated from soil by different workers in the following places; various parts of the United States, Hawaii, Panama, Nigeria and Canada by Ajello (4, 6); Cuba by Fuentes (14); Australia by Frey and Durie (9); and Ecuador by Rodriguez (10). Ajello reported a recovery of 31.9% for soil samples from Tennessee and Georgia, Frey and Durie reported 12.5% from Australian soil and Rodriguez isolated the fungus from 4 out of 10 soil samples from Ecuador. The recovery of *M. gypseum* from 22.1% of soil samples from the Philippines adds to the evidence now available which show that this fungus is prevalent in the soil throughout the world.

The ease with which *M. gypseum* can be isolated from the soil strongly suggests a saprophytic existence for this fungus.

Definite proof of saprophytism, however, was furnished by the report of Gordon *et al.* (7) on the demonstration of the characteristic macroconidia of *M. gypseum,* which never are produced on tissues of living animals, in a soil sample from Tennessee.

Infections with *M. gypseum* are rare and sporadic in occurrence and distribution. A review by Ajello (4) of reported cases gave only 155 instances of human infections in the United States and 115 cases distributed among the following countries: Argentina, Brazil, Canada, Panama, Puerto Rico, Uruguay, Austria, Belgium, Denmark, England, Finland, France, Germany, Hungary, Italy, Ireland, Netherlands, Spain, Switzerland and Australia. Among lower animals he found on record 61 instances of *M. gypseum* infections, 50 of which were in horses, 4 in monkeys, 1 in dog, 4 in cats, 1 in tiger and 1 in chicken. In our laboratory (16) only 6 human infections with this fungus were seen since 1950. Bocobo and Gutierrez (15) reported a case of *M. gypseum* infection in 1952. There is no local report of *M. gypseum* infection in lower animals.

Human infections by *M. gypseum* which are sporadic in occurrence and distribution can hardly be explained by transmission from one person to another. The rarity of infections in lower animals seems to minimize their importance as the primary source of human infections. The occurrence of *M. gypseum* in a large precentage of soil samples throughout the world has placed this natural habitat of the fungus as the more important source of infection of man and lower animals. This view is in accord with that of Ajello (4) who made the conclusion—"that soil must be considered the main source of human infections. Lower animals, thus, can no longer be implicated as the prime source of *M. gypseum.* They, like man, are infected from soil. Only infrequently are infections transmitted from animal to animal."

In spite of the prevalence of *M. gypseum* in soil, it is significant to note that infections with this fungus are rare. Because of this, one is led to consider *M. gypseum* a primarily soil inhabiting saprophyte, where—as suggested by other workers —it takes part in the breakdown of keratinaceous materials and only under certain special conditions can it bring about an infection.

So far only 2 species of dermatophytes have been isolated from soil, *M. gypseum* which is regularly obtained from soil and *T. mentagrophytes* which has been reported isolated by Lurie and Borok (11) from soil of caves and by Rodriguez (10) from Ecuadorian soil. There are indications that some other species of dermatophytes may also exist as saprophytes in soil but have remained undetected probably because the methods presently employed in searching for them are inappropriate.

SUMMARY

The pathogenic fungus *M. gypseum* was isolated from 23 out of 104 soil samples (22.1%) collected from various parts of Manila, Makati town, Rizal, Quezon City and the campus of the College of Agriculture at Los Baños, Laguna.

The method employed in isolating the fungus was described.

The implication of the presence of *M. gypseum* in a large percentage of soil samples was discussed.

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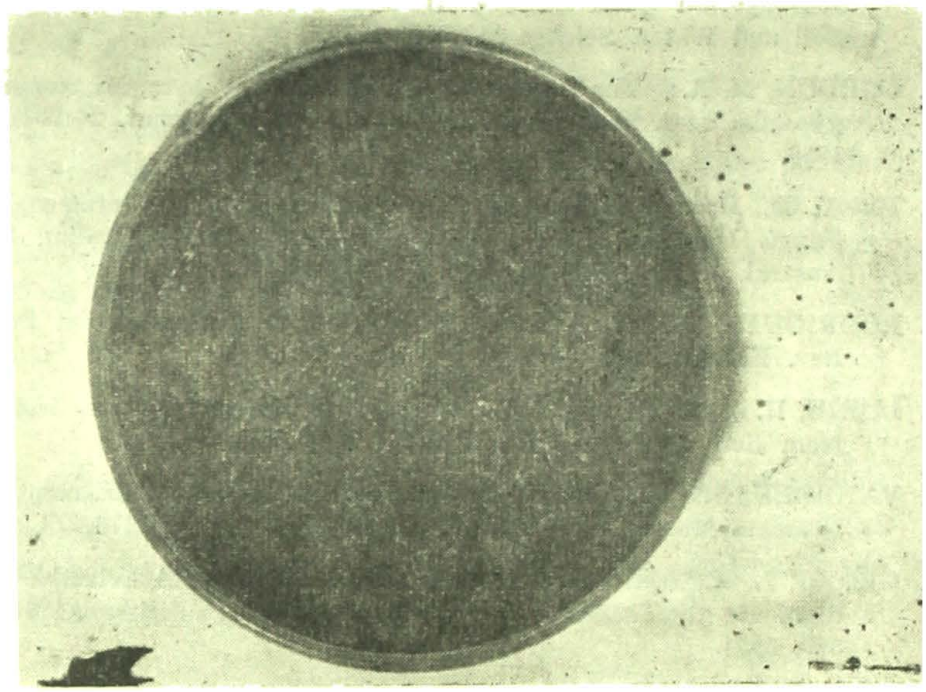

Figure 1

Soil plate showing appearance of Microsporum gypseum on hair filaments used as bait.

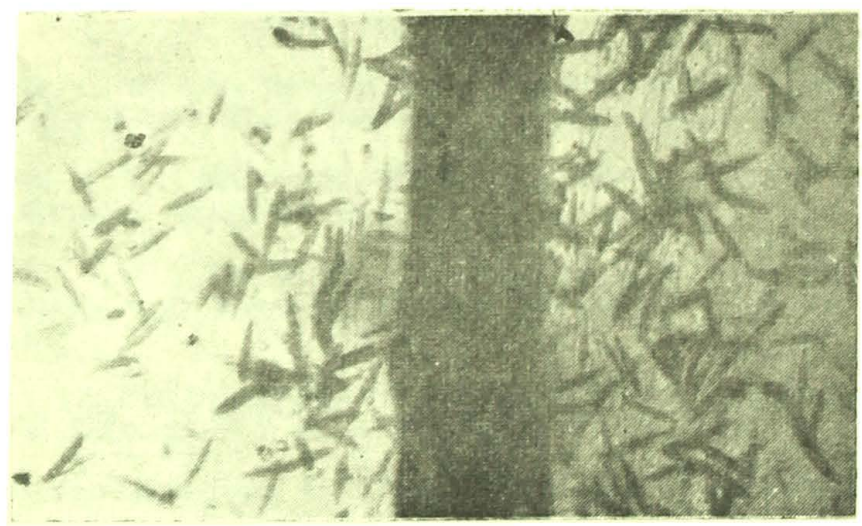

Figure 2

Profuse production of macroconidia by Microsporum gypseum on hair filament exposed to soil. (x 150)

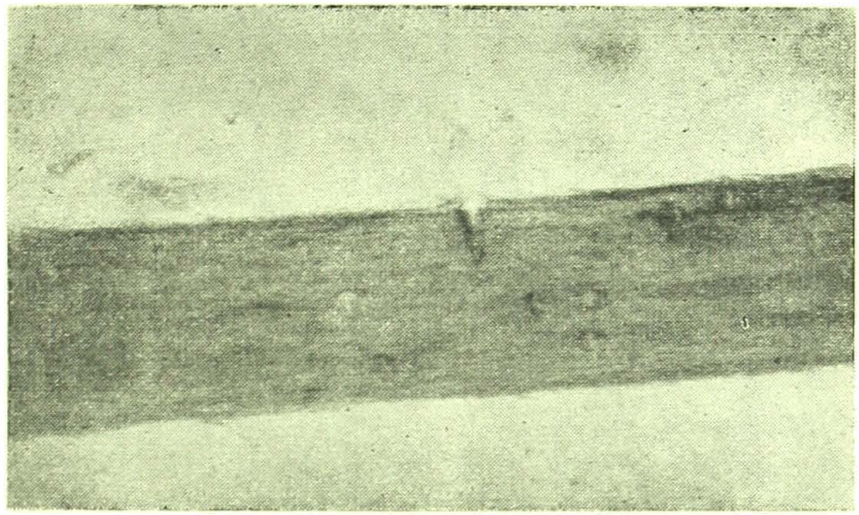

Figure 3

Perforation of hair filament by mycelium of Microsporum gypseum. (x 200)

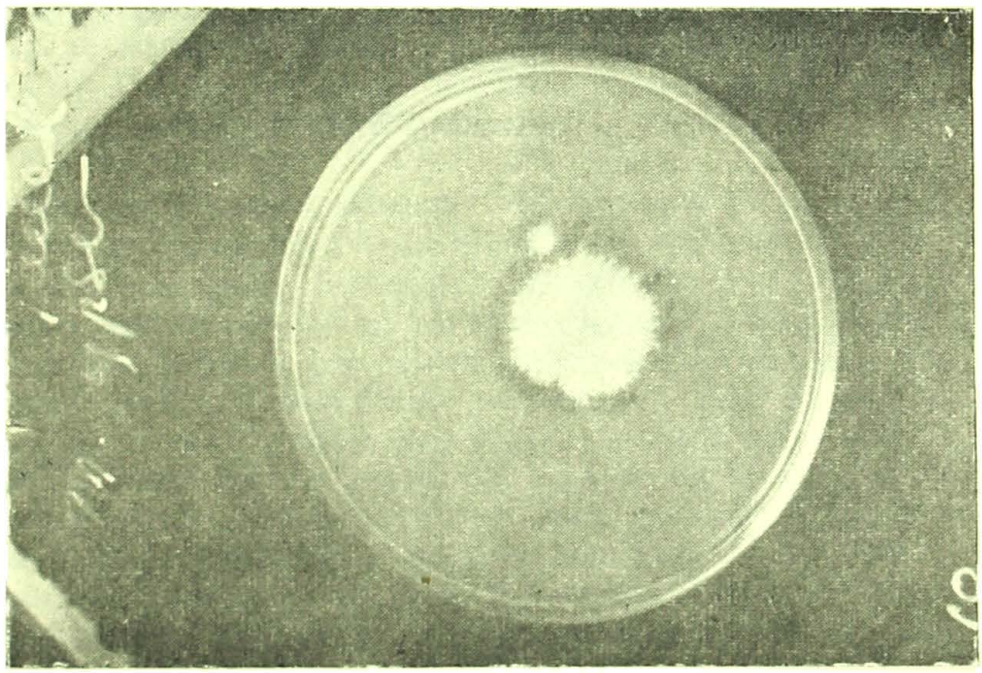

Figure 4

Cultural appearance of Microsporum gypseum on Sabouraud's glucose agar 1 week old. (x 314)

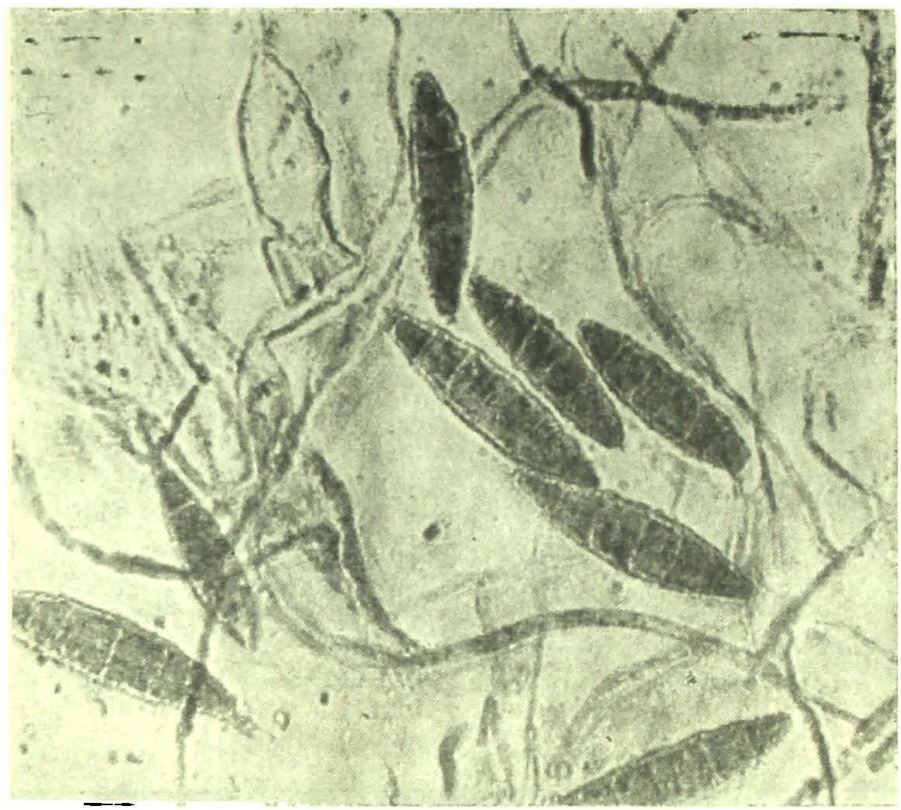

Figure 5

Macroconidia of Microsporum gypseum (x-500)