

mykosen

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Production of Antibiotics by Geophilic Dermatophytes

II. The antibiotic spectrum of crude filtrate

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In an earlier report (4) we demonstrated the antibacterial activity of the geophilic dermatophytes by observations of inhibitory zones around the fungus colonies in culture on solide medium with growth of various bacteria. Thereby *Microsporon cookei* showed a marked, *M. gypseum* and *Trichophyton terrestre* a slight inhibitory effect on 24 tested strains of *Staphylococcus aureus*. *Keratinomyces ajelloi*, however, was found to have no activity against the tested bacteria, which besides *Staphylococcus aureus* also included gram negative rods.

For further tests of antibacterial properties of the geophilic dermatophytes, the medium hitherto used, viz. Sabouraud's glyucose agar, is less suitable because of the lacking growth on that substrate of the bacteria being tested i. e. the streptococci. Therefore we have continued our studies by cultivating the fungi on a fluid medium and tested the crude filtrates for antibacterial activity according to the plate diffusion test method (2).

Material and Methods

10 strains of each. *T. terrestre*, *K. ajelloi*, *M. cookei* and *M. gypseum* isolated from Swedish soil were tested. The fungi were isolated according to the method described by PÅLSSON in 1968 (8). As controls were used 5 strains of *Epidermophyton floccosum*, recently isolated from skin lesions of infected men. A great care was taken not to use strains with signs of pleomorphism.

The fungi were cultivated in liquid medium of the following composition:

casein hydrolyzate Difco *)	10 g
glyucose	10 g
KH ₂ PO ₄	5 g
MgSO ₄ · 7 H ₂ O	1 g
aqu. dest. ad	1000 ml

final pH 5.1. Trace metals were added as a partial EDTA-chelate (7).

The bacteria used for the tests — species given in table 1 — were strains recently isolated from routine laboratory cultures and selected at random. The phage types of all the *Staphylococcus aureus* strains had previously been determined and 10 strains with and 10 strains without resistance against benzyl penicillium were used.

The medium for testing of the antibiotic activity of the fungi was agar, lacking pepton with 4 per cent horse blood.

For the production of antibiotic substances all the dermatophyte strains were grown submerged in shake cultures at room temperature. The flasks containing 100 ml of the fluent medium were inoculated with 3 mg comminuted mycelium from one to three weeks old Sabouraud glucose agar cultures. Samples of the culture broth were taken with a sterile pipette at intervals of three days tested for antibiotic activity against the bacteria. The fermentation was interrupted when the concentration of antibiotic substances did not seem to increase further i. e. on the 12th day.

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The broth was filtered through a Seitz filter — the filtrates tolerated storage at +4° C without loss of activity. The pH in the culture broth invariably rose during the growth of the fungi from originally 5.1 to values between 6.0—7.2.

The antibacterial activity was tested by the plate diffusion technique: cylindrical holes 8 mm in diameter were punched out in plates previously sown with bacteria and the holes were then filled with culture filtrate from the fungi. The inhibition zones were measured after 20 hours incubation of the plates at 37° C. The surface of the medium was flooded with a suspension of bacteria in broth, the excess fluid removed and the plates briefly dried. The concentration of bacteria in the suspension was chosen so as to give a dense but not confluent growth of bacterial colonies (1,2).

Table 1: Size of inhibition zones with filtrates of the dermatophytes

Microbe	Dermatophyte species				
	Mean inhibitory zones (mm)				
	K. ajelloi	M. cookei	M. gypseum	T. terrestre	E. floccosum
<i>Staphylococcus aureus</i>	—	28.4	11.6	12.7	24.3
β -hemolytic streptococci	—	—	—	—	—
Enterobacteriaceae spp.	—	—	—	—	—
<i>Pseudomonas aeruginosa</i>	—	—	—	—	—
<i>Bacillus</i> spp.	—	—	—	—	—
<i>Clostridium</i> spp.	—	—	—	—	—

Results

The results — see table 1 — showed that the filtrates of *M. cookei* and to a less extent those of *E. floccosum* had a uniform inhibitory effect on *Staphylococcus aureus* (both strains which were resistant and not resistant to benzyl penicillin). Some of the species of *M. gypseum* and *T. terrestre* had a slight effect which appeared first on 12 days filtrates.

The antibacterial activity of the filtrates increased from day to day and reached its maximum on the 12th day. No inhibitory effect of all the fungi was registered on β -hemolytic streptococci, *Escherichia coli*, *Proteus morgani*, *Pseudomonas aeruginosa*, *Bacillus* spp., and *Clostridium* spp.

Discussion

As in our previous report (4) we could demonstrate the antibacterial activity of some of the geophilic dermatophytes including *M. cookei*, *T. terrestre*, *M. gypseum* on strains of *Staphylococcus aureus*. The antibiotic spectrum of the crude filtrate of *E. floccosum* is reminiscent of that obtained from data in the literature (5, 6) and as WALLERSTRÖM (9) we found that 12 days filtrates of the fungi reached the highest potency in producing antibiotics. Interesting is that all the strains of *M. cookei* had a more marked activity than those of *E. floccosum*. The possible influence of serum in some of the media has not been taken into consideration in this study, but this will be studied further. According to our opinion, it is of importance that the studies on antibiotic properties of *M. cookei* must be continued and intensified also including the determination of the purified active substance, and its minimum inhibitory concentrations and toxicological data.

Summary

10 strains each of *Microsporon cookei*, *Keratinomyces ajelloi*, *Microsporon gypseum*, *Trichophyton terrestre* and *Epidermophyton floccosum* were cultivated submerged in shake culture and culture filtrates were examined for antibiotic activity. All the strains of *M. cookei* and to a less extent those of *E. floccosum* showed such activity on *Staphylococcus aureus* strains, both resistant and not resistant to benzyl penicillin. Some of the species of *M. gypseum* and *T. terrestre* had a slight antibacterial effect, which appeared first on 12 days filtrates.

Zusammenfassung

Die geophilen Dermatophyten *Trichophyton terrestre*, *Keratinomyces ajelloi*, *Microsporon cookei* und *Microsporon gypseum* wurden hinsichtlich ihrer antibakteriellen Fähigkeiten gegen *Staphylococcus aureus* und einige gramnegative Stäbchen getestet.

M. cookei zeigte eine deutliche, *M. gypseum* und *T. terrestre* zeigten eine schwache Hemmwirkung gegenüber allen getesteten Stämmen von *Staphylococcus aureus*. Nach Auffassung der Autoren ist dies der erste Bericht über antibakterielle Eigenschaften von *M. cookei*.

Bei weiteren Untersuchungen wurden je 10 Stämme von *Microsporon cookei*, *Keratinomyces ajelloi*, *Microsporon gypseum*, *Trichophyton terrestre* und *Epidermophyton floccosum* in submersen Schüttelkulturen gezüchtet. Die Kulturfiltrate wurden dann auf antibiotische Wirkung geprüft, und zwar gegenüber *Staphylococcus aureus*, β -hämolytischen Streptokokken, Enterobacteriaceen, *Pseudomonas aeruginosa*, *Bacillus*-Arten und *Clostridium*-Arten.

Alle Stämme von *M. cookei* und in etwas geringerem Grade auch die von *E. floccosum* zeigten eine deutliche Aktivität gegen die *Staphylococcus aureus*-Stämme, sowohl gegen Benzylpenicillin-resistente als auch nichtresistente Stämme. Einige der *M. gypseum*- und *T. terrestre*-Stämme hatten eine schwache antibakterielle Wirkung, die sich in 12 Tage alten Filtraten zuerst bemerkbar machte.

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