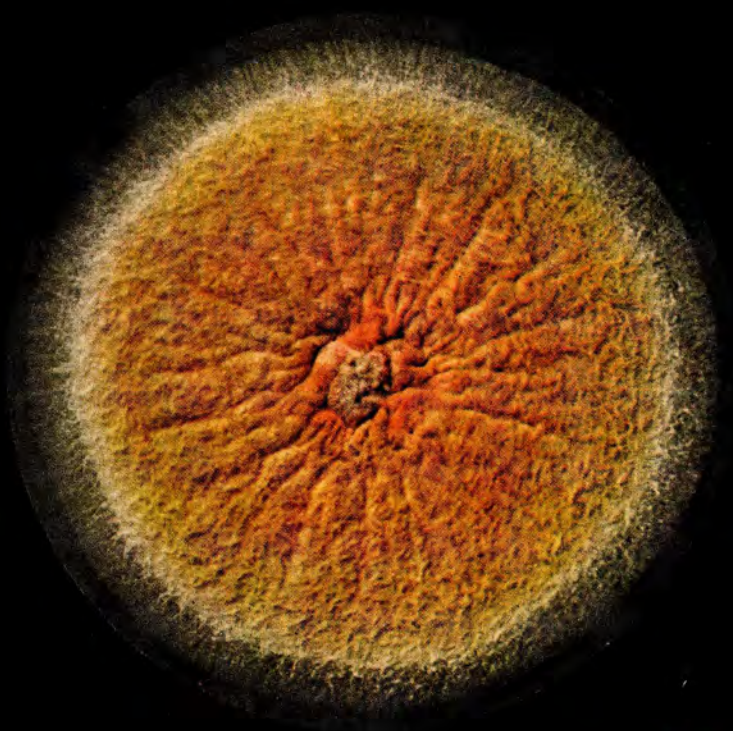
A 2547 E

mykosen



8/1970

1. August

Grosse Verlag GmbH. · 1 Berlin 31 (Halensee) · Kurfürstendamm 152

mykosen 13 (8) : 397—400 (1970) Eingegangen am 23. März 1970

Department of Dermatology, Karolinska sjukhuset, 104 01 Stockholm, Sweden and
Department of Bacteriology, Royal Veterinary College 104 05 Stockholm, Sweden

Production of Antibiotics by Geophilic Dermatophytes

I. Screening test of antibacterial activity

Lennart Gip and Göran Pålsson

The search after new antibiotics is important and necessary with respect to therapy and general biology. In therapy, discovery of new antibiotics may prove useful partly with regard to the acquired resistance of microbes to previous ones and the consecutive restrictions of their clinical efficacy and partly because of inadequacies of the antibiotic spectra of the agents being presently at our disposal. The development of resistant strains makes it necessary always to be in advance in discovering microorganisms producing antibiotics possessing an identical spectrum as the ones hitherto known, but with a different mode of action.

In regard to the great interest of the antibiotic capacity of some of the strains of *Penicillium, Aspergillus* and bacteria it is surprising that the biologic properties in this respect of the dermatophytes have been so relatively poorly studied.

The first description of antibiotic activity of dermatophytes are apparently those of Nakamura in 1932 (5) and Honda in 1936 (3). They found antibacterial properties of some *Trichophyton* species. During the last decades similary observations have been published by a series of other authors. A review of these findings is given by Götz 1962 (2). In most of the cases screening tests had been performed resulting in registered activity in strains of *Trichophyton mentagrophytes, Microsporon gypseum* and/or *Epidermophyton floccosum* on *Staphylococcus aureus.* Peck & Hewitt in 1945 (7) believed the antibiotic factor of these dermatophytes to be a penicillin-like substance, mainly because of its "spectrum of activity and behaviour toward penicillin-resistant organisms". In 1955 Úri et al. (9, 10) demonstrated the production of benzyl penicillin by *T. mentagrophytes* and reported that strains of *E. floccosum* inhibited not only the staphylococci, but also *Escherichia coli.* The effect of this fungus on various species of genus *Bacillus* and *Corynebacterium diphtheriae, Clostridium* as well as a weak effect on *Pneumococci, hemolytic Streptococci, Mycobacterium tuberculosis* and *Neisseria catarrhalis* was described by Katagiri et al. (4), Nishio (6) and Wallerström (11). The last author reported no inhibitory effect of *E. floccosum* on gram negative rods.

Wallerström is the first to touch the problem of the antibiotic effect of the *geophilic dermatophytes, Trichophyton terrestre* (Durie and Frey, 1957) and *Keratinomyces ajelloi* (Vanbreuseghem, 1952). They were found to be inactive or at most slightly active against some staphylococci. Unfortunately, no closer details from these experiments have been given in his paper.

The aim of the present series of investigations is to study the antibiotic properties of the geophilic dermatophyte species namely *T. terrestre, K. ajelloi, M. cookei* (Ajello, 1952) and *M. gypseum* (Bodin, 1907). It will be the first time *M. cookei* is examined in this respect.

This paper deals with a screen test of the antibacterial activity of these dermatophytes by observations of inhibitory zones around dermatophyte colonies in cultures with growth of various bacteria.

Table 1: Mean inhibitory zones (mm) around the dermatophytes tested against Staphylococcus aureus

Dermatophytes species (25 of each)	Staphylococcus aureus											
	Resistent to benzylpenicillin											
	Phage types											
	52		80	75	80	NT	KS6	52	52	81	83A	6
	80	187	81	77	KS6			52A	80	KS6		47
	81		KS6					80	81			53
	KS6							81				
K. ajelloi	0	0	0	0	0	0	0	0	0	0	0	0
M. cookei	70	70	74	73	71	70	75	74	70	71	71	73
M. gypseum	25	25	22	23	25	24	21	22	22	24	26	25
T. terrestre	20	21	19	21	21	21	20	20	19	22	21	22
E. floccosum	73	74	74	75	77	77	76	78	76	76	77	77

Dermatophytes species (25 of each)	Staphylococcus aureus											
	Not resistent to benzylpenicillin											
	Phage types											
		71	53			3B		3B	75	29	42D	83A
	3A		75	47	71	7	81	3C	77	52		
										80		
K. ajelloi	0	0	0	0	0	0	0	0	0	0	0	0
M. cookei	72	71	72	70	70	71	70	70	74	74	71	70
M. gypseum	26	24	23	24	24	26	23	25	25	24	23	23
T. terrestre	21	22	21	20	21	21	22	20	22	21	21	22
E. floccosum	75	76	74	75	76	76	77	75	78	76	77	76

Material and Methods

25 strains of each *T. terrestre, K. ajelloi, M. cookei* and *M. gypseum* isolated from Swedish soil were tested. For the detailed description of the isolation methods of these actual strains is referred to the report of PÅLSSON in 1968 (8). As controls were used 5 strains of *E. 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 cultured on Sabouroud's glucose agar (Difco)*). The substrate was dispensed on Petri dishes of 9 cm diameter, each filled with 33 ml of the medium.

The bacteria used for the tests — species given in **table 1** — were strains isolated from routine laboratory cultures and selected at random. The phage types of all the *Staphylococcus aureus* strains had previously been determined and strains, both with and without resistance against benzyl-penicillin were used **(table 1).** For the inoculation of each dermatophyte strain was used about 1 mg aerial mycelium. 10 days cultures incubated at + 22° C of each 25 strains of the four dermatophyte species were used. At this moment the diameter of the aerial mycelium varied between 15—17 mm and the weight of total culture was about 3 mg. The strains were exposed to bacterial suspensions which were floaded over the agar surface, whereafter the excess fluid was removed. The test plates were incubated at + 37° C. The preparation of the bacterial suspensions and the performance of the antibacterial tests were done according to the description given by ERIKSSON (1). Five control tests for each bacterial species were run on Sabouraud's agar not inoculated with fungi.

Results

The results are presented in **table 1.**

Of the geophilic dermatophytes *Microsporon cookei* showed a marked, *Microsporon gypseum* and *Trichophyton terrestre* a slight inhibitory effect on all tested strains of *Staphylococcus aureus* (**fig. 1**). However, they were all inactive against the tested gram negative rods (**fig. 2**). *Epidermophyton floccosum* had an antibiotic spectrum similar to that of *Microsporon cookei.* A uniform growth of bacteria was registered on all control plates.

Fig. 1: Inhibitory zone around a colony of *Microsporon cookei* growing on Sabouraud's glucose agar inoculated with *Staphylococcus aureus*

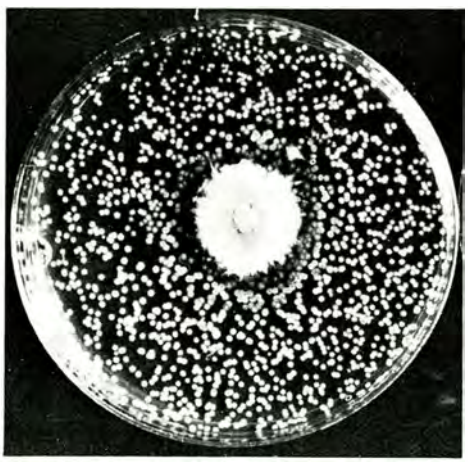


Fig. 2: No inhibitory zone around a colony of *Microsporon cookei* growing on Sabouraud's glucose agar, inoculated with *Pseudomonas aeruginosa*

*) Difco Laboratories, Detroit, Michigan, USA

Discussion

As far as we are aware, this is the first observation of antibiotic activity of *M. cookei* against *Staphylococcus aureus*. This dermatophyte has until now been the object of a very poor interest partly because of its scant occurrence in soil partly because of its slight pathogenicity. However, higher figures for its frequency have recently been obtained from soil in Sweden than from soil in other countries (8).

From botanical point of view it is surprising that such great differences in antibiotic properties can be obtained for the morphologically closely related dermatophyte species *M. cookei* and *K. ajelloi* with their large thickwalled cylindrofusiform macroconidia and their similar pigment production.

Noteworthy is that all 25 *M. cookei* strains showed the same effect against *Staphylococcus aureus* strains both resistant and not resistant to benzylpenicillin. As earlier investigators (4, 6, 11) we also found that *Epidermophyton floccosum* had antibiotic activity against *Staphylococcus aureus* but that it was inactive against some gram negative rods. The methods used did not allow any informations of the antibiotic properties of *Microsporon cookei* against bacteria other than those mentioned above, for example the streptococci, because of the unsuitability of the medium used for their cultivation. Of that reason we cannot exclude the geophilic dermatophytes *K. ajelloi, T. terrestre* and *M. gypseum* in our further tests of antibacterial activity. These will primarily include cultivation of the fungi in fluid medium with filtration and plate diffusion tests of the filtrates.

Summary

The geophilic dermatophytes *Trichophyton terrestre, Keratinomyces ajelloi, Microsporon cookei* and *Microsporon gypseum* were tested for antibacterial capacity against *Staphylococcus aureus* and some gram negative rods. *M. cookei* showed a marked, *M. gypseum* and *T. terrestre* a slight inhibitory effect on all tested strains of *Staphylococcus aureus*. This is, as far as the authors are aware, the first report on antibacterial properties of *M. cookei*.

References

1. ERICSSON, H.: Lappmetodens användning för kvantitativ bestämning av bakteriers känslighet för antibiotica. Från Karolinska sjukhusets klinisktbakteriologiska laboratorium, antibioticasektionen, Stockholm.
2. GÖTZ, H.: Die Pilzkrankheiten der Haut durch Dermatophyten. Handbuch der Haut- und Geschlechtskrankheiten. Bd. IV Teil III.
3. HONDA, S.: Über das antagonistische Phaenomen zwischen verschiedenen pathogenen Schimmelpilzen und Bakterien. Nippon Hifuka Gakkai Zasshi (Jap. J. Derm.) **39**, 97—98, 1936.
4. KATAGIRI, K. and SATO, K.: Studies on antibiotics produced by Epidermophyton floccosum II. On properties of fraction C. J. Antibiot. (B) Tokyo **14** : 1, 15—19, 1961.
5. NAKAMURA, T.: Über die antagonistische Wirkung des Trichophytonpilzes gegen Heubacillus. (I. Mitteilung) Nippon Hifuka Gakkai Zasshi (Jap. J. Derm.) **32** : 126, 1932.
6. NISHIO, K.: Epidermophyton inguinale in der Antibioticum-Forschung. Derm. Urol. **20**, 221—239, 1958.
7. PECK, S. M. and HEWITT, W. L.: The production of an antibiotic substance similar to penicillin by pathogenic fungi (dermatophytes). Publ. Hlth. Rep. **60** : 1, 148—153, 1945.
8. PÅLSSON, G.: Geophilic dermatophytes in the soil in Sweden. Studies on their occurrence and pathogenic properties. Acta vet. Scand. Suppl. 25.
9. ÚRI, J., SZABÓ, G. and OLÁH, D.: Production of antibiotics by dermatophytons. Acta Physiol. Hung. **5** : 1—2, 255—259, 1954.
10. ÚRI, J., JUHÁSZ, P. and CSOBÁN, G.: Über die Penicillinbildung von Trichophytonmentagrophytes-Stämmen. Pharmazie **10**—**12**, 709—713, 1955.
11. WALLERSTRÖM, A.: Production of antibiotics by Epidermophyton floccosum. I. The antibiotic spectrum of crude filtrate. Acta path. microbiol. scand. **71**, 287—295, 1967.

Address for reprint requests: Karolinska sjukhuset, 104 01 Stockholm, Sweden