THE PRODUCTION OF AN ANTIFUNGAL ANTIBIOTIC BY STREPTOMYCES GRISEUS

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Streptomycin, which is produced by strains of *Streptomyces griseus*, exhibits antibiotic activity against various gram-positive and gram-negative bacteria

TABLE 1

The highest dilution of 1 gram of streptomycin or of the antifungal antibiotic from S.griseus giving complete inhibition of growth of the test organism*

TEST ORGANISM†	ANTIFUNGAL ANTIBIOTIC FROM *S. griseus*	STREPTOMYCIN‡
Saccharomyces cerevisiae ATCC 918	2,500,000	<1,000
Cryptococcus neoformans	1,300,000	<1,000
Rhodotorula sp.	1,300,000	1,000
Hormodendrum pedrosoi 275	20,000	<1,000
Monosporium apiospermum	20,000	<1,000
Phialophora verrucosa	20,000	<1,000
Blastomyces dermatitidis 930	<10,000	<1,000
Candida albicans	<10,000	<1,000
Coccidioides immitis 819	<10,000	<1,000
Epidermophyton floccosum	<10,000	<1,000
Geotrichum sp.	<10,000	<1,000
Hormodendrum compactum	<10,000	<1,000
Nocardia asteroides 653	<10,000	<1,000
Sporotrichum schenkii	<10,000	<1,000
Trichophyton rubrum	<10,000	<1,000
Bacillus subtilis	<10,000	28,000,000
Staphylococcus aureus FDA 209	<10,000	21,000,000
Escherichia coli	<10,000	3,500,000
Pseudomonas aeruginosa ATCC 9027	<10,000	350,000

* Antibiotic diluted in agar medium (peptone 0.5%, glucose 1.0%, yeast extract 0.1%, agar 2.0%; pH 8.0) for the fungal spectrum and in liquid medium (peptone 0.75%, yeast extract 0.25%; pH 7.25) for the bacterial spectrum. Fungi incubated for 72 hours and the bacteria for 24 hours at 30 C.

† The cultures of fungal pathogens were obtained from Dr. N. F. Conant of Duke University.

‡ Seven hundred and eight micrograms per milligram.

(Schatz *et al.*: Proc. Soc. Exptl. Biol. Med., **55**, 66) but is ineffective against the fungal pathogens of man (Robinson *et al.*: Proc. Soc. Exptl. Biol. Med., **57**, 226; Reilly *et al.*: J. Bact., **49**, 585). We have found, however, that the growth of the fungal pathogen, *Cryptococcus neoformans*, is inhibited in a 1:100 dilution of a beer of *S. griseus* containing 130 μg per ml of streptomycin but that this same organism is not inhibited by 285 μg per ml of highly purified streptomycin. This ability of the beer of *S. griseus* to inhibit the growth of *C. neoformans* sug-

gested to us that *S. griseus* was producing, in addition to streptomycin, an antibiotic with antifungal activity.

The antifungal antibiotic was produced by Waksman's no. 4 strain of *S. griseus* in shaker flask cultures on the medium recommended by Waksman for the production of streptomycin (glucose, 1 per cent; meat extract, 0.5 per cent; peptone, 0.5 per cent; and NaCl 0.5 per cent). The preparation of this antibiotic that was used in determining its antibacterial and antifungal properties was obtained by extracting the beer of *S. griseus* with chloroform, removing the chloroform *in vacuo*, and dissolving the residue in methanol.

In contrast to streptomycin, the antifungal substance from *S. griseus* exhibits a high order of antibiotic activity against a number of yeasts and very little or no activity against the bacteria tested (table 1). It should be noted that of the fungal pathogens only *Cryptococcus neoformans* is highly sensitive to the action of this antibiotic. Furthermore, our antifungal antibiotic differs from streptomycin in its chemical properties. Streptomycin is chloroform- and ether-insoluble, whereas the antifungal antibiotic is chloroform- and ether-soluble; although both are water-soluble and thermostable.

At present, not enough information is available regarding the "second antibiotic of *S. griseus*" reported by Waksman (J. Bact., **51**, 753) and the antifungal agent from *S. griseus* to determine whether or not these two ether-soluble antibiotics are the same. Indicative of their being different is the relatively high degree of activity of Waksman's antibiotic against *Bacillus subtilis* (growth inhibited at 1:800,000 dilution). Strain differences between Waksman's and our *B. subtilis* test organisms, however, may explain this discrepancy in the anti-subtilis activity of the two antibiotic preparations.

A MODIFICATION OF HENRICI'S VEGETABLE-JUICE SPORULATION MEDIUM FOR YEASTS

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During a conference on alcohol production held at the Northern Regional Research Laboratory in February, 1943, the late Dr. A. T. Henrici discussed with us the infusion of four vegetables used by Mrak, Phaff, and Douglas (Science, **96**, 432) to obtain sporulation in yeasts. He informed us that he was using a commercially available blend of eight vegetable juices.[2] All that was required was the addition of sufficient agar to make a solid medium.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] The blend of vegetable juices used by Professor Henrici and by the writers was manufactured by Standard Brands at Terre Haute, Indiana, and marketed under the trade name, "V-8." We presume that other brands of mixed vegetable juice would prove equally satisfactory, and it is not our purpose to endorse any particular product.