SYNTHESIS OF AN ANTIBIOTIC CLOSELY RESEMBLING FUSIDIC ACID BY IMPERFECT AND PERFECT DERMATOPHYTES

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The antibiotic fusidic acid (Fusidin) was first found to be produced by the imperfect fungus *Fusidium coccineum* (TUBAKI) reported by GODTFREDSEN *et al.*[1] Recently, NICOT reported that fusidic acid was produced by *Paecilomyces fusidioides*[2]. This antibiotic was shown to be an acidic steroid[3] which was biogenetically and structurally related to helvolic acid and cephalosporin P_1[4]. Fusidic acid, characterized by low toxicity and oral activity, was shown to be clinically effective against penicillin-resistant staphylococci[5] and appeared to be synergistic with benzylpenicillin against strains of *Staphylococcus aureus in vivo* and *in vitro*[6].

Several years ago a program was initiated in our laboratories to screen dermatophytes for the synthesis of cephalosporin or cephalosporin-like antibiotics. The dermatophytes studied were obtained from the Communicable Disease Center, Atlanta, Georgia and Columbia University Medical School.

Dermatophyte cultures were tested for antibiotic activity following fermentation in media previously formulated for production of benzylpenicillin and cephalosporin C. No evidence of cephalosporin C activity was observed for any of the cultures, although a number of cultures were shown to be producers of penicillin and 6-aminopenicillanic acid, a property common to a number of dermatophytes[7,8,9,10,11]. *Trichophyton verrucosum* CDC 29 produced an antibiotic characteristic of a penicillin which was inhibitory to gram-positive bacteria, was stimulated by precursor, and was inactivated by penicillinase. This organism can be added to the number of antibiotic-producing *Trichophyton* species.

As seen in Table 1 the fermentation broth from *Trichophyton mentagrophytes* contained penicillin; there was increased activity when precursor was added to the fermentation medium and the antibiotic activity was rapidly destroyed by penicillinase. In contrast, neither *Microsporum gypseum* nor the two mutant strains of *Nannizzia incurvata*[12], the perfect (sexual) stage of this dermatophyte, showed a significant increase in antibiotic activity when precursor was added. Furthermore, the antibiotic produced by the imperfect and perfect forms of this fungus was not inactivated by penicillinase. In addition, the gram-positive activity produced by these fungi was not lost following treatment with cephalosporin β lactamase. Antibiotic activity from the three enzyme-treated broths could be extracted at pH 3.0~5.0 with any of several organic solvents (butanol, ethyl acetate, methylisobutylketone, or benzene). The activity could be further concentrated by re-extraction first into dilute sodium hydroxide, and then, after acidification, extraction into ethyl acetate. The ethyl acetate solution was then chromatographed on a silica gel (Grace type 62) column.

Appropriate column fractions (as indicated by thin-layer chromatography on silica gel) were combined, concentrated, and compared with cephalosporin P_1, helvolic acid, and fusidic acid on several paper and silica gel thin-layer chromatographic systems**. Subsequent bioautographs of chromatograms from each of these systems on plates seeded with *Bacillus subtilis* ATCC 6633 revealed strong zones of inhibition. Chromatographic fractions prepared from fermentations of each of three dermatophytes displayed Rf values identical to fusidic acid in all the thin-layer systems listed. The comparative Rf values for four solvent systems used with paper chromatography are seen in Table 2.

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** Silica gel thin-layer chromatographic systems used were: 1) ethyl acetate, 2) ethyl acetate saturated with water, and 3) ethyl acetate-methanol (9 : 1).

Table 1. The influence of precursor and penicillinase on the antibiotic activity found in dermatophyte fermentation broths.

Organism[1]	Pre-cursor[2]	Penicil-linase	Zones of inhibition (mm)				
			Staphylococcus aureus 3055	*Staphylococcus aureus* X-238[3]	*Bacillus subtilis* X-12	*Bacillus subtilis* X-12.1[3]	*Escheri-cheae* X-161
Trichophyton mentagrophytes (granulare) CDC 27[4]	+	−	24	25	23	14	−
	+	+	Tr[5]	−	Tr	−	−
	−	−	16	12	18	12	−
	−	+	−[6]	−	−	−	−
Microsporum gypseum CDC 22	+	−	18	Tr	−	−	−
	+	+	15	−	−	−	−
	−	−	27	24	14	16	−
	−	+	26	25	13	14	−
Nannizzia incurvata S10-1	+	−	20	22	14	20	−
	+	+	18	21	12	18	−
	−	−	19	20	15	18	−
	−	+	17	20	14	17	−
Nannizzia incurvata G12-4	+	−	10	6	−	8	−
	+	+	9	7	−	7	−
	−	−	8	8	−	7	−
	−	+	7	8	−	6	−

1) Fungi were grown at 25°C for 216 hours on a medium containing Nutrisoy flour (1.5 %), NH_4SO_4 (0.1 %), $CaCO_3$ (0.3 %), corn meal (3 %), and methyloleate (1 %) in H_2O.
2) Phenoxyacetic acid (+ indicates addition of precursor or penicillinase).
3) Erythromycin resistant.
4) Penicillin identified by paper chromatography.
5) Tr=zone of inhibition detected but too small for measurement.
6) −=no antibiotic activity detected by plate agar diffusion assay.

Table 2. Comparison of the Rf values of an unknown antibiotic produced by dermatophytes with fusidic acid, helvolic acid, and cephalosporin P_1.

Culture	Antibiotic	Solvent systems (Rf values)			
		A	B	C	D
**Microsporum gypseum* CDC 22	Fusidic acid	0.11	0.50	0.50	0.65
	Helvolic acid	0.45	−	−	−
	Cephalosporin P_1	−	0.72	0.65	0.78
	*Unknown	0.12	0.52	0.53	0.62
**Nannizzia incurvata* S10-1	Fusidic acid	0.11	0.5	0.52	0.63
	Helvolic acid	0.45	−	−	−
	Cephalosporin P_1	−	0.69	0.68	0.75
	*Unknown	0.13	0.52	0.58	0.61
**Nannizzia incurvata* G12-4	Fusidic acid	0.11	0.51	0.48	0.66
	Helvolic acid	0.45	−	−	−
	Cephalosporin P_1	−	0.75	0.60	0.78
	*Unknown	0.13	0.52	0.48	0.64

Solvent system
A—Methylisobutylketone saturated with H_2O, plus 2 % piperidine
B—Methylethylketone - benzene - H_2O (1 : 5 : 1)
C—7 % NaCl plus 2.5 % methylethylketone in water
D—17.4 g K_2HPO_4 plus 30 ml ethanol per liter water
* Indicates culture producing unknown antibiotic.

Recently, WALLERSTROM[13] reported that an *Epidermophyton floccosum*-filtrate, active against staphylococci, indicated cross resistance with fucidin. The discovery of an antibacterial activity closely resembling fusidic acid in dermatophyte cultures adds to the number of antibiotics produced by these fungi. Moreover, this study has revealed antibiotic production for the first time by a dermatophyte and its associated ascigerous stage.

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