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DESFERRITRIACETYLFUSIGEN, AN ANTIBIOTIC FROM *ASPERGILLUS DEFLECTUS*

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Aspergillus deflectus CBS 109.55 when grown on an iron-free medium produces several antibiotics; one of these was isolated and identified as desferritriacetylfusigen. It inhibits the growth of bacteria, whereas yeasts and fungi are not or only weakly affected.

Triacetylfusigen was reported recently among the sideramines produced by several species of *Aspergilli*[1] and one species of *Penicillium*[2]. Triacetylfusigen is a cyclic triester composed of three molecules of N^{α}-acetyl-N^{δ}-(*cis*-5-hydroxy-3-methylpent-2-enoyl)-N^{δ}-hydroxy-L-ornithine. We found desferritriacetylfusigen to occur among the antibiotics present in the culture broth of *Aspergillus deflectus*. So far, none of the desferrisideramines produced by fungi has been described to exhibit antimicrobial activity. On the other hand, several trihydroxamates excreted by actinomycetes *e.g.* sideromycins and nocardamine (desferrioxamine E) are well known for their antibiotic activity.[3] Desferrioxamine B has also been reported to be slightly active against *Proteus vulgaris*.[4]

In the present paper we will describe the isolation, characterization and the biological properties of desferritriacetylfusigen.

Production

Aspergillus deflectus was grown at 24°C on an asparagine medium composed of: 10 g, asparagine; 50 g, glucose; 5 g, KH_2PO_4; 5 g, $MgSO_4$; 1 ml, HOAGLAND mineral solution[5]; 1,000 ml, H_2O; pH 3.8. After incubation for 5～7 days on a rotary shaker, the cells were harvested when the pH had reached values between 5 and 6.

Isolation and Characterization

The mycelia were separated on a BÜCHNER funnel and discarded. The filtered broth was concentrated to one tenth of its original volume and extracted with ethyl acetate. After drying over $CaSO_4$ (sikkon, Fluka), the ethyl acetate was evaporated *in vacuo* and the residue was extracted with diethyl ether. The ether was discarded. The remaining oily brown paste was dissolved in methanol, applied to a Sephadex LH-20 (Pharmacia Fine Chemicals Inc.) column which was eluted with methanol. The fractions containing desferritriacetylfusigen were detected by addition of iron (III) chloride to a small aliquot and combined. After removal of the solvent the dry residue was dissolved in water and applied to a Sephadex G-10 column. The colorless fractions containing the antibiotic were pooled and lyophi-

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lized, yielding a white powder. The average yield of pure product from one liter of culture broth was about 150 mg.

The product was identified by thin-layer chromatography and its UV- and IR-spectra. After hydrolysis (6 N HCl; 12 hours), only N^{δ}-hydroxy-L-ornithine and 3-methylpent-2-eno-5-lactone were found.

N^{δ}-Hydroxy-L-ornithine was identified by electrophoresis at pH 4.5 and by paper chromatography in phenol - water (4: 1). The chromatogram and the electrophorogram were stained with ninhydrin as well as with triphenyltetrazolium chloride.[6] When applied to an amino analyser (Beckmann Unichrom) only one peak corresponding to hydroxyornithine could be detected.

3-Methylpent-2-eno-5-lactone was extracted from the hydrolysate with chloroform and identified by thin-layer chromatography on silica gel. The chromatogram was developed in propanol - ammonia (100: 26) and spotted with $KMnO_4$[7] or hydroxylamine-ferric chloride.[8] The analytical data thus obtained were found to be identical with those of triacetylfusigen kindly provided by Dr. H. DIEKMANN.

Biological Properties

Table 1 shows the spectrum of activity of desferritriacetylfusigen. Only bacteria are sensitive. The antibiotic activity depends to a great extent on the medium. When grown on a minimal medium most organisms are sensitive, whereas a few strains are inhibited also when grown on a complex medium, for example *Bacillus brevis, Clostridium pasteurianum, Pseudomonas fluorescens* and *Streptomyces viridochromogenes*. Even yeasts become sensitive to some extent when grown on minimal media. The minimal inhibition concentrations are more than one order of magnitude higher for yeasts than those for bacteria.

As shown in Table 2, the antibiotic activity of desferritriacetylfusigen may be abolished by addition of iron (III). The presence of some iron-containing triacetylfusigen does not affect the antibacterial activity, which is always proportional to the amount of the desferri compound in the mixture.

Table 3 shows the sensitivity of *E. coli* K 12 aroB AB 2847 against desferritriacetylfusigen. This mutant is not capable to synthesize its own iron-carrier enterochelin unless the precursor dihydroxybenzoic acid is added.[9] If the precursor is added to the medium, the M.I.C. in-

Table 1. Antimicrobial activity of desferritriacetylfusigen

Organism	M.I.C.* (μg/ml)	
	Complex medium	Minimal medium (iron free)
Bacillus brevis	1～10	ng**
Bacillus subtilis Tü*** 203	>300	1～5
Bacillus subtilis ATCC 6051	>300	5～10
Bacillus subtilis ATCC 6633	10～100	ng
Clostridium pasteurianum	10～50	
Escherichia coli K 12	>300	5～10
Lactobacillus casei	>200	ng
Leuconostoc mesenteroides	>200	ng
Proteus vulgaris	>200	5～10
Pseudomonas fluorescens	50	ng
Sarcina lutea	50	ng
Staphylococcus aureus	>200	10～50
Streptococcus faecalis	>200	
Streptomyces viridochromogenes	10～50	
Streptomyces sp. PRL 1642	>300	
Streptomyces sp. ATCC 23836	>300	
Aspergillus panamensis	>200	>200
Botrytis cinerea	>200	
Candida albicans	>200	>200
Fusarium cubense	>200	>200
Penicillium notatum	>200	>200
Rhodotorula rubra	>200	100
Saccharomyces cerevisiae	>200	

*Serial-dilution test[10]-Size of inoculum: 2×10^6 cells/ml

**no growth

***Culture Collection, University Tübingen, Lehrbereich Mikrobiologie I

Table 2. The antibacterial activity of desferritriacetylfusigen in the presence of triacetylfusigen. Test organism: *Streptomyces viridochromogenes*.

Desferri-compound (μg)	Ferri-compound (μg)	Inhibition zone (mm)
15	0	17
10	5	15
9	6	14
7.5	7.5	13
0	15	0

Table 3. The sensitivity of *E. coli* K 12 aroB AB 2847 in the presence of iron chelators.

Iron chelator	M.I.C. (μg/ml) for desferritriacetylfusigen
None	1
Citrate (4×10^{-5}m)	2~5
Dihydroxybenzoic-acid (4×10^{-5}m)	5~10

The size of inoculum was 2×10^{6} cells/ml. The organism was kindly provided by Dr. K. HANTKE.

Table 4. Antagonism between desferritriacetylfusigen and various iron chelators.

Iron chelator	*Bacillus brevis*	*Bacillus subtilis*	*E. coli*	*Streptomyces viridochromogenes*
Fe-Citrate	+	+	+	+
Triacetylfusigen	0	0	0	0
Ferrioxamine B	++	++	+	++
Ferrioxamine E	++	0	+	++
Coprogen	+	0	++	+
Ferrirubin	++	0	++	++
Ferricrocin	++	0	++	++
Ferrichrysin	++	0	++	++
Desferricrocin	++	0	++	++
Nocardamine	++	−	−	++

Antagonism test according to ZÄHNER *et al.*[11]
Bacillus subtilis and *E. coli* were seeded in minimal medium; *Bacillus brevis* and *Streptomyces viridochromogenes* in complex medium. All tested solutions had the same molarity (10 mM).
++: strong antagonism,
+: weak antagonism,
0: no antagonism,
−: inhibition by both substances.

creases more than five-times. The addition of citrate, a less potent iron-carrier, increases the M.I.C. twofold.

Among the tested sideramines, ferrioxamine B and the hexapeptide sideramines are the most effective antagonists of desferritriacetylfusigen. Table 4 shows the results of the antagonism test. Depending on the iron transport system, each organism shows its own pattern of antagonism. Therefore it appears to us that desferritriacetylfusigen could be of some value in elucidating the various iron uptake systems of different microorganisms.

The antagonistic properties of the iron complex (triacetylfusigen) resemble those of fusigen. The antibacterial activity of albomycin or A 22765 is not influenced by triacetylfusigen. Mutants of *E. coli* which were resistant against albomycin proved still to be sensitive against desferritriacetylfusigen. The effect of nocardamine on *E. coli* and *Bacillus subtilis*, on the other hand, was antagonized by triacetylfusigen.

All these findings indicate that the iron chelated by triacetylfusigen is no longer available to bacteria. The inhibition of growth is thus the result of a strong iron deficiency. It remains to be investigated whether triacetylfusigen is capable of permeating the cells or not, or whether bacteria lack the enzymes to transfer the iron from the chelate to cellular macromolecules for metabolic use in a similar fashion as found by ARCENEAUX and BYERS for *Bacillus megaterium* and the iron complex of desferal.[12] On the other hand, fungi are hardly affected by desferritriacetylfusigen. This can be explained by the wide distribution of this iron chelator which is produced by many *Aspergilli*, the *Penicillium* species investigated by EMERY[2] and by several other *Penicillia* (G. WINKELMANN personal communication), as well as by some Basidiomycetes (P. BLANZ personal communication). EMERY[13] isolated from his strain of

Penicillium an enzyme which hydrolyzes the ferric hydroxamate chelate of triacetylfusigen, thus making the iron available for the cell. These results suggest that triacetylfusigen is an important component in the iron transport systems of the many species of Ascomycetes and Basidiomycetes which produce this compound.

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