NOTES

ISOLATION AND IDENTIFICATION OF ALTHIOMYCIN FROM *CYSTOBACTER FUSCUS* (MYXOBACTERALES)*

BRIGITTE KUNZE, H. REICHENBACH, H. AUGUSTINIAK and G. HÖFLE

Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1 D-3300 Braunschweig, F. R. G.

(Received for publication December 14, 1981)

In the course of a screening program for new antibiotics from gliding bacteria, *Cystobacter fuscus* strain Cb 685 was found to produce a sulfur containing, acid and alkali labile antibiotic. The antibiotic was identified as althiomycin.

Cystobacter fuscus strain Cb 685 was isolated in March 1977 from rabbit dung collected in Tenerife. For antibiotic production, the organism was grown in peptone liquid medium (1% peptone from casein, tryptically digested, Merck, Darmstadt; 0.1% $MgSO_4 \cdot 7H_2O$; pH 6.8). Antibiotic production was performed in type b 50 (total volume 100 liters) and type b 200 (total volume 365 liters) fermentors from Giovanola Frères SA, Manthey, Switzerland, equipped with a circulating pump stirrer system ("intensor system"). Silicone antifoam agent (Merck, Darmstadt) was added to the medium at a concentration of 0.01%. Cultures in type b 50 fermentors were started by inoculating 63 liters of medium with 7 liters, in type b 200 fermentors by inoculating 220 liters of medium with 10 liters of log phase shake cultures. The fermentors were maintained at 30°C and 500 r.p.m. The aeration rate was 0.1 v/v/m. The pH was not regulated and increased during growth from 6.8 to 8.2. Under these conditions, the antibiotic accumulated in the culture medium during the growth phase and reached its maximum after 2~3 days, when the pH had risen to 7.6. Thereafter the antibiotic activity rapidly decreased. Cultures maintained at a pH of 7.6 reached a higher cell density, but the antibiotic activity did not further increase. The maximum yield was around 2.7 mg/liter.

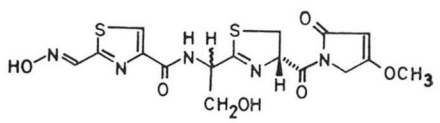
The cell free culture supernatant, obtained by centrifugation, was extracted with ethyl acetate. The extract was concentrated under reduced pressure at 40°C to a dark oily residue from which *n*-heptane precipitated the active material. Chromatography on Sephadex LH-20 (with methanol) and subsequently on reversed-phase silica gel RP-18 (Merck, Darmstadt; with 1/15 M phosphate buffer, pH 6.1 - methanol, 3: 2) yielded the pure antibiotic. Typically, 54 mg of antibiotic could be isolated from 70 liters of fermentation broth.

The antibiotic was obtained as microcrystalline white powder with a melting point of 181~183°C. It was soluble in methanol, acetone, dimethylsulfoxide, sparingly soluble in water, and insoluble in ether. In aqueous solution, it was reasonably stable only between pH 5.0 and 7.0, which complicated isolation. On thin-layer chromatograms (silica gel, Merck F_{254}, chloroform - acetone, 1: 1) the antibiotic was detected as a dark spot under the UV lamp (254 nm) at Rf 0.4. These data and the molecular formula, $C_{16}H_{17}N_5S_2O_6$, deduced from elemental analysis and an electron impact mass spectrum (m/z 421 = $M^+ - H_2O$) suggested the structure of althiomycin (Fig. 1)[4] for the isolated antibiotic.

This was proved by the ^1H and ^{13}C NMR spectra which were identical with those reported for althiomycin[2,4], including the doubling of some signals which is caused by the presence of two diastereomers in the ratio 1: 1. Interestingly, this doubling was not observed by BYCROFT and PINCHIN[1], who investigated a sample of althiomycin of indefinite origin. We were not able with a variety of chromatographic techniques to separate the diastereomers.

In addition to *Cystobacter fuscus* strain Cb 685 the following myxobacteria could be shown

Fig. 1. The chemical structure of althiomycin.



* Article No. 5 on antibiotics from gliding bacteria. Article No. 4: THIERBACH, G. & H. REICHENBACH, Biochim. Biophys. Acta 638: 282~289, 1981

by spectroscopic methods to also produce althiomycin: *Myxococcus virescens* strain Mx v12, isolated from sheep dung collected in St. Paul, Minnesota, USA; *Myxococcus xanthus* strain Mx x52, isolated from a soil sample (obtained by courtesy of Dr. J. LEHMANN) from the environments of Tokyo, Japan; and *Myxococcus virescens* strain Mx v54, isolated from a soil sample (obtained by courtesy of Dr. D. GERTH) from Syria. It thus seems that there are different genera and species of myxobacteria that produce althiomycin, as well as different species of *Streptomyces*. In this connection we would like to mention that we obtained from *Streptomyces matensis* (type strain DSM 40188=ATCC 23935) an antibiotic which could unequivocally be identified with althiomycin by its chromatographic behavior and by ^{1}H NMR spectroscopy. There was a second antibiotic activity which also inhibited Gram-positive and Gram-negative bacteria including *E. coli*, but migrated close to the front of the chromatogram. However, the data given for matamycin leave no doubt that the former compound was meant in the description of the antibiotic[5], so that the identification of matamycin with althiomycin is obviously correct. On the other hand, we were not able to isolate althiomycin from *Streptomyces bellus* (type strain DSM 40185=ATCC 14925) supposed to produce matamycin[6]. The cultures contained another, unidentified antibiotic activity, which inhibited Gram-positive bacteria only. As expected, cultures of *Streptomyces althioticus* (type strain DSM 40092=ATCC 19724) yielded althiomycin without any problems.

There are now many examples known for antibiotics which are produced by totally different organisms[3]. Still it is astonishing that such a complex structure like althiomycin is found in *Streptomyces* sp. and in different myxobacteria. It would be interesting to know more about the biosynthetic pathways leading in these totally unrelated organisms to the same secondary metabolite, and about the genetic background controlling these pathways.

Acknowledgements

The fermentations for antibiotic production were performed by W. WANIA and his collaborators in the fermentation service of the GBF. The mass and NMR spectra were run by Drs. L. GROTJAHN and V. WRAY. Mrs. S. LUCKA and Mrs. O. EBELMANN provided careful technical assistance. Dr. H. IRSCHIK discovered althiomycin in Mx v54. We wish to thank all these persons for their collaboration.

References

1) BYCROFT, B. & R. PINCHIN: Structure of althiomycin, a highly modified peptide antibiotic. J. Chem. Soc., Chem. Commun. 1975: 121~122, 1975
2) KIRST, H.; E. SZYMANSKI, D. DORMANN, J. OCCOLOWITZ, N. JONES, M. CHANEY, R. HAMILL & M. HOEHN: Structure of althiomycin. J. Antibiotics 28: 286~291, 1975
3) LECHEVALIER, H. A.: Production of the same antibiotics by members of different genera of microorganisms. Adv. Appl. Microbiol. 19: 25~45, 1975
4) SAKAKIBARA, H.; H. NAGANAWA, M. OHNO, K. MAEDA & H. UMEZAWA: The structure of althiomycin. J. Antibiotics 27: 897~899, 1974
5) SENSI, P.; R. BALLOTTA & G. G. GALLO: Matamycin, a new antibiotic. II. Isolation and characterization. Antibiot. Chemoth. 9: 76~80, 1959
6) UMEZAWA, H. (*Ed.*): Index of Antibiotics from Actinomycetes. Tokyo University Press, and State College, Pennsylvania: University Park Press. pp. 122, 404, Tokyo, 1967