THE DETECTION OF MEMBERS OF THE OLIVANIC ACID FAMILY IN *STREPTOMYCES GEDANENSIS*

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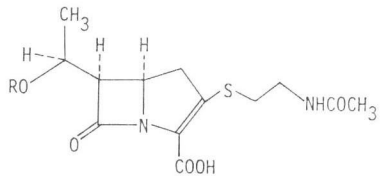
During investigations into the production of β-lactamase inhibitory activity by microorganisms we studied the culture *Streptomyces gedanensis* ATCC 4880. This culture has been reported by Hata *et al.*[1] to produce β-lactamase inhibitory activity, the active component designated KA107 was shown to be a high molecular weight substance, probably a protein. Our studies have shown that *S. gedanensis* produces, in addition to KA 107, a series of low molecular weight inhibitors which are all members of the previously described[2,3] family of antibiotics, with β-lactamase inhibitory activity, the olivanic acids (Fig. 1).

In our preliminary studies *S. gedanensis* was grown in a range of fermentation media. The β-lactamase inhibitory activity of the resulting culture filtrate samples was determined by an automated method[3] using a cell free R_{TEM} enzyme preparation with penicillin G as substrate. Samples were also assayed using the previously described[4] agar plate method for detecting β-lactamase inhibitors. In this method, test samples were placed in holes cut in the agar plates seeded with the test organism *Klebsiella pneumoniae* ATCC 29665. Significant levels of inhibitory activity were detected in both test systems. This result was unexpected since *K. pneumoniae* produces an intracellular β-lactamase, and a high molecular weight compound would not be able to diffuse through the bacterial cell wall to inhibit the β-lactamase. This suggested the presence of a low molecular weight β-lactamase inhibitor in the culture filtrate samples and studies were undertaken to characterise this inhibitor.

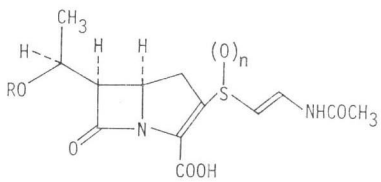
S. gedanensis was inoculated into seed stage medium (50 ml in 250 ml Erlenmeyer flasks), the medium had the following composition: Dextrin, 2%; yeast extract paste, 1%; NaCl, 0.5%; KH_2PO_4, 0.5%; adjusted to pH 7.0. The seed stage was incubated at 26°C on a gyrotary shaker for 48 hours and used to inoculate (5%) the production medium (50 ml in 250 ml Erlenmeyer flasks) of the following composition: Medium A, Glycerol, 2%; soybean flour, 1%; $CaCO_3$, 0.02%; Na_2SO_4, 0.05%; $CoCl_2 \cdot 6H_2O$, 0.0001% prepared in deionised water and adjusted to pH 7.0. Medium B as medium A but without Na_2SO_4. The fermentations were carried out at 26°C on a gyrotary shaker.

Samples of culture filtrate were assayed by ion exchange thin-layer chromatography on weakly

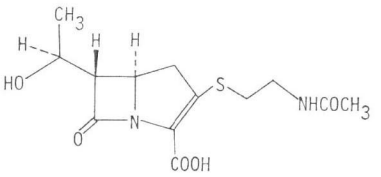
Fig. 1. The structures of the olivanic acids.



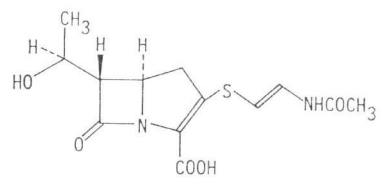
MM 22380 R = H
MM 17880 R = SO_3H



MM 22382 R = H n = 0
MM 13902 R = SO_3H n = 0
MM 4550 R = SO_3H n = 1



MM 22381



MM 22383

Fig. 2. Thin-layer chromatography of culture filtrate samples from *S. gedanensis*.
Support : DEAE cellulose
Solvent : 0.1 M NaCl in 0.05 M potassium phosphate buffer pH 7.0
Assay organism: *B. subtilis*

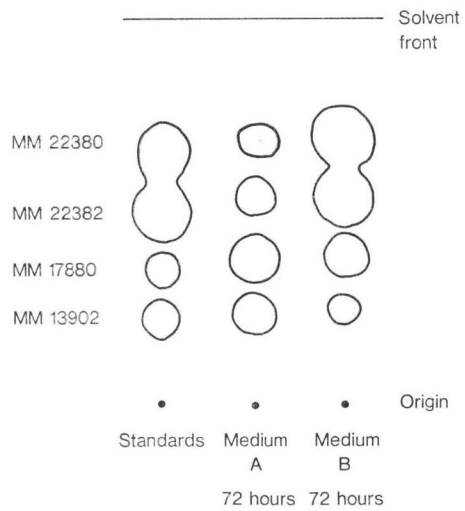


Table 1. High performance liquid chromatography of culture filtrate samples from *S. gedanensis*.

Compound	Retention time (minutes)	Peak height (mm) in culture filtrate (72 hours)	
		Medium A	Medium B
MM 4550	2.9	60	32
MM 17880	4.4	20	8
MM 22380	5.5	6	18
MM 22381	7.7	2	2
MM 13902	9.9*	16	26
MM 22382	10.0*		
MM 22383	12.5	14	20

* These compounds were not adequately resolved. Peak height represents combined levels.
Chromatography on a Waters C_{18} μBondapak column eluting with 3 % acetonitrile in 0.05 M ammonium phosphate buffer pH 4.7 at 2 ml/minute. Effluent monitored for UV absorption at 300 nm.

basic diethylaminoethyl cellulose[3] using 0.1 M NaCl in 0.05 M potassium phosphate buffer as eluant. The presence of antibiotic activity was detected by laying the thin-layer chromatography plates on agar seeded with *Bacillus subtilis* ATCC 6633. Standard solutions of the olivanic acids were chromatographed on the same thin-layer chromatography plates as markers. Results of the chromatography of 72 hours old fermentation samples are shown in Fig. 2. The results demonstrated that *S. gedanensis* produced at least four antibacterially active components with Rf values consistent with four members of the olivanic acid family, MM 13902, MM 17880, MM 22380 and MM 22382. Further identification of the compounds was achieved by high performance liquid chromatography of culture filtrate samples. Samples were chromatographed on a C_{18} reversed phase column eluting with 3% acetonitrile in 0.05 M ammonium phosphate buffer (pH 4.7). The results of these assays on the same culture filtrate samples as described above are summarized in Table 1. These results confirmed the presence in the culture filtrate of *S. gedanensis* of the seven previously described olivanic acids, MM 4550, MM 13902, MM 17880, MM 22380, MM 22381, MM 22382 and MM 22383. The ratio of the sulphated to nonsulphated derivatives being determined by the presence or absence of sodium sulphate in the medium in a similar manner to that described for these antibiotics in *Streptomyces olivaceus*[3]. The ability of *S. gedanensis* ATCC 4880 to produce the olivanic acids was of particular interest in view of the previously described ability of the culture to produce the high molecular weight β-lactamase inhibitor.

References

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