

Technical method

A rapid method for identifying bacterial enzymes

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Biochemical tests for characterizing bacteria are often time-consuming. They generally involve detecting one or more bacterial enzyme by using colour changes, coagulation, turbidity or gas formation as indices of enzyme activity. These visual changes depend on the conversion of relatively large amounts of substrate by the enzyme. Qualitative tests for bacterial enzyme activity could be made much more rapid by increasing the sensitivity of detection of the reaction products by the human eye.

We describe here a simple and rapid method for detecting bacterial enzymes that depends on the release of a fluorescent reaction product (methylumbelliferone) from a non-fluorescent substrate (methylumbelliferyl substrate).

The following substrates were obtained from Koch-light Laboratories Ltd, Bucks, England.

- A 4-methylumbelliferyl- β -D-glucopyranoside (for β -D-glucosidase)
- B 4-methylumbelliferyl- β -D-galactopyranoside (for β -D-galactosidase)
- C 4-methylumbelliferyl- β -D-glucuronide (for β -D-glucuronidase)
- D 4-methylumbelliferyl-2-acetamido-2-deoxy- β -D-glucopyranoside (for N-acetyl- β -D-glucosaminidase)
- E 4-methylumbelliferyl- α -D-mannopyranoside (for α -D-mannosidase).

Fifteen μ mol of each substrate except B (5 μ mol) were dissolved in 0.2 ml of dimethylsulphoxide, and the volume of each solution was made up to 10 ml with phosphate buffered saline pH 7.3 (Dulbecco 'A', Oxoid Ltd, London).

Two standard bacteria were studied, *Pseudomonas aeruginosa* (NCTC 10490) and *Escherichia coli* (NCTC 10418). These were maintained on Dorset egg slopes and cultured on DST agar (Oxoid Ltd, London) and McConkey agar respectively.

To test for the presence of a bacterial enzyme, a sample of a colony was taken with a glass rod and rubbed vigorously onto a Whatman No. 1 qualitative filter paper (Balston Ltd, England). The bacterial smear was then covered with 2 drops (30 drops per

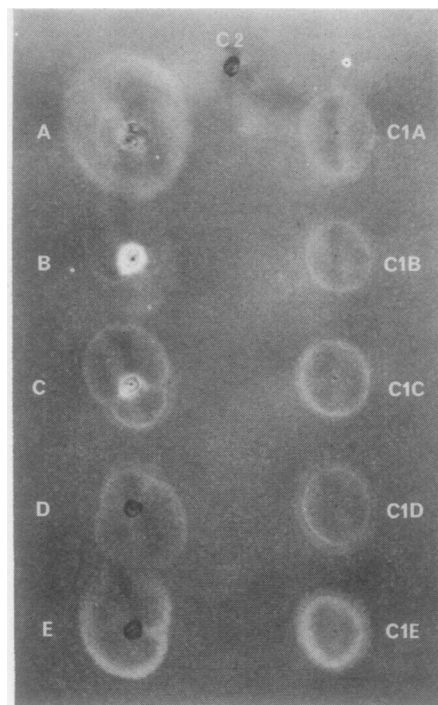


Figure Effect of *Esch. coli* (NCTC 10418) on methylumbelliferyl substrates for: (A) β -D-glucosidase, (B) β -D-galactosidase, (C) β -D-glucuronidase, (D) N-acetyl- β -D-glucosaminidase, and (E) α -D-mannosidase. C1 A to E: controls of methylumbelliferyl substrates only C2: control of *Esch. coli* + substrate solvent

ml) of substrate, and a further 2 drops of substrate were placed alongside to serve as a control. Another bacterial smear covered by 2 drops of the solvent in which the substrate was dissolved served as a second control. This procedure was performed with each of the five substrates using *Ps. aeruginosa* and *Esch. coli*. After incubation at 37°C for 10 minutes the spots on the filter paper were covered with 2 drops (30 drops per ml) of 0.1N NaOH. Alkali increases the fluorescence intensity of methylumbelliferone and also washes it away from the bacterial smear so that it can be more easily seen. The spots on the filter paper were viewed in a dark room under ultraviolet light (366 nm).

The results with *Esch. coli* (NCTC 10418) are shown in the accompanying figure. There was light blue fluorescence of methylumbelliferone with substrates A, B, and C, indicating that the organism possessed the enzymes β -D-glucosidase, β -D-galactosidase, and β -D-glucuronidase respectively. The intensity of the fluorescence was in the order B > C > A. Tests with substrates D and E gave no

light blue fluorescence and neither did any of the controls. The *Esch. coli* smears gave a light brown fluorescence.

With *Ps. aeruginosa* (NCTC 10490) there was no light blue fluorescence of methylumbelliferone with any of the substrates A-E, showing that the tests for enzyme activity were negative. However, the organism had a green fluorescence, presumably due to fluorescein, and possibly this could have masked methylumbelliferone fluorescence.

This simple method for identifying bacterial enzymes can be performed within minutes compared with 18 to 24 hours using standard biochemical

tests. Obviously the scope of this method depends on the availability of suitable substrates. There are, however, 22 derivatives of methylumbelliferone available commercially which are enzyme substrates, and doubtless others could be synthesized should there be a demand for them. We consider that the rapidity of this method might be important both diagnostically and economically and justifies its further evaluation.

We are grateful to Mr. P. Blake for photography. When this work was carried out M.J.G. was a pupil of Atlantic College, St. Donat's Castle, Glam.

Letters to the Editor

Orientation of Blocks from Cervical Cones

For many years we have used a method for orientation of blocks and sections from unbisected cervical cones which is more simple than that outlined by Sanerkin and Fraser (*J. clin. Path.*, 1975, **28**, 202). They identify the anterior lip of the cervix by transfixing it with a needle and infiltrating the tissue with Indian ink. A quicker way, and just as effective, is to make a superficial incision across the ectocervical surface of the anterior lip with a razor blade. After parallel slicing of the cervix, at right angles to the razor cut, it is easy to identify the cuts on the anterior lip of the cervix in the subsequent sections.

There is an additional procedure which we find useful. The purpose of the serial blocking of the cervix is to ensure a step-wise examination of the whole specimen. Therefore it is essential to make sure that the blocks are all orientated satisfactorily in the paraffin wax. This is facilitated by applying a spot of Indian ink on the surface of the block opposite to the side from which sections are to be cut. The first spot goes on the rounded surface of the first block at 9 o'clock. The only block which is not examined in serial fashion is the final block from the edge of the cervix at 3 o'clock, which is blocked on the flat surface.

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The authors have commented as follows:

Making an incision across the anterior lip of the ectocervix readily comes to mind and was certainly tried out at our laboratory at St. David's Hospital before we decided to search for a more satisfactory procedure. We found the incision method quite unacceptable because, in our experience, many cones have numerous transverse surgical nicks on both lips, and in examining the final sections we could not be certain which 'nick' was ours and which the surgeon's.

Marking the 'upper surface' of tissue blocks is a routine procedure at most laboratories, and certainly at St David's Hospital, to ensure that the correct ('lower') surface is trimmed and sectioned. Instead of a spot, however, we use a cross with Indian ink.

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Book reviews

Frontiers of Radiation Therapy and Oncology, Vol. 9. The Relationship of Histology to Cancer Treatment. Proceedings of the 9th Annual West Coast Cancer Symposium, San Francisco, 1973. Edited by J. M. Vaeth. (Pp. viii + 316; 111 figures; 75 tables. £20.60.) Basel, Munich, Paris, London, New York, and Sydney: S. Karger AG. 1974.

On the whole, pathologists have been slow to integrate modern experimental techniques into their field with the aim of improving the prognostic and diagnostic accuracy of histopathology, although recent developments in the lymphoma field, particularly the use of lymphocyte surface markers, show a promising trend in this direction. With this objective in mind and given the considerable developments of modern cell biology it is perhaps a little disappointing to find in this volume such a complete dichotomy between experimental and routine histopathological clinical considerations. Although the difficulties are not to be minimized, it is clear that careful observation of the behaviour of untreated and treated tumours may illuminate histopathological interpretation while, on the other hand, the consideration of histological characteristics in the light of the current concepts of cell biology, and particularly cell population kinetics, may also be a valuable exercise as far as assessing the relevance of experimental findings to the human situation are concerned. In this book the first few chapters touch on these