Education Development Fund report

Proteus mirabilis: a protean tool for teaching microbial physiology

Charles Penn

A ten-credit, six-week course in microbial physiology at second year BSc level required a concise, coherent laboratory element that would excite students and illustrate basic principles. Model organism(s) had to be intellectually and visually interesting, with some strong phenotypic characters, easy to handle, and preferably reasonably harmless! One organism that met these criteria well was *Proteus mirabilis*: it is motile, with an intriguing swarming mode that is easily demonstrated, robust in the lab, fast-growing for growth experiments, and has a strong, inducible urease activity that is susceptible to experimental demonstration at student level. While not highly virulent, the organism has some medical interest as a urinary tract pathogen, sufficient to interest students with its relevance to their own (hopefully not too personal!) or friends' experiences.

The SGM grant enabled me to run through some of the experiments with an undergraduate student 'guinea pig', and make some improvements in light of student limitations of technical ability and understanding.

Five experiments were devised. First, the well-known swarming motility on plates was examined macroscopically, but more excitingly by microscopy. About 100 µl of molten LB agar was allowed to set on a microscope slide (about a 2 cm diameter area), inoculated in the centre with a light touch from a loop touched to the swarming edge of a plate culture, covered with a coverslip and left for 1 hour at 37 °C before microscopic examination by phase contrast. At least half the groups observed the fascinating reptile-like behaviour of swarming *Proteus* (in stark contrast to the rapid random motility of stationary-phase liquid cultures) and the best preparations were videotaped for playback to the class in a later debriefing session.

A growth curve could be constructed in an afternoon by inoculating a 1/50 dilution of exponential-phase culture, started in the morning, and after 3 hours shaking with half-hourly sampling, a growth curve could be plotted showing exponential growth increase (can be used to derive growth rate constant), and the beginning of entry into stationary phase at about 2·5 hours could be seen on a semi-log plot of optical density against time.

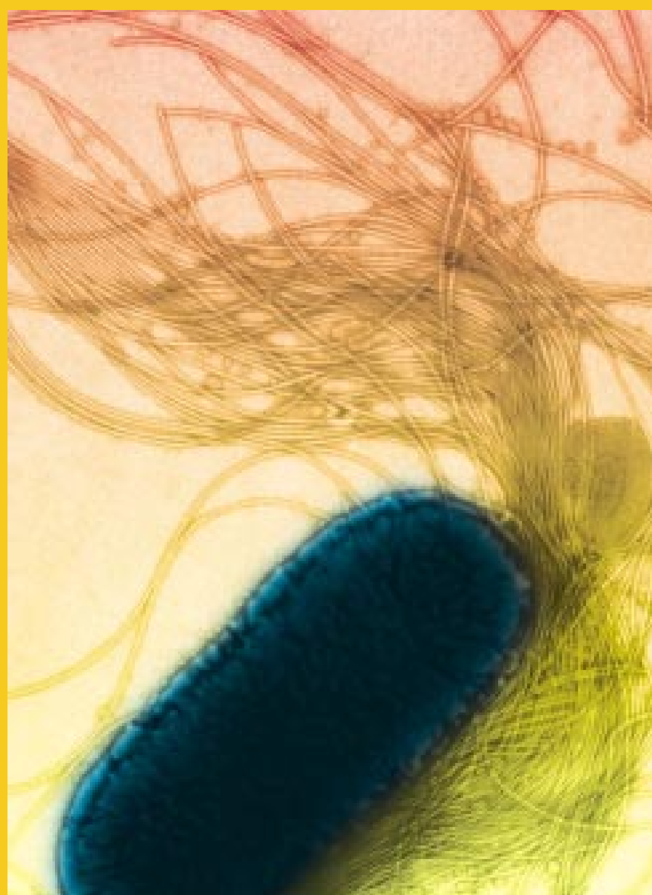
Urease activity was examined by means of a test solution containing 0·1 M phosphate buffer at pH 7, 10 mM urea and phenol red indicator, mixed with equal volumes of serial dilutions of cell suspensions in microtitre plates. After a few minutes the colour changed to magenta where significant urease activity was present and an end point could be determined for semi-quantitative determination of enzyme activity. Readings could be taken with a plate reader. It proved possible to induce urease activity in cultures supplemented with urea after about 30 minutes of exposure – again an easy objective for a 2- to 3-hour class.

It was possible to exploit the huge level of expression of flagellin in swarming cells, in a simple demonstration of the prominence of this protein by SDS-PAGE. Extraction of a loopful of actively swarming cells from an agar plate in 100 ml glycine buffer (pH 2), spinning out residual cells and neutralizing with NaOH resulted in an almost pure preparation of flagellin protein that could be seen easily on a mini-SDS-PAGE gel. By comparing swarming cultures scraped from the edge of a spreading colony with stationary-phase broth-grown cells and running whole-cell proteins alongside the flagellin, the prominence of the flagellin band in swarming cultures compared with its weakness in non-swarming cells was obvious.

Additional experiments dealt with the effect of cell size and shape (modulated by growth phase or swarming versus swimming mode) on quantitation by optical density and plate count. Because the cell size is dramatically altered not just between swarming and non-swarming mode, but also between exponential-phase and stationary-phase broth cultures, it was easy to demonstrate its effect on different methods for measuring biomass. The options are limited only by the time available and the ingenuity of the teacher. Also, it is feasible to select a small number of students to do some simple electron microscopy by negative staining – preparation of grids is very quick, and a small number of students can be taken to examine their grids in, say, a 1 hour session. Again, the results can be demonstrated to the class in a debriefing session.

Experience has shown that this series of experiments, set up on a 'circus' basis so that each student or group (we have tried groups or three or four – smaller is better!) moves round the lab to do a different experiment each week, provides a meaningful exploration and overview of an organism that seems to bring a real appreciation of the scope of the subject.

Charles W. Penn is Professor of Molecular Microbiology, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK. Tel. 0121 414 6562; email c.w.penn@bham.ac.uk



ABOVE: False-colour micrograph of *Proteus mirabilis* clearly showing the numerous flagella produced when the organism swarms. The cell is 1 µm wide. COURTESY C. PENN

Information about the SGM Education Development Fund is available on the Society website **www.sgm.ac.uk**