SHORT COMMUNICATIONS

A Simple Device for Obtaining Axenic Cultures of Phototactic Unicells

By K. J. ADAMS

*Department of Plant Biology and Microbiology, Queen Mary College
(University of London), Mile End Road, London, E. 1*

(Accepted for publication 5 September 1969)

A reliable technique was required for the cleaning of a contaminated stock culture of *Euglena gracilis* which would preclude the selection of subtle physiological mutants and provide sufficient euglenas for an inoculum in as short a time as possible.

The phototactic response exhibited by many unicells and coenobia has been exploited for years with varying success as a means of inducing cells to swim away from non-motile contaminants. The success of the simple device illustrated (Fig. 1) depends on the ease with which organisms can be injected into sterile media (with a minimum of disturbance) and then withdrawn under sterile conditions, following accumulation as a dense axenic suspension in a narrow beam of light. As the tube has a narrow bore, and is held perfectly still during separation, the organisms swim along it without inducing sufficient convection to contaminate the solution farther along the tube, permitting a high yield of 'cleaned' organisms. Thus the tendency to select physiological mutants does not arise to the same extent as with dilution separation techniques, particularly where these rely on establishing new clones from single-cell isolates. Originally devised for removing a persistent actinomycete from a laboratory stock clone of *Euglena gracilis*, this technique has been found equally suitable for 'cleaning up' the following: *Euglena gracilis* (two strains), *E. spirogyra*, *Ochromonas danica*, *Cryptomonas ovata*, *Amphidinium carteri*, *Chlamydomonas* sp., and the coenobia *Gonium sociale* and *Pandorina morum*.

Operation. The apparatus is made of Pyrex glass with the area shown solid in the diagram permanently masked with heat-resistant black enamel paint.

Before operation the ports A and B are plugged with new 'Suba-seal Turn-over Closures' (William Freeman & Co., Barnsley) the culture solution poured in, taking care to displace all the air, and the open end plugged with cotton-wool. The unit is then supported horizontally in an autoclave and sterilized intact.

To use the tube it is aligned horizontally on a burette stand, and left for a few minutes for convection to cease. A suitable quantity of the contaminated organism is then slowly injected with a syringe through seal A. The ports are the same diameter as the main tube so that any small bubbles or buoyant contaminants become trapped in the port. As the tube is masked except for the narrow zone around port B, the phototactic cells will accumulate as a dense suspension within a light beam shone onto this region. A sterile syringe is used to withdraw the cleaned suspension through seal B, after the top of the seal has been sterilized with ethanol.

The depression below port B will be found useful for accumulating sluggish species such as *Euglena spirogyra*, which tend to settle out after arriving in the light beam. That below port A will trap heavy contaminants.

Fig. 1. Tactic Unicell Separator. For explanation see text.

The dimensions are probably not very critical but a ratio of 10:1 for the tube length/diameter has given satisfactory results. With a 10 cm. swimming path, *Euglena gracilis* should arrive in the collecting zone after 30 min. A single run is generally adequate to provide bacteria- and fungus-free cultures, but if several runs are necessary new culture solution is required. This is most readily achieved by preparing a number of the units to be used in sequence. In *E. gracilis* and possibly other organisms the phototactic response is lost in continuous light; a 6 to 10 hr dark pretreatment may be required to induce a maximum response.

Other uses. This device could be used for a variety of phototactic and chemotactic separations. Phototactic species with differing swimming rates could be separately concentrated by repeated passes through the tube. Negatively chemotropic responses could be utilized by injecting the contaminated suspension at B, the inducing agent at the open end, with final axenic recovery at A. For a positive chemotactic response inject the suspension at A, the attractant at the open end and recover at B.

Where a culture is heavily contaminated a sample should be withdrawn as soon as the first organisms arrive at the recovery port. For cleaning organisms with contaminated mucilage sheaths a dilute agar solution might prove suitable as a swimming medium.