Affinity chromatography of *Euglena:* A novel means of sorting phytoplankton species

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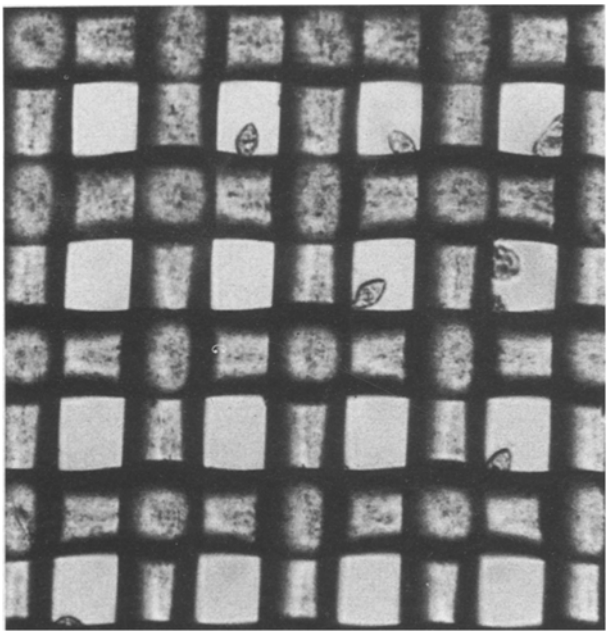
Summary. Euglena species can be selectively bound to nylon using an E. gracilis strain Z antiserum. This affinity chromatography procedure isolates living cells and is genus-specific.

Phytoplankton ecology, in contrast with terrestrial ecology, rarely includes the physiological analysis of individual species in situ[2]. A technique is needed to isolate monospecific fractions of living algae from a plankton sample so as to permit biochemical analysis of individual species. The utilization of affinity chromatography in cell sorting[3] suggests that this biologically specific technique might be adapted to separate a given phytoplankton species from the diverse particulate matter present in plankton samples. To examine whether this immunological technique can specifically isolate an algal species, the following procedure was developed, using Euglena gracilis.

E. gracilis strain Z was cultured axenically[4] and a total of 50 mg (dry wt) of either living or 0.2% formalin-fixed cells were injected i.m. into rabbits over a 6-week-period. Serum was inactivated at 56 °C for 30 min and stored frozen. Sera generated against either the living or formalin-fixed cells immobilized Euglena at dilutions of $^1/_{600}$ to $^1/_{2000}$; the cells shed their flagella and assumed an immobile, spherical shape[5], but after 2 h they regenerated the flagellum and regained normal activity.

In order to use anti-Euglena serum for affinity chromatography isolation of Euglena, a double antibody method was employed. Anti-rabbit IgG (Cappel Laboratories, Downington, PA) was covalently linked to 1 cm discs of fine nylon screen (Nitex, 62 μm mesh) following the procedure of Inman and Hornby[6]; 24 screen discs were incubated with 2.8 mg of protein and the screens were stored frozen. Euglena samples containing $1–2 \times 10^4$ cells were suspended in 1.0 ml of a $^1/_{10}$ dilution of anti-Euglena serum in physiological buffered saline, pH 7.5 (PBS) and incubated at 25 °C for 1 h. The cells were then washed in PBS using a 0.45 μm membrane filter, resuspended, and 0.25 ml containing $2–4 \times 10^3$ cells was gently pipetted on to an antibody-coated screen disc in 0.75 ml of PBS in a depression slide. After 30 min incubation, the screens were gently agitated to displace unbound cells. As a control, cells were incubated with rabbit sera taken prior to immunization and similarly applied to anti-rabbit IgG-treated screen discs.



Antiserum-treated Euglena gracilis attached to nylon screen, following affinity chromatography. Screen mesh diameter averages 40 μm.

Microscopic inspection of the screens revealed many antiserum-treated Euglena cells bound to the nylon (figure), but none of the control cells were attached. The firmness of the binding varied; some cells could be detached by shaking and others remained bound after several transfers of the discs from one depression well to another. The percentage of antiserum-treated cells retained on the screens ranged from 30 to 70% and the higher values were achieved by reducing the flow rate with which cells were applied to the screens. After 1 h, many of the cells had released themselves from the screen surface and were swimming normally.

To test the specificity of binding, E. gracilis strain Z was added to various mixtures of cultured algae and field-collected plankton which were then treated with anti-Euglena serum and processed as above. These samples included Scenedesmus, Ankistrodesmus, Pediastrum, Closterium, Chlorella-like organisms, Anabaena, Synedra, and miscellaneous diatoms. In all cases, Euglena was specifically removed from the mixtures and no other cells or particulate matter were bound to the screens. It is noteworthy that detritus, including detritus from Euglena cultures, was not retained on the antibody-coated screens. Testing the specificity of this method within the euglenoid flagellates revealed that the antiserum is genus-specific and that serotypic differences are not apparent between strains of E. gracilis. The antiserum developed against E. gracilis strain Z allowed affinity chromatography of 5 strains of E. gracilis (2 Z strains from different cultures, E. gracilis var. bacillarius, E. gracilis Klebs, and E. gracilis isolated from an aquarium), E. viridis, E. deses and E. acus. In contrast, Phacus pleuronectes, P. longicauda and Trachelomonas sp. were not bound to the screens using the anti-Euglena serum.

Since living Euglena cells are selectively bound by this technique and other organisms, detritus and inorganic particles are excluded, affinity chromatography offers a means of fractionating plankton samples into taxonomically defined subsamples. This procedure should allow a physiological analysis of individual phytoplankton species under natural conditions.

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