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Sample Collection

For coastal and oceanic sites plankton is usually concentrated by gentle towing with a plankton-net, e.g. 10 minutes with a 200µm and a 600µm mesh net. For hypersaline lakes the algal cell concentrations may be sufficiently high for picking out cells directly. Some species may also be collected from rock pools or the shoreline. Gentle handling and time are important factors as damaged and dying cells will not produce viable cultures.

Equipment

An inverted microscope with a long-working distance condenser and good range of lenses (x4, x10, x20 and x40 magnification) to provide easy access for pipettes is essential. A mechanical stage designed for both multiwell plates and slides is very desirable. Borosilicate glass has been used traditionally but is now being replaced by plasticware. Advantages of plasticware are that it is pre-sterilised in ready-to-use packages and some multiwell plates can be coated with growth substances which may enhance growth of many algae.

Culture Medium

Many chlorophytes, diatoms and dinoflagellates can be isolated into full strength medium. We usually isolate into Erd-Schreiber or Keller's medium. However, some species are quite sensitive so isolating into very dilute culture medium may be required. The strength of the medium can then be increased incrementally to improve growth of the culture. For halotolerant algal species such as *Dunaliella salina*, isolation directly into culture medium with added sea salt has been successful in our laboratory.

Single-Cell Isolation

This is carried out using a micropipette. Traditionally a fine glass Pasteur pipette or a glass capillary tube has been used. We usually use a sterile extended fine tip plastic transfer pipette (Alpha Laboratories Ltd. Cat. No. LW4231). The aim of micropipette isolation is to pick up a cell from the sample and deposit it, without damage, into a sterile droplet of medium. Various cell isolators (Fig. 2) have been developed to help with this process. We use a multitest slide (Fig.1) for the initial isolation.

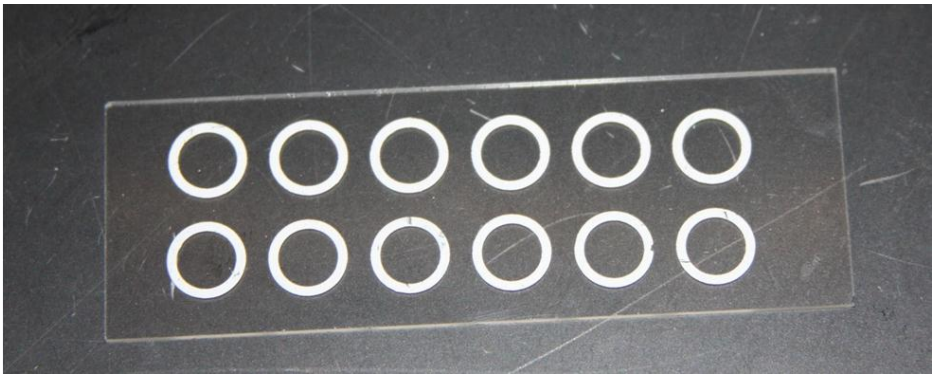

Fig1. A multitest slide for isolating individual cells

The sterile droplet containing the target cell, and probably other cells, is examined under the microscope. Using a clean pipette, the target cell is transferred to a second drop of medium. This is repeated until a single cell can be transferred into the culture vessel. We usually use sterile plastic 24-well tissue culture plates for initial culturing (Fig. 3). The process needs to be carried out enough times to ensure clean isolation of a single cell but without causing cell damage by excessive handling.

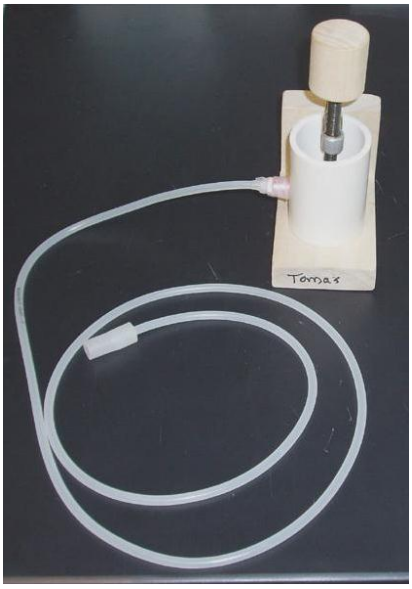

Fig 2. Algal cell isolater

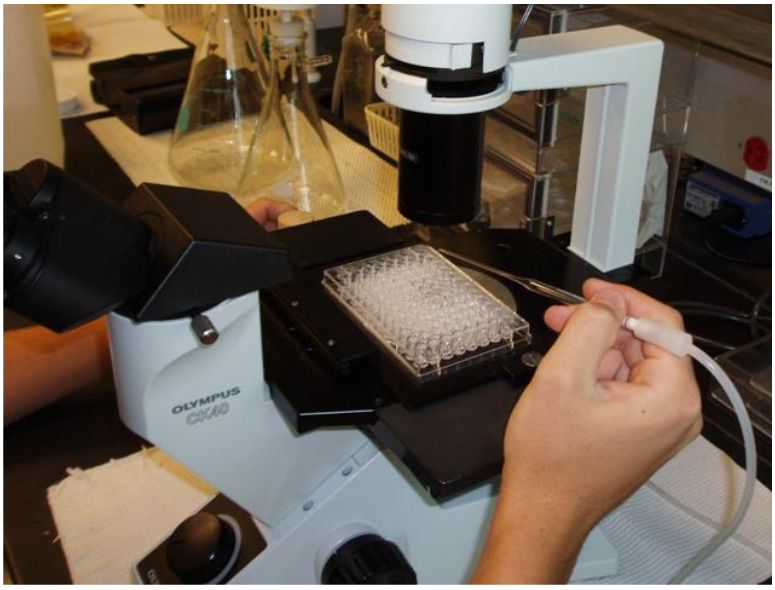

Fig 3. Using isolater to pick out cells.

Daily microscopic examination should be carried out and when the culture is growing it can be transferred to a 100ml glass flask. Once the culture is established it can be grown in larger quantities.

Other methods of isolation include; the use of agar, dilution techniques, centrifugation and phototaxis (see Andersen, 2005, Algal Culturing Techniques, Elsevier Academic Press).

Culturing Dunaliella salina

Dunaliella salina is a halotolerant species which grows relatively easily over a wide range of salinities and temperatures. In our laboratory we grow it at 15°C in Erd-Schreiber medium with added sea salt (50g l^{-1}). We use a 14 hour light 10 hour dark regime and sub-culture each strain every 2 months.