Isolation and Culture of Algae

Algal cultures are essential when conducting competition studies, bioassays, assessment of zooplankton food preferences, and determination of algal life histories. They are also necessary for molecular systematic work. Algal cultures may be "unialgal," which means they contain only one kind of alga, usually a clonal population (but which may contain bacteria, fungi, or protozoa), or cultures may be "axenic," meaning that they contain only one alga and no bacteria, fungi or protozoa. There are four major techniques for obtaining unialgal isolates: streaking, spraying, serial dilution, and single-cell isolations. Streaking and spraying are useful for single-celled, colonial, or filamentous algae that will grow on an agar surface; cultures of some flagellates, such as *Chlamydomonas* and *Cryptomonas* may also be obtained by these procedures. Many flagellates, however, as well as other types of algae must be isolated by single-organism isolations or serial-dilution techniques. We will practice spraying and single-organism isolations.

Spraying. In this technique, a stream of compressed air is used to disperse algal cells from a mixture onto the surface of a petri plate containing growth medium solidified with agar. Hold a petri plate about 18 inches from the touching tips of two Pasteur pipettes. One of these is attached to an airline via a hose, and mounted onto a ringstand. The other pipette is suspended tip-up into a container holding the algal mixture. The airflow from the first pipette creates a vacuum that draws a stream of algae-containing liquid up from the container through the second pipette. The airflow also sprays the suspended algae through the air, where they can be intercepted by the agar plate.

Single-cell/colony/filament isolations. The first step in this procedure is to prepare a number of "micropipettes" (very fine-tipped pipettes) from glass Pasteur pipettes. Hold a pipette in both hands; the tip end is held with a forceps so that the glass near the tip is within the flame of a bunsen burner (gas flame). The pipette is held in the flame only until the glass becomes slightly soft. This is determined by testing for flexibility by moving the tip with the forceps. Then the pipette is removed from the flame and pulled out straight. or at an angle so that there is a bend. **If you pull the pipette while it is still in the flame, it will seal up, so don't do this.** Always remove the pipette before pulling it! Use the forceps to break the tip. You can vary the diameter of the finely pulled tip by changing the speed of pulling; the diameter of a slowly-pulled tip will be greater than that of a rapidly-pulled tip. You would want a narrow diameter tip if you are trying to isolate very small algae, but a larger diameter tip is required for large cells. Try to match the diameter of the pipette tip to the size of the algal cells to be isolated.

Prepare a multiwell plate with sterilized media in each well. Place multiple drops of sterilized media or water onto the inside surface of a sterile petri plate. Attach a micropipette to a length of rubber tubing, attach a ethanol-sterilized mouthpiece to the the other end of the tubing, and put the mouthpiece in your mouth. Place a petri dish of algae on the stage of a dissecting microscope and locate the single cell/colony/filament to be isolated. Then find the tip of the micropipette and move it to the vicinity of the alga, then suck it up into the pipette tip, then stop the suction. Try to avoid sucking up any other algae. Now remove the pipette from the dish, then blow the liquid+alga into one of the drops of water on a petri plate). Break off and dispose of the portion of the micropipette tip that contained liquid; this has been contaminated. The micropipette can continue to be used until all of the pulled portion has been consumed. Now use the micropipette

to transfer the isolated alga from the first drop into a series of fresh drops. This is a washing procedure that helps remove contaminants. After transfer through 5-10 drops, transfer the alga into a well of the multiwell plate holding liquid growth medium suitable for that particular species. Repeat the procedure. Usually several attempts are made because not all isolated algae will continue to grow, or some may be contaminated with other algal cells.

A particularly effective means of obtaining unialgal cultures is isolation of zoospores immediately after they have been released from parental cell walls, but before they stop swimming and attached to a surface. Recently-released zoospores are devoid of contaminants, unlike the surfaces of most algal cells. But catching zoospores requires a steady hand and experience.

Filaments can be grabbed with a slightly curved pipette tip and dragged through soft agar (less than 1%) to remove contaminants. It is best to begin with young branches or filament tips which have not yet been extensively epiphytized.

Antibiotics can be added to the growth medium to discourage growth of contaminating cyanobacteria and other bacteria. Addition of germanium dioxide will inhibit growth of diatoms.

Axenic cultures (beyond the scope of this course) can be obtained by treating isolated algae to an extensive washing procedure, and/or with one or more antibiotics. Resistant stages such as zygotes or akinetes can be treated with bleach to kill epiphytes, then planted on agar for germination. It is usually necessary to try several different concentrations of bleach and times of exposure to find a treatment that will kill epiphytes without harming the alga.

Place the tubes/dishes with isolated algae into the culture room and allow growth to occur for 3-4 weeks. Examine them with the dissecting scope for signs of growth or contamination.

Freshwater Growth Media used in this class:

1) BBM is Bold's basal medium, chemically defined; good for many green algae.

2) Soil-water is undefined and used for algae whose nutritional requirements are unknown, or which will not grow on simple inorganic media. The soil should be loam from a site where herbicides have not recently been used. Sometimes it is advisable to add a dried pea to the medium before autoclaving.

3) SD11 is a defined medium that is somewhat more complex than BBM; it contains a vitamin mixture. Good for many green algae.

4) DYIII is a defined medium to which vitamins are often added, used for culture of chrysophytes and cryptomonads as well as some dinoflagellates.

Reference: Stein (ed.) 1973. Handbook of Phycological Methods. Culture methods and growth measurements. Cambridge University Press.