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Algae and cyanobacteria: isolation, culture and long-term maintenance

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INTRODUCTION

The word algae means different things to different people. To the layman, algae are commonly known as 'pond scums', 'green slime', 'seaweeds' and other non-specific terms. The scientific definition is rather elusive as they are such a diverse group. The definition given by Lee [3] is: 'The algae are thallophytes (plants lacking roots, stems, and leaves), which have chlorophyll *a* as their primary photosynthetic pigment and which lack a sterile covering of cells around the reproductive cells.' This definition includes the prokaryotic blue-green algae which are now recognized as cyanobacteria. The term 'algae' in this article will also include the cyanobacteria except where a distinction is required.

The algae are found world-wide in practically every type of environment including mountain snowfields, hot springs and desert soils. They are the major primary producers in aquatic systems, both freshwater and marine. Algae are extremely diverse in morphology and size ranging from unicells about one micrometer in diameter up to complex seaweeds many meters long. Some of the unicells and colonial forms are motile flagellates which resemble Protozoa. Most algal cells have a bounding cell membrane which may be composed of relatively pure carbohydrates or may be impregnated or layered with inorganic substances e.g. silica, calcium or magnesium carbonate [7]. Reproductive processes are highly varied and involve vegetative, asexual and sexual mechanisms. The culture of algae in the laboratory is a relatively new field. In the 103 years since Beijerinck obtained the first pure culture of *Chlorella*, only about 10% of the approximately 24 000 recognized species have been successfully maintained for long periods in culture collections. Most of these are microalgae and only this group will be considered here.

ISOLATION AND CULTURE

Cultures used in phycological research should be at least unialgal and for some studies, e.g. physiological, biochemical and genetic, the cultures should be axenic. The techniques for producing such cultures require a great deal of patience in addition to knowledge of the physiology, ecology and taxonomy of the algae and the appropriate techniques. It can take from one week to several months to produce an axenic culture; in some cases it appears to be impossible to achieve.

Early methods of isolation depended largely on chance. Agar plates enriched with various nutrients were streaked with field material, usually resulting in a mixed culture of a few 'weedy' species. The development of the capillary pipette method [6] enabled researchers to pick up single cells or filaments of a particular species, and establish clonal cultures. This works quite well for non-motile species but is a challenge with fast-swimming flagellates! The technique which I prefer uses a sterile Pasteur pipette drawn out to a fine tip which is bent at a 45° angle and has an opening of 3–4 times that of the alga being isolated. Attached to the pipette is about 30 cm of flexible Tygon tubing and a cotton-plugged glass mouthpiece. Cells are repeatedly picked up by gentle mouth suction and blown into medium contained in a series of wells in a microwell plate (well volume = 0.3 ml) to eliminate contaminants. Clonal cultures can be made in 4-well Multidishes (Nunc, Roskilde, Denmark) with a volume of 2.2 ml. Usually after 1–2 weeks in the Multidish the culture is ready for transfer to a test tube. A polystyrene tube with one flattened side (e.g. Nunc) is ideal as it allows for easy microscopic observation. Alternatively, cultures could be transferred to a glass screw-cap tube or an agar plate for further development and purification.

Other types of isolation and purification involve manipulation of the medium, e.g. adding cyclohexamide to prevent the growth of eukaryotes, elimination of Si to prevent diatom growth, elimination of N to encourage the growth of N-fixers [8]. The phototactic response has been used to obtain axenic cultures of some flagellated algae using a specially designed pipette [5]. Reactions to light have also been used to isolate cyanobacteria into pure culture using differential migration through an agar overlay on plates [2]. The serial dilution technique may also be useful to obtain non-clonal cultures [9]. Flow cytometry has the potential to be used for isolation and to select particular strains of algae [4].

Initially, algal media were based on solutions used for higher plants and used tap water as a base. Much progress

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has been made in media preparation and development, so that it is now possible to make a defined medium, custom-designed for a particular species. The use of water purification systems, high quality chemicals and filter-sterilization has made it possible to culture more fastidious species. Several inorganic elements are known to be universally required for algal growth and these are N, P, K, Mg, Ca, S (macronutrients) and Fe, Cu, Mn and Zn (micronutrients). Chelating agents, e.g. EDTA are often added to enable adequate amounts of trace metals to be held in solution. Some algae require Co, Mo, Na, Se, Si, V or vitamins and all require a carbon source, usually supplied by free CO_2 in the water or by adding $NaHCO_3$ or $NaCO_3$. Marine species require a medium based on natural or artificial sea water. Most algae are grown autotrophically but many strains of cyanobacteria and some green algae can be grown in the dark with the addition of organic substances such as acetate, sucrose and glucose. Soil extracts may be useful for some algae, particularly in the isolation stage. Liquid media are commonly used but the use of solid media (1–2% agar) is practical for many species, especially for long-term maintenance. The pH of media can be from 1 to 9 depending on the strain being cultured but the majority are in the range of 6–8. Hydrogen ion buffers, e.g. HEPES, MES are useful as they form only weak complexes with metal ions.

The physical conditions for culture growth are varied and depend on the preference of the alga and the rate of growth required. Controlled environment chambers with fluorescent lights (Cool-White, General Electric, Mississauga, Ont., Canada) are ideal but many cultures may be grown in a room with natural (e.g. a north window) or fluorescent light. A wide range of temperature and light can be tolerated by most algae but cultures are usually grown at 15–20 °C in moderate light. For cyanobacterial cultures, temperatures of 25–30 °C with low to moderate light levels are common. For some algae a day:night cycle is required for sexual reproduction; this may be from 8 to 16 hours light and from 8 to 16 hours dark. To prolong viability, cultures should be stored at low temperature e.g. 10 °C and low light.

LONG-TERM MAINTENANCE

Many algal cultures are valuable resources and are maintained indefinitely in culture collections. For example, the Culture Collection of Algae and Protozoa (CCAP) in the UK retains viable cultures of *Chlorella protothecoides* isolated by Kruger in 1892. The most common form of preservation is by serial transfer. An aliquot of culture is subcultured to fresh medium on a regular schedule, ranging from 2–4 weeks for liquid cultures and from 2–4 months for agar slants. For small collections this is the most practical and least expensive method of preservation; for many algae it is the only reliable one currently available. Some of the disadvantages are the high risk of contamination and of change in characteristics, as well as possible mislabelling or loss of cultures. At the University of Toronto Culture Collection (UTCC) we maintain all of our 260 cultures of algae and cyanobacteria by serial transfer but are considering other techniques such as cryopreservation (see below). We have been able to avoid any serious problems of loss by having a back-up system for the growth chambers so that there is always at least one viable culture in each of two chambers. Contamination problems are minimized by using a laminar flow hood, pre-sterilized by UV and cleaned with Virocidin-X (Fisher Scientific, Ottawa, Ont., Canada), for all transfers. Growth chambers are regularly cleaned with 10% javex water (bleach). All agar plates are sealed with a double layer of Parafilm to prevent the entrance of airborne contaminants. Regular microscopic examinations of cultures are made and sterility test plates are used to monitor axenic cultures.

A technique which can prevent loss of algal cultures or their characteristics is cryopreservation. Currently, it is used only by some of the large collections e.g. CCAP where one third of their 2000+ algal strains are stored under liquid nitrogen. The cryoprotectant is usually 5% DMSO but methanol and sucrose can be effective. The rate of recovery is often a problem and where loss of viability is severe, freeze-resistant mutants may actually be selected. Unicellular green algae and cyanobacteria are most successfully cryopreserved. For cyanobacteria, an alternative technique of lyophilization is very convenient for culture collections.

The microalgae maintained in service culture collections represent a resource that holds great potential for biotechnology. Many discoveries which have been made in basic phycological research using algal cultures, e.g. in systematics, ecology, physiology, and biochemistry could be applied to practical problems and result in the development of many commercial products [1,4]. Genetic manipulation is only commencing but holds promise for increasing algal growth rates and yield as well as improving product compositions. It is only recently, however, that microalgae have begun to be utilized for industrial processes.

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