

A NEW AND USEFUL METHOD FOR OBTAINING
AXENIC CULTURES OF ALGAER. Malcolm Brown, Jr. and Harry W. Bischoff¹

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The importance of obtaining and maintaining axenic cultures of algae, especially for study of their taxonomy, morphology, and physiology, is generally conceded. With increasing use of various physiological tests on algae to obtain supplementary data (Bold and Parker, 1962), the often painstaking procedures of purification into the axenic state have become accentuated. Therefore, briefly described is a new method by which many different unicellular, colonial, and filamentous green algae can be relatively easily obtained in axenic culture.²

This method employs an ultrasonic instrument³ operating in the frequency range of 90 kilocycles per second. The principle involved is a physical separation of most bacteria from the surface of the algae. Once this physical separation has been accomplished, the bacteria and algae are segregated by repeated centrifugation and washings with sterile distilled water. Following the ultrasonic treatment, the washed algae are plated-out in a suitable inorganic 2% agar medium such as modified Bristol's (Bold, 1949) and allowed to grow 2-10 days before isolation of bacteria-free colonies. Not all the developing colonies will be bacteria- or fungi-free, but generally one can (with patience) locate and isolate an axenic colony. The isolation procedure involves the use of a 30-gage micro-loop platinum wire for surface isolation, and a micro-spatula for sub-surface isolation. Disposable soft-glass Pasteur pipettes, drawn to a fine bore tip, can also be used for this procedure. The algae are transferred to 60 mm. Petri dishes with media favorable for their growth and that of any contaminants that may still be present. The entire isolation and transfer procedure is performed with the aid of a stereoscopic binocular microscope. Once it has been ascertained that the algae are free from contamination, they are transferred for maintenance to appropriate, slanted media in cotton-plugged tubes.

There are many variations possible, since each alga to be isolated may present special problems. The following further suggestions are presented to increase the efficiency and speed with which the axenic state may be achieved.

In the first place, a proper instrument must be employed for the ultrasonic treatment. The use of a high-intensity, closed system such as those used in cell fractionation work is to be avoided. The best is a low intensity, ultrasonic water bath used in cleaning small and delicate objects. Thick suspensions of algae are placed in heavy-walled, 13ml sterile centrifuge tubes, and the tubes are immersed by hand into the ultrasonic water bath for the treatment period.

The duration of the initial treatment is important. Such motile unicellular algae as *Chlamydomonas* may not survive ultrasonic vibrations longer than 60 seconds, while such algae as *Chlorococcum*, *Chlorosarcinopsis*, *Bracteacoccus*, and *Stigeoclonium* can withstand from 1-20 minutes of ultrasonic vibration without harmful effects.

The medium in which the algal cells undergo ultrasonic treatment is also important. The ultrasonic method has been most successful with sterile distilled water as the suspending medium. However, the use of soap or synthetic detergents

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³ "Disintegrator, System 80"; Model G-80C1. Manufactured by Ultrasonic Industries, Inc., 141 Albertson Ave., Albertson, L.I., New York.

during the ultrasonic treatment has aided in the separation of bacteria from the algae. Common soaps have not been as successful as the synthetic detergents as the high pH is detrimental to many algae. A non-ionic surface active agent with the trade name "Tween-80"⁴ has been successfully used at a concentration of 5%, the pH being 5-7. The "Tween-80" solution should be prepared immediately before use by adding the concentrate to sterile distilled water and heating gently to get it into solution. Variations with the detergent technique have been investigated. The method now most generally used in our laboratory is as follows: after initial ultrasonic treatment, the algae are allowed to remain in the detergent from 15 minutes to 4 hours; the length of time depends on the resistance of the organism to the detergent. If the immersion in the detergent is long, the algae are exposed frequently (every 15-30 minutes) to ultrasonic vibrations, the duration of each period not exceeding 10 seconds.

The degree of centrifugation and number of repeated washings with sterile distilled water must be carefully considered. Following the detergent treatment, the cells should be centrifuged only to the degree that they become loosely packed in the bottom of the centrifuge tube. Prolonged centrifugation will deposit many bacteria with the algae, and this is to be avoided. After centrifugation, the supernatant is discarded. Sterile distilled water is added, and the cells are re-suspended by short 10 second bursts of ultrasonic vibration. It is important that this procedure be repeated no fewer than 5 times, since the removal of the physically separated bacteria occurs at this stage of purification.

Careful consideration should be given to the media into which the algae are plated and to which they are subsequently transferred. The agar medium is cooled to 40° C. before pouring. The cooled, melted agar is poured into 100 ml Petri dishes to which a quantity, from a loopful to 0.5 ml, of thick algal suspension from the washed ultrasonic treatment has been added previously. The plates should be swirled in both clockwise and counter-clockwise directions to distribute the algae thoroughly in the liquid agar phase, particularly in the surface layer, as surface isolation is easier than a sub-surface isolation. The type of medium to be used for pouring and transferring depends on the organism's requirements for vitamins or other organic nutrients. Algae isolated from the poured Petri plates should be transferred to an organic-base medium (Soil-water extract agar, proteose agar; Starr, 1960) favorable for the growth of algae as well as bacteria, actinomycetes, yeasts, and fungi which may be present. The organic-base medium serves the dual purpose of supplying possible nutrient deficiencies of the algae and, at the same time, of revealing within a very short time the presence or absence of any contaminants.

The writers realize, of course, that these methods may not be applicable to all algae. They hope, however, that this method will be useful to others in shortening present algal purification procedures.

⁴ "Tween-80" is polyoxyethylene (20) Sorbitan monoleate, a non-ionic active surface agent and is manufactured by the Atlas Powder Company, Wilmington, Delaware.

BOLD, H. C. 1949. The morphology of *Chlamydomonas chamydogama* sp. nov. Bull. Torrey Bot. Club 76: 101-108.

BOLD, H. C. and B. C. PARKER. 1962. Some supplementary attributes in the classification of *Chlorococcum* species. Arch. Mikrobiol. 42: 267-288.

STARR, R. C. 1960. The culture collection of algae at Indiana University. Amer. Jour. Bot. 47: 67-86.