

Isolation of Blue-green Algae for Pure Culture

MARY M. AITKEN, Purdue University

Within the last few years, the study of Algology has been developed from a scientist's science, carried on by a relatively few physiologists and taxonomists to an active, rapidly-growing field of research. The sudden surge of popularity is due to the ever-widening search for organisms which might be capable of elaborating antibiotic substances. All groups of plants have been investigated; however, the Thallophytes have received the most careful examination so far because they have, up to the present, been the most prolific producers of antibiotics. The bacteria, from which are obtained eumycin, subtilin, and gramicidin, among the most prominent; the actinomyces, from which are obtained streptomycin and streptothrycin; the molds, which produce penicillin, flavacin, and Kojic acid; and the algae, producers of chlorellin. The higher plants, moreover, have not been neglected; some of them, for instance the common garlic, have been found to contain an antibiotic substance.

As was mentioned before, the algae have been investigated and still are being investigated. Most of the research has been, however, on the green algae, since *Chlorella vulgaris*, a green form, is known to secrete an antibiotic. The blue-greens, as a whole, have been neglected for several reasons:

1. Cultures have been considered difficult to secure; under most favorable conditions, the blue-greens do not produce as conspicuous or luxuriant growths as other forms. As a group, they are exceedingly small and tedious to work with.
2. Because of a gelatinous sheath which is secreted by the cell wall and harbors bacteria, bacteria-free cultures have been difficult to obtain.
3. Methods of cultivating the blue-greens for large-scale production have been difficult because of their nutritional idiosyncrasies and the difficulty of satisfying their light requirements.

In the face of so many difficulties, it may seem strange that an investigation of their antibiotic properties would be attempted; however, there are several reasons why such a project seems logical. First, the medicinal properties of antibiotics found so far (especially penicillin) have proved to be so unique that no group of organisms should be neglected in the search. Second, since so many bacteria have been found to elaborate antibiotic substances, it seems probable that organisms so closely related to the bacteria as these simplest algae may be capable of producing antibiotics. Third, blue-greens, simple as they are, are capable of living and reproducing in surroundings in which competition for *lebensraum* is keenest. They are found in drainage waters, pools rich

in organic matter, the alimentary tracts of higher animals (including man), and soils high in organic content—all situations in which other forms are numerous and vying for their share of space and food. It seems logical that their ability to compete against other forms may be due to a production by them of some substance that may inhibit or at least discourage growth of other organisms. It is a well known fact that some algae are capable of rendering water unfit for animal consumption and that others prevent normal root function of some of the higher plants. Fourth, the pigment phycocyanin, peculiar to the blue-green algae, may, like other plant pigments, such as chlorophyll, be capable of inhibiting growth. Five, the strains of bacteria which are consistently found associated with the algae may, in themselves, be antibiotic.

In isolating cultures of blue-greens for studies, in the past, bacteriological techniques have frequently been employed to obtain uni-specific cultures—and, when possible, bacteria-free. Streaking of a loopful of cell suspension in a grid pattern on nutrient solution plus 2% agar, followed by picking off and transfer to other media has frequently been employed. Agar dilution methods have been favorites also, both the usual bacteriological techniques and one which calls for flooding an agar plate with a dilute suspension of cells and allowing the water to evaporate, "stranding" isolated cells. This is followed by picking off the single cells or colonies and transferring them to other media. These methods, however, do not guarantee bacteria-free cultures as the bacteria remain in the sheaths and ride along with the algae.

Although the cells of the blue-greens are seldom completely devoid of their mucilaginous coating, there are times when this covering is reduced to a minimum. After adverse conditions, the cyanophyceae may produce endospores, exospores, or akinetes, which can be isolated by single-spore techniques with a micromanipulator or dilution methods. This is an exceedingly tedious task, however.

The author of this paper, in work with the blue-greens, has attempted first to isolate the algae in unialgal or uni-specific cultures. One method which can be employed in the laboratory has been worked out by Lewis Flint, algologist at Louisiana State University. It is applicable to isolation of algae from soil samples. The soil is sprinkled in a light layer over the bottom of a sterile petri dish and covered with a thick layer of silica sand. The sand is moistened with a nutrient solution, such as Krop's or Detmer's, and the plate is placed about 6-8 inches below the tubes of an ordinary fluorescent lamp. The plate is kept moistened and in from 2 weeks to 4 weeks, algal colonies appear on the surface. These colonies can be picked from the sand surface with the aids of dissection binoculars. After the colonies are picked from the sand surface, they are transferred to the top layer of a stack of 5-6 sterile, moistened coarse filter papers in a petri dish. Eventually, the algae penetrate the various thicknesses of the paper and by discarding the top papers, one can reach a level where only one colony has penetrated. A more convenient method than the above has been devised by the author for the isolation of uni-specific algal cultures from soil. Filter paper clippings (about 4 table-spoonsful) are placed in a petri dish, moistened with nutrient solution,

and autoclaved at 15 pounds pressure for 20 minutes. On top of this is sprinkled very sparingly the soil sample and the plate placed from 8-10 inches below the fluorescent tube. Colonies spread out from the soil particles; and since the filter paper holds the moisture, watering and therefore mixing of the algae is avoided. Filaments or colonies also are easier to pick from the clippings than from sand; very small portions can be removed with a sterile needle.

After isolation of uni-specific algal colonies, the next step is to free the isolate of bacterial contamination. Since bacteria prefer neutral or slightly acid media, acidification of the medium greatly inhibits their growth; unfortunately, most blue-greens will not grow in an acid medium.

In the isolation of blue-greens from solid substrates, such as rocks or pebbles, the author of this paper has found that by placing the pebbles in a sterile petri dish, covering with a thick layer of sterile 3% agar and inverting the plates near a light source, one gets abundant growth of algae on the bottom of the plate, while the molds and bacteria grow at the surface or slightly below. After illumination for 2 to 3 weeks, the surface of the plate is flooded with 1:1000 HgCl₂ solution for 10-15 minutes to kill surface contaminants, then the agar surface washed with sterile water 3-4 times. After this, with a right-angle needle, the surface agar can be lifted off and the alga removed to another medium.

Trelease and Selsam¹ have described a method to remove protozoan contaminants in which the cells are treated 12-24 hours with .45 M MgSO₄ solution. Allison and Morris² successfully employed ultra-violet rays to irradiate cultures, reasoning that the irradiations would have lethal effect on the bacteria present before the algae succumbed. The author has been eager to try this method; this has not been attempted up to the present.

The above methods outlined are only the beginning—only the first halting steps—in an attempt to develop techniques which can be employed in as satisfactory a manner as modern bacteriological methods.

¹ Trelease, S. F. and M. E. Selsam. Influence of calcium and magnesium on the growth of *Chlorella*. Am. Jour. Bot 26:339-341. 1939.

² Allison, F. E. and H. J. Morris. Nitrogen fixation by blue-green algae. Science 71:221-223, 1930.