Preliminary Results in the Laboratory Culture of Planktonic Blue-green Algae

William R. Eberly, Manchester College

During the school year 1963-64 the writer was granted a sabbatical leave to pursue a research project at the Institute of Limnology, University of Uppsala, Sweden, entitled "Environmental requirements of planktonic blue-green algae." The project was supported largely by the National Science Foundation through grant GB-900 in conjunction with partial salary allowed by Manchester College under a sabbatical contract. The facilities of the Institute of Limnology were made available through the great kindness of the director, Dr. Wilhelm Rodhe. Appreciation is expressed at this time to all who helped make this project possible.

A large number of temperate lakes have phytoplankton communities dominated by one or more members of the Cyanophyta, the "blue-green" algae. Though many sessile or benthic species of blue-green algae have been cultivated and a great deal learned of their ecology and physiology, planktonic forms are extremely difficult to grow in laboratory conditions. Consequently, little is known through controlled experimental work of the physiology of these forms. Within the last decade or so, some progress has been made in cultivating planktonic species, chiefly by workers at the University of Wisconsin and the National Research Council at Ottawa.

Until about 1950 no truly planktonic (euplanktic) species of blue-green algae had been successfully cultivated under laboratory conditions in purely inorganic media (1,9). Gerloff *et. at.* (3) reported 22 species in culture, of which at least 5 are bonafide euplankters: *Lyngbya Birgei, Aphanizomenon flos-aquae, Diplocystis aeruginosa (Microcystis aeruginosa), Anabaena circinalis,* and *Gloeotrichia echinulata.* Most of the other species are to be considered as tychoplanktic or facultative plankters. Gorham (4) reported 11 species under culture in the laboratories at Ottawa, of which 9 are unquestionably euplanktic: *Anabaena flos-aquae, Anabaena limnetica, Anabaena spiroides, Anabaena schermetievi, Anacystis cyanea f. minor, Aphanizomenon flos-aquae, Coelosphaerium kuetzingianum, Gloeotrichia echinulata,* and *Microcystis aeruginosa.* Considerable advances have been made in understanding the physiology and ecology of *Microcystis aeruginosa* (6, 7, 12), *Aphanizomenon flos-aquae, Gloeotrichia echinulata* (11), and *Anabaena flos-aquae* (5).

In addition to the above, Staub (10) has published an extensive account of the physiology and ecology of *Oscillatoria rubescens,* the first truly planktonic species of the genus to be cultured in the laboratory, and Drews and his co-workers (2) have described a new species of planktonic blue-green alga, *Synechococcus plancticus,* which they have produced in mass culture in their laboratory. To the writer's knowledge, this represents all the euplanktic Cyanophytes in laboratory culture until just recently.

The first stated objective of the work was to obtain cultures of some of the common planktonic blue-greens. This involved among other things

the successful isolation of available forms, development of suitable media, and determining satisfactory growing conditions. Stable unialgal cultures of 5 forms never before cultured were established, of which 2 are being described as new taxa. These include: *Oscillatoria agardhii* var. *isothrix*, *Oscillatoria baltica* nov. spec., *Oscillatoria limnetica*, *Anabaena delicatula* var. *robusta* nov. var., and *Synechocystis parvula*. The cultures are being maintained at the Institute of Limnology at Uppsala and will also be available in the laboratory of the writer. A number of other forms than those listed were isolated, but were not saved, since major interest was in previously uncultured forms and especially in deep water filamentous forms, such as the *Oscillatorias*.

Colonial forms such as *Microcystis* and *Aphanizomenon* were easily isolated by means of micro pipettes. It proved to be extremely difficult to isolate single trichomes of forms like the *Oscillatorias*, partly because the filaments were so small (generally under 5 micra in diameter). Single trichomes placed in test tubes rarely survived. Perhaps the filaments, because of their low density, tended to float to the surface where they became lodged in the concave meniscus and were consequently dried out with minute amounts of evaporation of the medium. Dense samples dominated by a single species of blue-green were purified with the addition of 50ppm of Actidione, an anti-biotic with selective anti-algal properties (13). All forms except blue-greens are inhibited and eventually killed by Actidione. Such cultures were termed "working cultures" and were shown by visual examination to be unialgal, though one cannot be positive that contaminants were wholly absent.

Motile forms such as the *Oscillatorias* were isolated by plating the culture on medium solidified by the addition of 1.5 percent agar. After several weeks, in petri dishes with one half covered with opaque paper, the trichomes crawled away from any contaminating algae into the lighted half and could easily be lifted out and transferred to a culture vessel. The extremely small *Synechocystis* species (0.7-0.9 μ) was purified by filtering a culture through a membrane filter type RA (pore size 1.2 μ) which removed all known extraneous forms.

A number of culture media were tried but most formulae which have been used successfully with other types of algae seemed too concentrated for the species with which this study was concerned. The ASM medium of Gorham and his associates (6) proved quite good for nearly every form studied. Some tentative conclusions seem warranted on the basis of limited nutrient studies. Blue-greens, at least the planktonic forms, do not need a full trace element supplement. The ASM formula provides only Fe, B, Mn, Zn, Co, and Cu. It also seems likely that the iron requirements are not as rigid as in various other algae that have been studied (9). Filamentous forms, such as *Oscillatoria* and *Anabaena*, seem to require more calcium than coccoid forms. It seemed that at least 10mg/1 Ca is needed for best growth. The Ca/Mg ratio by weight also seems to be critical, with best growth being obtained where this ratio did not deviate significantly from unity. Further studies seem to clarify the nutritional requirements are needed.

For the most part, material was grown at room temperatures, i.e., 20-25° C. Very rapid growth was observed under these conditions, which does not explain the occurrence of population maxima in nature at

depths where the temperature is below this level. Illumination was provided by both ordinary fluorescent lamps and by a combination of warm tubes (heavy in the reds) and cold tubes (heavy in the blues). This combination provided a much broader, flatter spectrum of wide range than the ordinary daylight bulb with a single pronounced peak near the center of the spectrum. The effect of various spectral combinations was not determined and awaits further study. Too high a level of intensity is harmful to planktonic Cyanophytes. Good average growth occurred at about 500 lux (50 ft. candles) at the level of the culture vessels.

The most critical factor observed by the writer concerns the mode of culture. Stationary vessels, regardless of size, shape, etc., proved unsuitable for these planktonic forms. The most successful culture methods required a shaking table. Rate of shaking can be balanced with the size, shape and capacity of culture vessel to give the desired amount of agitation (generally the minimum amount of agitation required to maintain full dispersion of the algae is best). A "fermentation tower" patterned after those used by Gorham (12) was constructed, in which a flow of air through the bottom of the tube produced turbulence throughout the length of the column. This was successful with several fialmentous and unicellular forms, but growth of colonial forms under these conditions was not so good (see also 11). I found that a much lower rate of flow of air than that reported by these workers (12) was adequate. Where they used 1000 cc/min, in our work 300 cc/min (which was the lowest rate that could be measured) was sufficient.

A few very preliminary experiments were carried out with 5 species to determine the photosynthetic response (a measure of growth rates) under different combinations of light intensity and temperature. Identical aliquotes were innoculated with C-14 (as bicarbonate) and exposed to 4 different levels of light intensity at 2 ranges of temperature (12° and 20°). The major difficulty encountered was the precise measurement of light intensity. In spite of this, photosynthetic response curves at the two temperature levels (using relative light values) indicate significant species differences. The results clearly indicate that this can be a fruitful field of inquiry as soon as suitable apparatus can be devised and constructed.

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