Proceedings of the Iowa Academy of Science

Volume 58 | Annual Issue Article 14

1951

Unialgal Cultures of Haematococcus lacustris (Girod) Rostaf.

R. L. Hulbary
State University of Iowa

Unialgal Cultures of Haematococcus lacustris (Girod) Rostaf.

By R. L. Hulbary

Introduction

Algae are being used more frequently in the attack on an ever widening variety of biological problems. We find them in many laboratories as experimental organisms in the study of the fundamentals of photosynthesis, mineral nutrition, and genetics. Their present uses and potential uses commercially such as, sources of food, in food processing, and in industrial processes are growing steadily in number. Because of the increasing interest in them experimentally and practically, a better understanding of their culture is imperative. Several forms such as, *Chlorella, Scenedesmus*, and some of the Cyanophyta have been cultivated for laboratory purposes with success. Others are regularly cultured for short periods to provide material for class use in schools. Only relatively few species have been grown in pure or unialgal culture, (Bold, 1942; Pringsheim, 1946; Brunel, Prescott and Tiffany, 1950).

The cultivation of *Haematococcus* (Sphaerella) *lacustris* was undertaken because it is one of the most attractive members of the Volvocales for use in general botany and general biology classes, because certain phases of the life cycle need clarification, and because it promises to lend itself to several genetic studies anticipated. In this preliminary investigation the principal object was to find a method of growing the organism in quantity and by a standardized procedure that could be repeatedly accomplished with relatively limited laboratory facilities.

Materials and Methods

Haematococcus lacustris is reported throughout Europe where it is found commonly from Scandinavia to Venice. In the U. S. it is distributed widely in shallow puddles along limestone ledges, in the marble urns of graveyards and in most any water-filled depression on rocky substrates where considerable nitrogenous organic material has collected. The material used in this investigation was collected from a cement bird bath in Iowa City. The surface of the cement was brick red with millions of small spherical unicells of

the haematocyst stage. The debris containing these cysts was scraped from the bottom of the bird bath and brought into the laboratory where it was placed in a small glass jar of distilled water. Twenty-four hours later the water was green with biflagellate macrozoospores which constitute the motile vegetative stage. Transfers in the form of cysts or macrozoospores have been made several times in the course of the now nearly five months since this original collection was taken.

In an effort to prepare unialgal cultures, single cell or multiple-celled isolations were prepared by a slight modification of the dilution method described by Bold (1942). Single cells in motile or cystous condition were isolated from the surface of the agar dilution plates, after five days of growth, and transferred to test tubes containing two types of media. The first medium was a modification of Detmer's (1888) prepared as follows:

$Ca(NO_3)_2$	1.0 g.
KCl	0.25 g.
$MgSO_4$	0.25 g.
KH_2PO_4	0.25 g.
Fe-citrate	"a few drops of a 1% aqueous solution"
Glass distilled water	1 liter

Before using this stock medium it was diluted with equal parts of distilled water and adjusted to pH 7.0.

For the second medium 5 cc. of rich garden soil was added to each of several clean test tubes. The soil was covered with 3 cc. of white sand. These dry tubes were sterilized in a hot air oven. Just before inoculation 10 cc. of distilled water was added to each tube. From the single cell isolates on the two different media, were produced, the unialgal stock cultures from which massive, quantity cultures were obtained.

The stock cultures on soil, sand, and distilled water contained thick green clouds of macrozoospores mixed with haematocysts. One half cc. of the rich stock culture was pipetted into the following media prepared in test tubes:

1. Casein—sterile soil—sterile sand—glass distilled water, pH 6.6.
2. Casein—sterile soil—sterile sand—Detmer's solution, pH 6.6.
3. Sterile soil—sterile sand—glass distilled water; pH 6.6—7.0.
4. Sterile soil—sterile sand—Detmer's solution, pH 6.6.
5. Detmer's solution pH 6.6—7.0.
6. Detmer's solution pH 5.1—5.4.
7. Soil extract (prepared by extracting rich garden soil for 3 days at room temperature with frequent agitation.)

Twelve test tubes of each medium were inoculated. Cultures were placed in test tube racks under artificial light from two 48 inch

fluorescent tubes, at temperatures varying from 15° C. to 22° C. Illumination was maintained over a period of 14 hours per day. Observations were taken during growing periods of 18 days. The experiment was repeated three times in two months.

Large glass cylinders containing 10 cc. of sterile garden soil, covered with 10 cc. of sterile sand and 500 cc. of glass distilled water were inoculated with one cc. of the thrifty stock cultures. These cylinders are 2.5 inches in diameter and 9 inches high. With this arrangement, an air space 2 inches in height remains above the growth medium. Each cylinder was covered with a thin glass plate and placed under the same environmental conditions as the test tube cultures.

Observations and Discussion

The results in terms of the gross increase in number of macrozoospores are included in Table I. Where growth occurred in the seven media used, the alga multiplied by divisions of the green protoplast of motile macrozoospores, first into two then into four daughter cells. Division frequently began before motility ceased in the dividing cell. Each of the four daughter cells developed two flagella before breaking through the mother cell membrane. After their release young macrozoospores became motile and increased in size. The size of the nearly spherical, mature macrozoospores varies from 24 microns to 37 microns with an average of 31.17 microns. As a culture grew older macrozoospores ceased to swarm, secreted a thick wall, and produced abundant haematochrome. Some of the red cysts floated in the liquid but many of them settled to the bottom or became attached to the sides of the culture vessel near the surface. They form a brick red "paint" over any solid surface to which they attach. When transferred to fresh medium cysts germinate readily producing usually four, sometimes two or eight, macrozoospores.

Table I.

Relative value of media used in the culture of *H. lacustris*

Medium	Exp. A.	Exp. B.	Exp. C.
1. Casein, soil, sand, water	—	—	
2. Casein, soil, sand, Detmer	+	—	
3. Soil, sand, water	—	+++	++
4. Soil, sand, Detmer		++	+++
5. Detmer, pH. 6.6—7.0	+		
6. Detmer, pH. 5.2—6.4	—		
7. Soil extract			+++

As illustrated by Table I, best growth is obtained on media containing sterile soil or soil extract at pH of 6.6 or above. Casein in the form of a dry powder was used in small quanities or only enough to cover the bottom of the test tube. Where it was present with the soil and sand, *H. lacustris* could not grow for long in competition with the extreme numbers of bacteria that form dense white clouds. In media containing sterile soil with water or with Detmer's solution enough cells are present after 4 days to produce a light green color in the liquid above the white sand. An increase in greenness indicated an increase in cell number which reached a maximum in 10 to 14 days in test tube cultures. Following the climax of this period of rapid cell division, macrozoospores were transformed into cysts as the medium attenuated.

The fact that the organisms failed to grow well on the modified Detmer's medium would suggest that it may require some organic carbohydrate or protein (nitrogen) source. Further experiments are necessary to establish this, of course. Pringsheim (1946) reports that many Volvocine species will grow on inorganic salts but frequently grow better when either acetate or peptone, or both, are present in the culture medium. Macrozoospores did divide, and slight growth did occur in Detmer's solution, but only in the higher pH range.

It was learned in earlier attempts to grow *Haematococcus lacustris* that a pH range of 6.5 to 8.5 is compatible with good growth. When the pH drops below 6.5, growth is definitely inhibited. This early observation is supported by results obtained with the seven different media used in the present investigation.

The glass cylinders utilized to grow larger quantities of *Haematococcus* were of the type first used by Behlau (1935) who obtained massive cultures of *Chlamydobotrys* in them. Another advantage of these larger vessels is that, living thrifty cultures can be maintained without transfer for a month to six weeks. In ten days, under the conditions described, a usable unialgal culture was obtained from which hundreds of inocula of *Haematococcus* macrozoospores could be taken. In these vessels sterile soil covered with sterile sand and distilled water adjusted to pH 7.0 proved to be a medium sufficient to maintain cultures without attenuation for more than a month.

Conclusions

1. Healthy unialgal cultures of *Haematococcus lacustris* may be grown with ease on soil or soil extract media to which either distilled water or an inorganic nutrient solution such as Detmer's is added.

2. In this type of medium only the two stages, haematocysts and macrozoospores, are found regularly.

3. The optimum pH for any of the effective media ranges from 6.5—8.5.

4. Behlau cylinders are useful in growing large quantities of macrozoospores and cysts, and for maintaining cultures for long periods without transfers or addition of nutrient.

Literature Cited

Behlau, J. 1935. Die Spondylomoraceen Gattung *Chlamydobotrys*. Beitrag. Biol. Pfl. 23: 125-166.

Bold, H. C. 1942. The cultivation of algae. Bot. Rev. 8: 69-138. (with 503 references).

Brunel, J., G. W. Prescott, and L. H. Tiffany. 1950. The culturing of algae, a symposium. Antioch Press, Yellow Springs, Ohio.

Detmer, W. 1888. Das Pflanzenphysiologische Practikum.

Pringsheim, E. G. 1946. Pure cultures of algae. Cambridge.

Department of Botany
State University of Iowa
Iowa City.