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Methods of a Unialgal Culture of Pandorina

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The study of Algae has for many decades involved great numbers of very important problems. To try and solve certain phases of these problems, it is first necessary to develop standardized techniques in the handling and culturing of certain forms. It is with this fact in mind that the author has experimented with a controlled set of environmental conditions and a series of culture media for the maintenance in culture, pure and unialgal, of *Pandorina morum*, a member of the Volvocales.

METHODS AND RESULTS

Pringsheim (1946) and Fritsch (1935) state that *Pandorina* (Fig. 5) is very difficult to culture in the laboratory and that it often disappears from seemingly health cultures in a very short period of time. This was found to be true, and as a safeguard against losing all cultures they were kept on two separate media. The present clone has been kept in culture for over a year. It was collected from a roadside ditch three miles south of Iowa City in Johnson County, Iowa. To separate the colonies from other algae present, bacteriological techniques were used in which *Pandorina* was plated out on Bennecke's medium (Bold, 1936) containing one per cent agar. After four days the clumps of colonies are readily removed to sterile liquid media with either a micro pipette or micro needle.

In the search for a suitable liquid medium, soil extract was made by using one part of soil by volume to two parts of distilled water. This material was placed in a large Erlenmeyer flask and air was bubbled through it for 48 hours to keep the soil components in suspension. Aeration was then suspended and the material left until the soil particles could settle out. The supernatant liquid was then poured off and filtered. The filtrate was autoclaved at fifteen pounds presslure for thirty minutes and thereafter kept in a cool place. This soil extract was always used in full strength.

Other media tried were those proposed by Bold (1936). They consisted of Detmer's, Bennecke's, Schulze's, and Knop's media which were used in a series of cultures to determine which was the best (Fig. 1). As a result of this comparative study, Detmer's medium was selected for further use. The effect of adding small traces of peptone to this medium was tried with good results (Fig. 2).

Modified Detmer's Medium

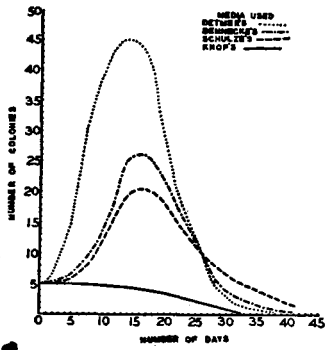
KH_2PO_4 ..... 18 cc. of 2% solution
K_2HPO_4 ..... 5 cc. of 2% solution
$Ca(NO_3)_2$ ..... 35 cc. of 2% solution
$Mg\ SO_4.7H_2O$ ..... 22 cc. of 2% solution
$Fe_2(SO_4)_3$ ..... 2 drops 2% solution
Plus 1900 cc. of distilled water.
Plus .25 grams of peptone.

In order to determine the best environmental conditions, a series of cultures was grown varying only one factor with that variant retained which gave the maximum amount of growth. Growth throughout this paper is measured in numbers of colonies per low power field under a compound microscope. As a result of these comparisons, the following method is suggested for culture work with *Pandorina*.

An inverted standard fluorescent light shade utilizing two 40-watt bulbs was used as the light sources (Fig. 9). Glass plates were fastened to the top of the shade on which the cultures were kept. The optimum light period was seventeen hours and measured 200-250 foot candles at the base of the cultures. This period was regulated by an automatic time switch.

A series of cultures indicated the optimum temperature to lie between 8-18°C. with the maximum at 37°C. Similarly the optimum pH was found to be 6.4, the maximum 7.7, and the minimum 4.4.

A combination of longer lighting periods and of higher temperatures tend to have a detrimental effect on colony growth. Within a



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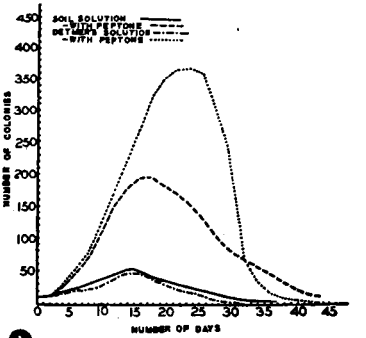

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STRUCTURE	MEASUREMENTS* IN MICRONS MINIMUM	AVERAGE	MAXIMUM
COLONY DIAMETER	8	40	70
COLONY LENGTH	20	65	120
FLAGELLA LENGTH	15	32	38
SHEATH THICKNESS	2.4	5.6	8.7
PYRENOIDS	3.2	6.8	7.1
EYESPOTS	1	2.8	3.3

*BASED ON 500 COLONIES

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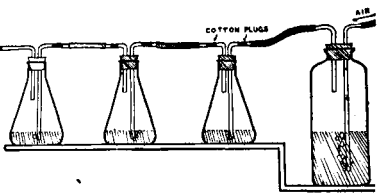

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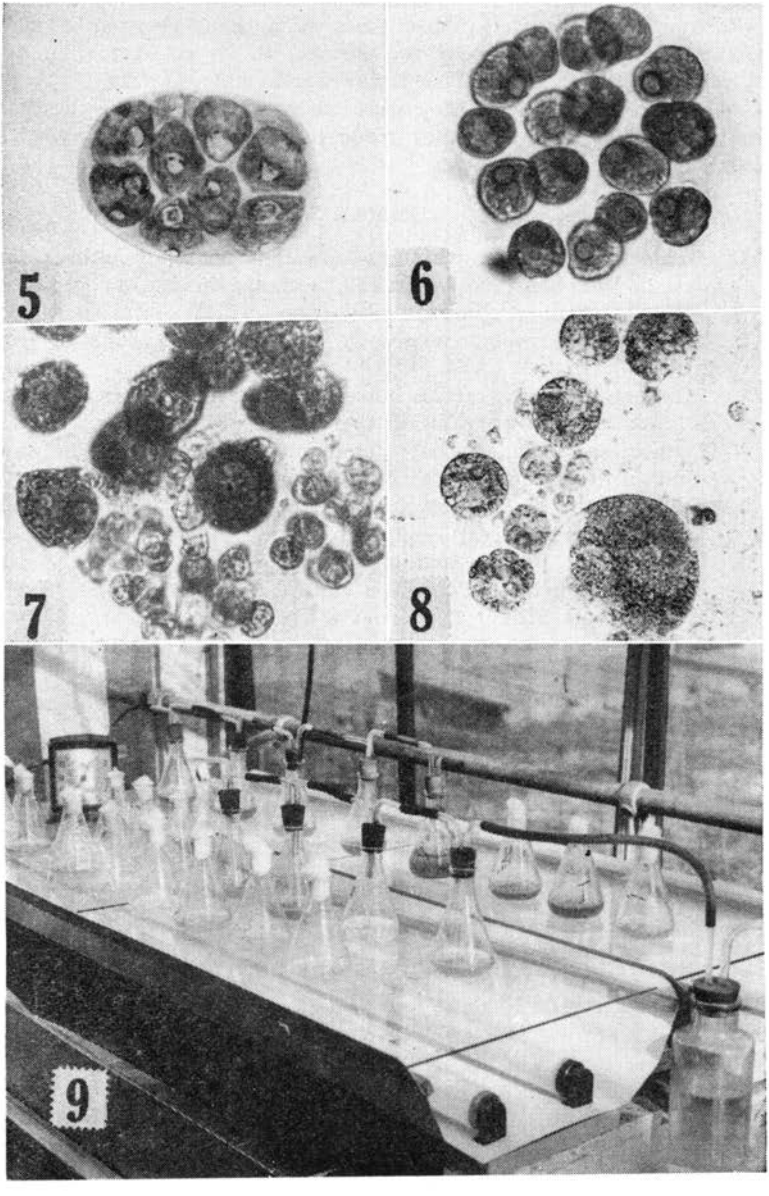
few days the colonies become very irregular. Certain cells disintegrate within a colony leaving units of from one to sixteen cells (Fig. 6, 7, 8). It is not uncommon to find the individual cells of these units reproducing independently of each other at different times (Fig. 7). Normal colonies have the cells reproducing at the



same time. Certain of these cells were recorded as having eight eye spots.

Acting on a suggestion by Cooper (1941) compressed air was used in the method of aeration (Fig. 4). The number of colonies was increased five times over that of non-aerated flasks. This increase may be in part due to the agitation of the cultures by the bubbling.

It was often observed in test tubes of vigorous growing colonies that they concentrated near the surface. In an effort to increase this area, 125 cc. Erlenmeyer flasks were employed. These proved to be very successful as they were easily adapted to the aeration system used and in that they made it possible to do away with cumbersome racks.

SUMMARY

1. *Pandorina* has been maintained in rich unialgal cultures for over a year with no observable decrease in vitality on both soil extract and Detmer's solution.
2. The addition of peptone greatly increases the growth of cultures.
3. Dilutions of soil extract, Schulze's medium and modified Knop's solution were of no value in the culture of *Pandorina*.
4. Bennecke's medium with 1% agar added proved to be the best solid medium on which to culture *Pandorina*.
5. Best growths of *Pandorina* are obtained with a light period of 17 hours at a temperature of 8-18°C.
6. Longer light periods and higher temperatures tend to cause abnormal growth in *Pandorina* cultures.
7. The optimum pH for *Pandorina* is 6.4.
8. Aeration increases the number of colonies several times over that of non-aerated cultures.
9. A simple technique for the culturing of *Pandorina* is presented.

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