REPRODUCTION AND GROWTH OF *CHLOROTYLIUM CATARACTARUM* KUTZ

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ABSTRACT. — *Chlorotylium cataractarum* Kutzing was cultured in soil water and on soil water agar at ± 21-22°C. The alga reproduced asexually by zoospores produced from the short cells of a filament. Each zoospore was pale green, elliptical, and contained two photoreceptive bodies. Released zoospores attached themselves to the substrate by their flagella. The alga exhibited two prostrate vegetative growth patterns. The first growth pattern resulted in either straight or slightly curved filaments; the second was decidedly curved and produced pseudoparenchymatous colonies. Older cultures contained numerous unicells that had very heavy layered cell walls.

Kutzing (1843) characterized *Chlorotylium cataractarum* by its unilateral and lime encrusted filaments. Collins (1909) reported that two types of cells composed the unilaterally branched filaments. One cell type was round, contained a large quantity of chloroplast material, and functioned in asexual reproduction; the other was elongated, hyaline in appearance, and vegetative in function. Mature filaments were composed of 2-3 round reproductive cells that alternated with 3-7 elongated vegetative cells. Each cell had a plate-like or band-like chromatophore that contained a pyrenoid. Asexual reproduction of *C. cataractarum* was by zoospores that possessed 2-16 flagella and originated from the round cells. Printz (1927) reported that red colored cells were also produced by the alga and these were identified as either akinetes or aplanospores. He also stated that bi-flagellated zoospores were produced from the terminal cells and that the palmelloid stage of the alga had a peculiar coloration.

The investigation was undertaken to clarify and elaborate on the reproduction and growth of *C. cataractarum*.

MATERIALS AND METHODS

A culture of *Chlorotylium* was obtained from Silver Lake, Iowa. Francis Drouet, University of Arizona, later identified the species as *C. cataractarum*.

Stock cultures of the alga were maintained in test tubes of either soil water (SW) or 2% soil water agar (SWA). Soil water was prepared by using a one gallon bottle that was filled one-fifth of its total volume with a rich soil, and distilled water was added to the half-gallon mark. The mixture was then autoclaved for 15 minutes at 15 psi. One week later the supernatant liquid was decanted and diluted 1:1 with Chu's (1942) number ten solution. The final liquid was filtered to eliminate all foreign material. Soil water agar was prepared in a similar fashion but 20g Difco agar was added to every liter of amended SW. Both culture media were then sterilized according to Bold (1942).

Algal samples used in this study were

obtained from bottle cultures. Each bottle was capable of holding 8 ounces of liquid and contained a 3″ x 1″ glass microscope slide. The bottles were filled with four ounces of SW and inoculated with two loops of algal material from stock cultures. All of the inoculation procedures were carried out under sterile conditions to avoid contamination of any type. After inoculation the bottles were placed in a north facing window according to Beyerinck (1890) and Bold (1942). No attempt was made to grow the alga under a 12-12 light/dark photoperiod.

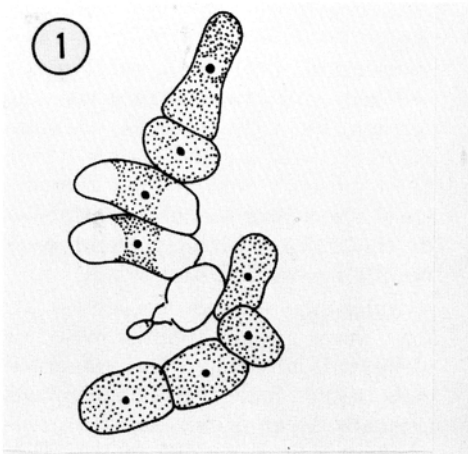
Microscopic observation of algal reproduction and growth was accomplished by the placement of glass slides from the 8 ounce SW bottles onto a continuous-drip apparatus according to Kulfinski, Pappelis, and Monoson (1963). The various growth stages of the alga were recorded as photomicrographs using an Exacta camera.

Results

Zoosporogenesis occurred in *C. cataractarum* 6-8 days after the alga was inoculated into SW bottles. A green ring was noted along the meniscus of the SW solution and suggested that reproduction or growth had taken place. Microscopic observation of the SW slides showed many unicells and small algal colonies on the glass surfaces. The SW slides were then transferred to the continuous-drip apparatus for further observations. Zoospores developed in the round cells of a filament two days after a SW slide was placed on the continuous-drip apparatus. The round cells functioned as zoosporangia and contained 6-8 zoospores. The bi-flagellated zoospores were elliptical, pale green, 9 x 6μ, and contained a cup-shaped chloroplast. An obscure pyrenoid was located in the median portion of the chloroplast. Two photoreceptive bodies were located on either side of the flagellar insertions (Fig. 1). The nucleus and vacuoles of the zoospore were not observed. Zoospores were released from the zoosporangium through a pore in the cell wall and swarmed in a circular fashion for 2-20 minutes. Attachment of these asexual spores to the glass substrate was by their flagella. Once a zoospore was firmly attached to the substrate the flagella were readily reabsorbed by the cell.

Individual cells of *C. cataractarum* were either round, oval, or elongated in shape. The elongated cells often had their chloroplasts centrally located and the end areas of these cells appeared empty (Fig. 2). Short two-, three-, four-, and five-celled filaments were often curved (Fig. 3). A red colored substance was noted along the inner surface of the curved cells. All the algal growth that occurred in SW solutions consisted of one primary filament from which secondary filaments had developed. The secondary filaments were divided into smaller tertiary branches that gave the mature colony a pseudoparenchymatous appearance (Fig. 4).

Zoospores that were inoculated onto SWA plates developed into round, elevated colonies that were composed of short, dark green cells. One-week-old algal colonies on SWA formed unilateral branched filaments that were composed of elongated and hyaline cells. Once again a red colored substance was noted on the inner surface of the curved filaments (Fig. 5). Two-week-old colonies of the alga maintained on SWA plates had disassociated into single and very thick-walled cells (Fig. 6). Microscopic observations were not continued after two weeks and, therefore, it was not possible to determine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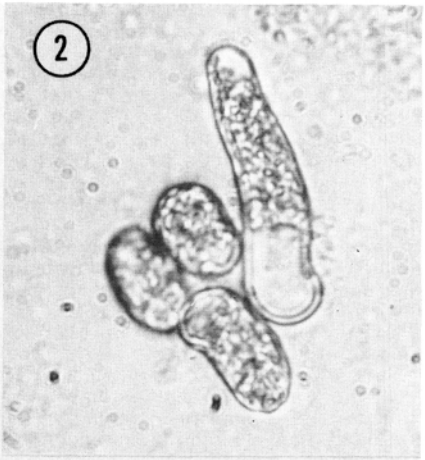
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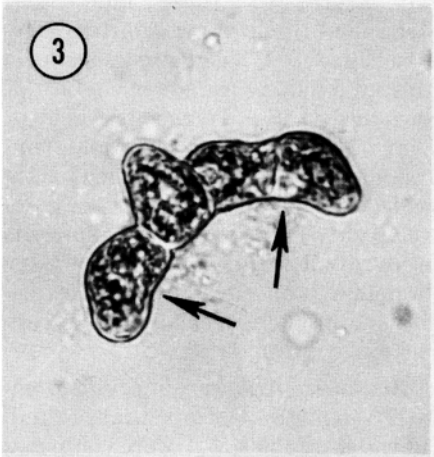
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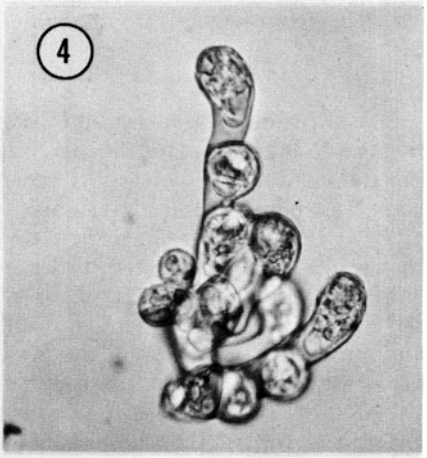
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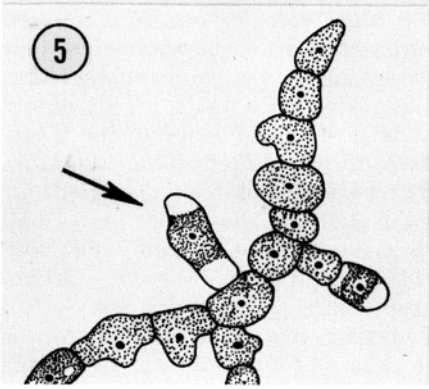
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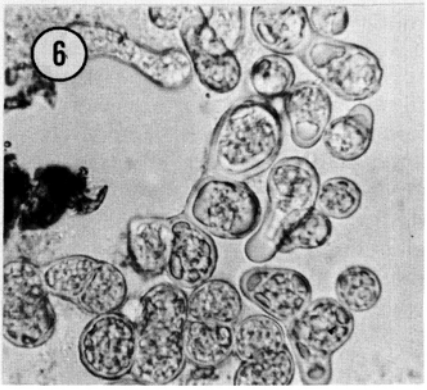
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whether the thick-walled cells were either akinetes or aplanospores.

Discussion

Growth patterns exhibited by *C. cataractarum* were quite different than those reported by other investigators (Kutzing, 1843; Collins, 1909; Printz, 1927; Smith, 1933). The observations of this study have suggested that certain isolates of the alga are heterotrichous. If this were the case the previous reports were of the upright mature filaments and this study dealt with the prostrate anchorage stage from which these upright filaments had originated. At no time during the study were zonal colonies observed as reported by Kutzing (1843).

The development of the algal colony proceeded with the enlargement and division of the original zoospore. Unilateral branching was common in the early stages of development but bilateral branching was more common in older colonies. This type of branch development was again in direct contrast with that described by Collins (1909).

The shape of the cells and filaments were affected by a red colored internal secretion that hitherto had not been reported in the literature. Accumulation of this red substance on one side of the cell resulted in a more rapid growth on the opposite side and curvature of the cell or filament resulted.

Two-week-old colonies contained cells with very thick and layered cell walls. These thick-walled cells readily separated and were possibly the akinetes reported by earlier workers (Collins, 1909; Printz, 1927). If these were in fact akinetes, then any cell of the filament has the potential of becoming an akinete as the culture ages. In the present study, it was impossible to determine whether these thick-walled cells were either akinetes or aplanospores.

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Figures 1-6.—The growth stages of *Chlorotylium cataractarum* Kutz.

1.—Mature filament with zoospore next to a round reproductive cell. X 556.

2.—Young individual algal cells. X 1400.

3.—A curved four-celled filament. Arrows point to the inner cell wall that has a red colored secretion. X 330.

4.—Compact growth pattern due to the early secretion of red colored substance. X 360.

5.—A colony grown on soil water agar. Arrow points to a hyaline cell. X 430.

6.—Two-week-old algal colonies on soil water agar. Note the numerous single cells with heavily layered cell walls. X 1480.