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HETEROCHLAMYDOMONAS, A NEW ALGA FROM TENNESSEE[1]

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ABSTRACT

The new monotypic chlorophycean alga *Heterochlamydomonas* was isolated into axenic culture from soil collected in a cedar glade in Cedars of Lebanon State Forest, Wilson County, Tennessee, and studied in culture over a period of several months. The distinguishing characteristics of the new genus include: the protoplast of free-swimming vegetative cells is closely adpressed to a thin but distinct cell wall; the two closely inserted anterior flagella are of distinctly unequal length; reproduction is by endogenous bipartition of the protoplast of immobile cells to form 2, 4, or 8 daughter cells. *H. inaequalis* is designated as the type species.

INTRODUCTION

The alga described herein was isolated from surface soil collected aseptically in a cedar glade in Cedars of Lebanon State Forest, Wilson County, Tennessee. This paper represents a portion of an extensive investigation of the soil algal flora of cedar glades in the above area (Cox 1967, Cox and Deason 1968). The primary objective of the study was to augment our knowledge of the taxonomy of the soil algae. The obvious need for adequate characterization of algae found in soil has been clearly indicated in recent investigations (Deason and Bold 1960, Chantanachat and Bold 1962, Mattox and Bold 1962, Bishoff and Bold 1963, Brown and Bold 1964, Hofstetter 1968).

MATERIALS AND METHODS

Heterochlamydomonas inaequalis was isolated from algal growth which appeared 2 weeks after 10 grams of aseptically collected soil were added to 50 ml of Bristol's solution (Deason and Bold 1960) in a 125 ml Erlenmeyer flask. This culture vessel as well as all subsequent subcultures were maintained at 22-25 C, under illumination approximating 350 ft-c provided by "cool white" fluorescent bulbs on a cycle of 12 hr. light and 12 hr. darkness. The alga was obtained in axenic culture using the spray method of Wiedemann *et al.* (1964). Most morphologic observations were made on cultures grown on Bristol's agar for 2 weeks under the "standard conditions" of light and temperature indicated above. A Wratten number 48 filter was used as suggested by Friedmann (1966).

An axenic culture of the alga has been donated to the Culture Collection of Algae, Indiana University, and a herbarium specimen has been sent to the Chicago Natural History Museum, Chicago, Illinois.

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DIAGNOSIS

Heterochlamydomonas gen. nov.

Cellulae solitariae libere natantes ellipsoideae ovoideaeve. Protoplastus ad membranam cellulae propriam adpressus. Flagella 2 longitudine inaequa approximataque antice inserta. Chloroplastus parietalis, dissectus, pyrenoide unica praeditus; stigma unicum perspicuum. Vacuolae contractiles 2 anteriores.

Reproductio asexualis per bipartitionem endogenam, in plerumque 2 vel 4, raro 8, cellulas-filiales effecta.

Heterochlamydomonas inaequalis sp. nov.

Cellulae libere natantes ellipsoideae, plerumque antice truncatae, aut ovoideae, 5.6–9.8 x 2.8–5.6 microns. Cellulae immobiles sphaericae ad diametron maximam 14.0 microns amplificantur. Papilla nulla; membrana cellularum mobilium levis tenuisque; membrana cellularum immobilium usque ad 3.0 microns spissescens, cellulis senescentibus. Cellulae mobiles 2 flagella inaequa antice inserta habentes; flagella longitudine aequa corpori cellulae aut paulo longiora. Chloroplastus parietalis, cellulam partim circumdans, maxime dissectus, etiam segmentatus; pyrenoides aequatoria ad paululum anteriorem; stigma convexo-concavum anterius. Vacuolae contractiles 2 anteriores; nucleus posterior. Cellulae solitariae intra vaginas gelatinosas inclusae. Vaginae in cellulis aggregatis confluentes.

Reproductio asexualis per bipartitionem endogenam cellularum immobilium, ad 2 vel 4, raro 8, cellulas-filiales formandas, effecta.

Reproductio sexualis non observata.

Culturae 2 hebdomadum aetate in agaro Bristolii dilute virides viscidaeque, in 2-3 menses atroviridescentes.

Origo: e solo e loco Cedars of Lebanon State Forest, Wilson County, Tennessee dicto, m. Aug. 1964 lecto.

The Latin diagnosis was prepared by Dr. Hannah Croasdale of Dartmouth College.

OBSERVATIONS

Free-swimming cells of this alga are ellipsoidal (usually truncate or rounded anteriorly) or ovoidal (Fig. 1, 2). They range from 5.6-9.8 microns in length

Fig. 1. General view of 2-week-old culture showing cell shapes.
Fig. 2. Vegetative cells becoming motile; note dissected chloroplast and prominent stigma (indicated by arrows).
Fig. 3. Solitary cell with gelatinous sheath.
Fig. 4. Motile cells; note unequal flagella.
Fig. 5. Quiescent spherical cell.
Fig. 6. Bipartition to form daughter cells.
Figs. 1-6. X970.

and from 2.8-5.6 microns in width. When cultivated on Bristol's agar, individual cells may produce gelatinous sheaths which measure as much a 3.0 microns in thickness (Fig. 3). The individual sheaths become confluent when cells form loose aggregates. Many cells from agar cultures have flagella and stigmata and become motile immediately upon transfer to distilled water. Those without flagella produce them within a short time and also become motile. The protoplast of motile cells is adpressed to a smooth, thin, distinct cell wall. No papilla is present. Two flagella of distinctly unequal length are inserted close together anteriorly and approximate or slightly exceed cell body length (Fig. 4, 7). Each cell contains 2 large anterior contractile vacuoles and a posterior nucleus. The chloroplast is parietal, partially encircles the protoplast, and has a very irregular surface dissected by numerous fissures. In some cells, the chloroplast appears to be segmented. A single pyrenoid surrounded by starch plates occupies an equatorial to slightly anterior position, and a large convexo-concave stigma is always anterior (Fig. 7, 8). A test for starch is positive (a blue-black color when stained with I_2KI).

Motility is usually of long duration, lasting for 24 hours or more. As the cells become quiescent, they withdraw their flagella and begin to enlarge. During enlargement, they may retain their ellipsoidal shape, or become subspherical to spherical (Fig. 5). Some of the spherical cells may become relatively large with age, attaining a diameter as great as 14.0 microns and a cell wall thickening to 3.0 microns.

Asexual reproduction is by endogenous bipartition of the protoplast of immobile cells (this includes the large subspherical to spherical cells also) to form 2 or 4, rarely 8, daughter cells (Fig. 6) which are liberated by gradual gelatinization of the parent cell wall.

Sexual reproduction was not observed for this species.

Colonies on Bristol's agar are light-green after 2 weeks growth, becoming dark-green in 2-3 months. The surface of colonies examined at a magnification of 20X is shiny and smooth. They are of viscid consistency at 2 weeks, and become more viscid with age.

Discussion

This unicellular chlorophycean alga is clearly a member of the Chlamydomonadaceae (Volvocales) since the protoplast of free-swimming vegetative cells is closely adpressed to a thin but distinct cell wall. It differs from other members of the family in that the 2 closely inserted anterior flagella are of distinctly unequal length. Accordingly, the alga has been described as the new taxon, *Heterochlamydomonas inaequalis.*

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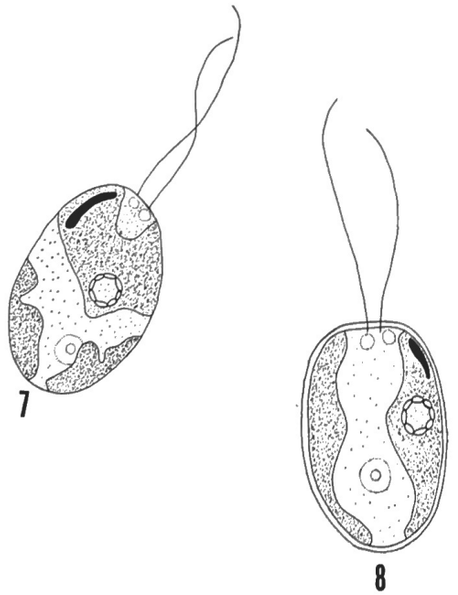

Fig. 7. Vegetative cell in surface view showing unequal flagella, dissected chloroplast, and large curved stigma.
Fig. 8. The same in median optical section.
Figs. 7 and 8. X3000.

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SOME PROPERTIES OF CERTAIN HYPER-SOLIDS

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Introduction

We shall discuss some metric relationships which characterize certain hyper-solids of n dimensions and result in some well-known elementary formulas being recognized as special cases of much more generalized forms. For instance, the perimeter and area of an equilateral triangle, as well as the surface and volume of a regular tetrahedron are four of the specific cases which are obtained from a coverall relationship.

The Hyper-cube:

The definition of this solid is obtained by following an iterative procedure. Start with a point, a O-dimensional entity. Apply straight line motion to it over a distance of a units. Then its trace forms a line segment, a 1-dimensional magnitude. By moving this line segment out of its 1-dimensional space in a direction perpendicular to that space over a units a square, a 2-dimensional element, is obtained. Similar motion of the square creates a cube, a 3-dimensional manifold. Likewise with the cube, a tesseract (the 4-dimensional hyper-cube) will be formed. Continue this process indefinitely and it will lead to a set of hyper-cubes of n dimensions, where $\epsilon \{0, 1, 2, \ldots\}$.

Relationships:

1. Number of boundary manifolds:

$$M_{n,i} = 2^{n-i}\binom{n}{i} \qquad (1)$$

where $\binom{a}{b} \underset{\text{def.}}{=} \frac{a!}{b!(a-b)!}$ and $\binom{a}{b} = 1$ whenever $b = 0$.

Since n means the dimension of the solid under consideration, and i represents the dimensionality of the boundary manifold in question, $i \geq n$.

Proof:

To obtain relationship (1), note first that, owing to the definition of the n-dimensional hyper-cube, the recurrence formulas

$$M_{n,i} = 2M_{n-1,i} + M_{n-1,i-1} \quad ; \quad \begin{matrix} n > 0 \\ n \geq i \end{matrix}$$

$$M_{n,o} = 2M_{n-1,o} = 2^n \quad ; \quad n > 0 \qquad (0)$$

$$M_{o,o} = 1$$

hold. These formulas enable one to construct Table I quite rapidly.

But

$$\binom{n}{i} = \binom{n-1}{i} + \binom{n-1}{i-1} \quad ; \quad (a),$$

as may be seen from the definition of these symbols.

Superimposing this relationship on formulas (0), relationship (1) results. (1)

Formula (1) may now be used for some specific values of n and i to obtain a whole array of otherwise unrelated facts: a line segment has two points as its extremities ($M_{1,0}=2$); a square has four vertices ($M_{2,0}=4$) and four edges ($M_{2,1}=4$), and a cube has eight vertices ($M_{3,0}=8$), twelve edges ($M_{3,1}=12$) and six faces ($M_{3,2}=6$).

2. Relationships among these manifolds:

An horizontal summation in Table I results in:

$$\sum_{i=0}^{n} M_{n,i} = 3^n \qquad (2)$$

Proof:

Explicitly stated, relationship (2) shows that

$$M_{n,o} + M_{n,1} + \cdots + M_{n,i} + \cdots + M_{n,n} = 3^n,$$

or—according to (1)—

$$2^n + 2^{n-1}n + \cdots + 2^{n-i}\binom{n}{i} + \cdots + 2\binom{n}{n-1} + 1 = 3^n.$$

However, since the left member of this statement represents the binomial expansion of $(2 + 1)^n$, the conclusion follows immediately.

3. Euler's generalized relationship:

In 3-dimensional space, V, the number of vertices, E, the number of edges and F, the number of faces of any solid are interconnected by the well-known Euler-formula

$$V + F = E + 2$$

(1) Multiply both sides of (a) by 2^{n-i}, and rewrite the resulting equation as

$$2^{n-i}\binom{n}{i} = 2 \cdot 2^{n-1-i}\binom{n-1}{i} + 2^{n-1-(i-1)}\binom{n-1}{i-1}.$$

This, however, becomes interpreted as:

$$M_{n,i} = 2M_{n-1,i} + M_{n-1,i-1}.$$

and from 2.8-5.6 microns in width. When cultivated on Bristol's agar, individual cells may produce gelatinous sheaths which measure as much a 3.0 microns in thickness (Fig. 3). The individual sheaths become confluent when cells form loose aggregates. Many cells from agar cultures have flagella and stigmata and become motile immediately upon transfer to distilled water. Those without flagella produce them within a short time and also become motile. The protoplast of motile cells is adpressed to a smooth, thin, distinct cell wall. No papilla is present. Two flagella of distinctly unequal length are inserted close together anteriorly and approximate or slightly exceed cell body length (Fig. 4, 7). Each cell contains 2 large anterior contractile vacuoles and a posterior nucleus. The chloroplast is parietal, partially encircles the protoplast, and has a very irregular surface dissected by numerous fissures. In some cells, the chloroplast appears to be segmented. A single pyrenoid surrounded by starch plates occupies an equatorial to slightly anterior position, and a large convexo-concave stigma is always anterior (Fig. 7, 8). A test for starch is positive (a blue-black color when stained with I_2KI).

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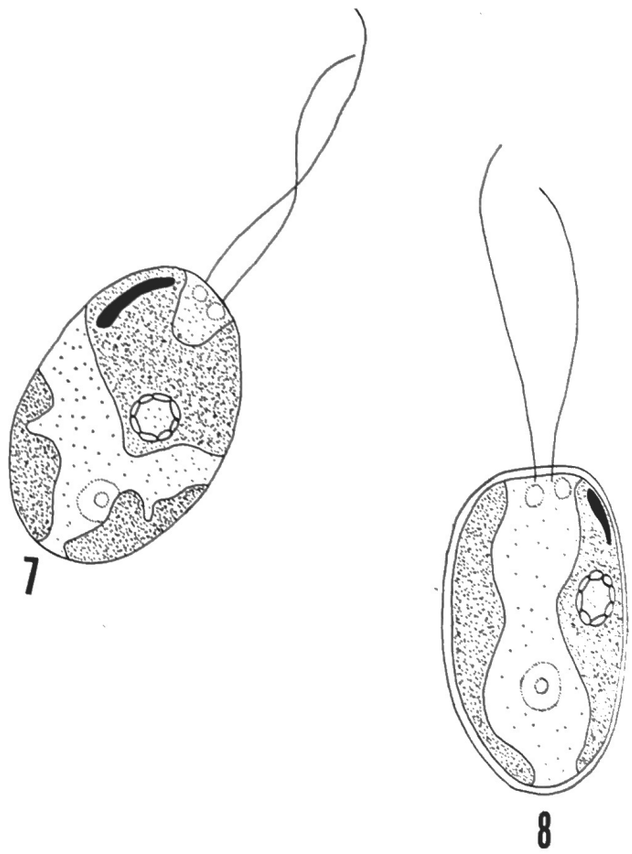

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