AKINETES, REPRODUCTION, AND COLONY FORM IN THE GREEN ALGA *SORASTRUM*

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ABSTRACT. — One-month-old cells of *Sorastrum* in a defined mineral medium enlarged slightly and became thick-walled, yellow-green, and packed with yellow fat droplets in which carotenoid pigments were dissolved. These akinetes survived desiccation for at least one month. When placed in a fresh culture medium, they germinated and produced daughter colonies as do vegetative (non-akinete) cells. Reproduction in *Sorastrum* is described, and the author advances an hypothesis to account for colony shape.

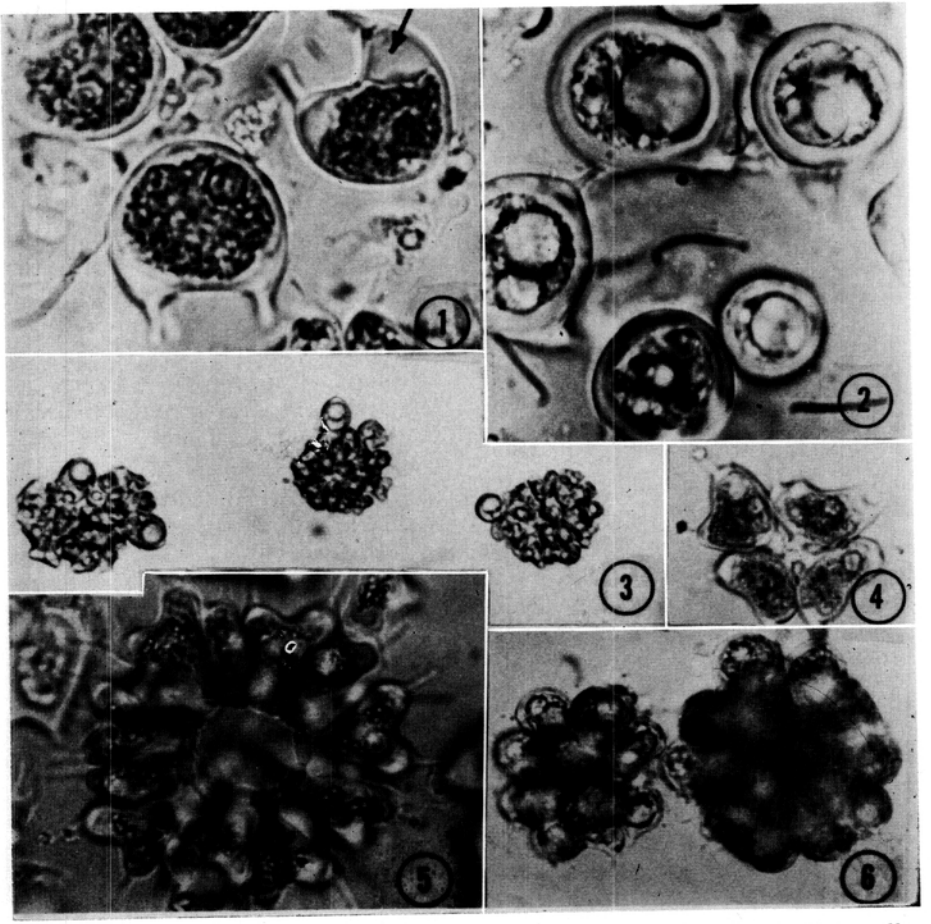
Although reproduction in the colonial green alga *Sorastrum spinulosum* has been described by several workers, their papers give conflicting reports. De-la-Rue (1873) observed that colony cells separate, enlarge, but may become thick-walled cysts. Colony cells usually divided into two parts, each of which became typically shaped cells. With further development these cells became cysts. The contents of the cysts divided into two to several cells which united to form a colony. The colony was released when the cyst wall disappeared or when it broke apart. Palik (1936 also observed the resting stages (cysts) of *Sorastrum spinulosum*. These were thick-walled, yellow cells containing red oil droplets. He demonstrated that the resting stages were able to survive four months of desiccation and freezing temperatures. Probst (1926) stated that during reproduction the cell contents divided into 8-64 multinucleate "plasmaballen" which were released in a vesicle. Each of these divided again and gave rise to groups of 4-32 biflagellate zoospores which became separate colonies. Palik also observed several daughter colonies in one vesicle. Geitler (1924) reported that reproduction begins with the successive division of the chloroplast and of the multinucleate protoplast into parts each of which becomes a zoospore. The zoospores were released in a vesicle through a break in the wall of the mother cell. Zoospores moved slowly for a while, stopped, flattened, and formed one hollow spherical colony. The colony of zoospores became united at its center. The vesicle disappeared, and each cell of the young colony developed four horns toward the outside and a stipe toward the center of the colony. The stipes of all cells were embedded in a sphere of gelatin.

I have seen the complete process of reproduction many times. I have also obtained cells (akinetes) similar to the cysts of De-la-Rue, and to the yellow, thick-walled cells (resting stages) of Palik. The present study describes my observations of reproduction, colony formation, and akinete formation and germination.

Materials and Methods

A unialgal culture of *Sorastrum* (LB 785) was obtained from the Culture Collection of Algae at Indiana University. The author determined that the alga was *Sorastrum spinulosum* Näg. The culture was used to inoculate five test tubes containing 15 ml of sterile mineral Medium C (Davis, 1963), and four 125 ml Erlenmeyer flasks containing 75 ml of sterile Medium C. The algae in the tubes were subcultured every 10 days; those in the flasks were not subcultured. The cultures were under fluorescent "cool white" lights of 350 ftC with a 16/8 hour light/dark cycle. Temperature was maintained at 20± 2 C. Nuclear staining was done with hematoxylin stain prepared and used according to Bowen (1963).

Reproduction and colony form in vegetative cells—Colonies from the ten-day-old culture were transferred to the fresh culture medium. After one or two days the uninucleate protoplasts divided in many planes so that the cell contents were progressively cleaved into many



Figures 1-6. — Cells and colonies of *Sorastrum spinulosum*. 1. Ten-day-old cells; arrow is in body at base of horns; 900X. 2. Thick-walled akinetes containing fat droplets, 900X. 3. Daughter colonies from akinetes; projecting cells contain fat droplets. 4. A 4-celled colony with solid center. 5. Hollow-centered 32-celled colony. 6. Colonies of akinetes. (3-6, 450X).

small parts. The nucleus of such cells also divided so that each of these small parts contained one nucleus. The small parts separated and became rounded zoospores. Cells containing such zoospores were spherical and considerably larger than cells not forming zoospores. After one to three days the outer wall of the mother cell cracked in several places and the thick middle wall adjacent to the cracks disappeared. Then the outer wall split off, the middle wall disappeared completely, and the zoospores were left in a hyaline vesicle. Before the outer wall split off, the vesicle was the innermost layer of the cell wall. Occasionally the vesicle was extruded through a wide crack in the outer wall, and the outer wall with its horns and stipe did not break apart. Usually 16 or 32 zoospores were in each vesicle, occasionally eight. Each zoospore was spherical with two flagella, and contained a parietal chloroplast which occupied the entire contents except for a small clear portion from which the flagella emerged. Just after release the vesicle was the same size as the mother cell, and the confined zoospores began to wriggle slowly within it. The vesicle then expanded slowly as the zoospores moved faster and arranged themselves in a spherical colony one cell thick and in contact with the vesicle. After moving for 3-5 minutes, the zoospores slowed down and stopped. The clear parts of the zoospores with the flagella pointed toward the center of the colony. Each zoospore produced a stipe toward the center of the colony and four horns toward the outside. The stipes were produced by the clear part of the zoospore; the horns grew from the wall adjacent to the chloroplast. Stipe and horn formation began a few seconds after the zoospores stopped moving and was completed in three to five minutes. In four and eight-celled colonies the stipes grew together and filled the center of the originally hollow colony (Fig. 4). In sixteen and thirty-two-celled colonies, and in some eight-celled colonies, the base of the stipes broadened slightly into disks parallel to the colony surface (as in Fig. 5). The disks of neighboring zoospores grew together and fused. Just after fusion each disk was hexagonal. The bases of the stipes thus formed a hollow central sphere bounded by the hexagonal disks. Except in aberrant colonies, the vesicle expanded so that the young colony floated free within it. These events (protoplast cleavage through vesicle expansion) occurred within one day. The vesicle disappeared after one or two more days, and the young colony reached full size (35-40μ dia) in ten to fifteen days. Cells of ten-day-old colonies contained a large central vacuole completely surrounded by a parietal chloroplast, one nucleus, and a single pyrenoid. Neither the nucleus nor the pyrenoid occupied a fixed location.

Each cell had two pairs of horns. Often each mature cell contained one or two solid, clear, lenticular bodies (approx. 6X8μ) at the bases of the pairs of horns (Fig. 1). Frequently the body under one pair of horns of a cell was considerably larger than that under the other pair. After the release of the vesicle these bodies usually remained attached to the outer surface of the vesicle, or they remained in place attached to the remnants of the mother cell. An aqueous solution of aniline blue stained these bodies blue, thus indicating they were of callose (Johansen, 1940, p. 185).

Some ten-day-old cells and all older cells developed a thick middle wall between the vesicle and the outer cell wall. The pectin stain ruthenium red (Meyer, Anderson & Swanson, 1955, pp 165-166) colored the thick middle walls red; the cellulose stain chloroiodide of zinc (Sass, 1958, p 97) colored them blue. An aqueous methylene blue solution (recommended by Bold, 1957, p 630, for algal sheath staining) colored them blue, and stained the inner layers most deeply. This stain failed to reveal any gelatin in the center of the colony.

There were a few aberrant colonies on almost every slide prepared for observation. These colonies always occurred in a constricted space: either the vesicle failed to expand, or it failed to be released and thus the colony formed within the mother cell. Zoospores forming these aberrant colonies did not move. Zoospores of such colonies usually formed fewer than four horns, and generally not all of the horns projected toward the outside of the spherical colony. These zoospores, however, did produce stipes.

Multiple colonies were observed within a vesicle on three different occasions. Two 16-celled colonies in one vesicle were observed twice. Another vesicle contained 4 colonies; two 8-celled and two 16-celled. All these colonies and their cells were typically shaped.

Akinete formation—Algae in the flasks formed akinetes. After inoculation and reproduction, these algae grew to full

size in about ten days. Very little reproduction occurred thereafter. During the next 20 days each cell gradually deposited a thick middle wall of several layers. In addition, a clear, solid, lenticular body formed between the middle wall and the inner wall on each side of the cell at the bases of the pairs of horns.

As the thick middle wall was deposited, the cells became almost spherical and somewhat larger than ordinary cells. At the end of the third week in the culture medium, many small yellow droplets appeared in the cells. In one more week these became larger and coalesced to form 2 or 3 large droplets (FIG. 2). After the cells had developed their thick middle walls, a slight pressure on the coverslip would separate the cells of a colony. By the end of the fourth week the mass of colonies was yellow-green. Except for color, the individual colonies looked like ordinary vegetative colonies (FIG. 6). The culture solution was decanted at the end of the fourth week and the colonies, now akinetes, were allowed to dry completely. When dry, they were a bright orange. Dry colonies and cells shrank slightly, but otherwise did not change their shape. Most of the cells contained one nucleus; a few contained 2 or more nuclei.

After several months in the culture solution, the akinetes became orange and enlarged slightly. Some of the akinetes enlarged markedly to become almost twice the diameter of vegetative cells.

The akinetes reacted to stains like vegetative cells. The orange droplets within the akinetes were fat colored by carotenoid pigments, for when concentrated H_2SO_4 was dropped on akinetes the orange droplets became light blue. After removal of the acid and treatment with Sudan IV, the droplets became red, thus demonstrating that they were of fat. Evidence of the presence of carotenoid pigments is given by the change of color from orange to light blue with the treatment of sulfuric acid (Johansen, 1940, p 201). This is further supported by using ethyl ether to obtain a yellow extract from the akinetes, which extract, when placed in a DU spectrophotometer gave an absorption curve displaying in the visible spectrum a single maximum at 470 $m\mu$.

Akinete germination — A small quantity of akinetes which had been dry for one month was placed in fresh Medium C. These akinetes became green in one day, and in two to three more days they produced daughter colonies in the same manner as vegetative cells. Colonies formed from akinetes, however, often contained large, fat-filled cells (FIG. 2). As an akinete formed zoospores, its fat droplets were incorporated into one to several zoospores. Zoospores containing fat droplets were larger and moved more slowly than those formed from zoospores without droplets. Most cells containing droplets failed to form all four horns; some formed no horns at all.

DISCUSSION

Although Probst (1926) observed that *Sorastrum* in solutions whose mineral concentration was over 0.3% produced thick-walled cells, he reported that such cells would degenerate unless soon transferred to a suitable medium. Thus he may have observed akinetes without recognizing them as such.

Akinetes and old vegetative cells have three distinct walls: the innermost wall which becomes the vesicle; the thick middle wall which has a lamellar construction; and the outside wall, parts of which form the horns and stipes. Probst, (1926) using congo red stain, deduced that the outside wall of the mother cell and the stipes were cellulose. He assumed that the middle wall contained pectin since congo red did not stain it. In my work, the thick middle wall of akinetes and the thick middle wall of vegetative cells reacted to the strains for cellulose, pectin, and gelatin (methylene blue). Since the middle wall quickly dissolved after the outer wall broke away, it is not cellulose, but more likely a combination of pectin and gelatin, as both of these are water soluble. West (1916, p. 127) stated that the gelatinous substances of

algal cell walls stain with both methylene blue and ruthenium red.

Those zoospores produced from akinetes and containing fat droplets often failed to produce all four horns. Such zoospores were larger and less active than the other zoospores in the same vesicle. If we accept with Geitler (1924) that all cells are polar, then the horn forming parts of such slow moving zoospores might become disposed against another cell and thus unable to form all four horns. A similar phenomenon occurred in *Pediastrum* (Davis, 1962).

Although Probst (1926) and Palik mention multiple colonies in one vesicle, such colonies were not mentioned in Geitler's report nor did I observe more than three vesicles containing multiple colonies. I also did not observe the central sphere of gelatin mentioned by Geitler (1924). However, my observations of reproduction agree essentially with those of Geitler (1924) and Probst (1916).

It is possible that all colonies observed by Probst (1926) and De-la-Rue were similar to the aberrant ones described in this study. Observations of colonies confined within the mother cell may account for De-la-Rue's failure to observe zoospores. The "plasmaballen" mentioned by Probst could have been zoospores in non-expanding vesicles each of which later developed into a colony.

Generally, large colonies were hollow while small colonies had solid centers. This is to be expected as zoospores of four and eight-celled colonies are close enough for their stipes to join at the center. This union probably occurred before the stipes reached full length and thus inhibited the formation of the disks observed in the 16 and 32-celled colonies. In these larger colonies, the stipes of the single layered colony were too short to join at the center of the colony. In such colonies, the stipes grew to full length and developed disks which joined and became hexagonally shaped from mutual pressure. *Sorastrum americanum* also produces a similar hollow central sphere made of the hexagonal bases of the stipes (Fritsch, 1935, p. 170, 49B after Schröder).

Probst (1916, 1926), Palik (1936), and the present author have observed two flagella on each zoospore. All three authors have noted that the flagellar end of the zoospores turned toward the center during colony formation while the opposite end formed the horns. Probst (1926) and the present author further agree that the flagella occurred at a clear portion of the zoospore wall and that this clear portion produced the stipes.

Davis (1964) and Moner (1953) have observed that the colonies of *Pediastrum* are formed in a lens-shaped vesicle and are flat. As is well known, the colonies of *Sorastrum* are formed in a spherical vesicle and are spherical. Pocock (1960) notes that when *Hydrodictyon reticulatum* reproduces asexually, the new colonies form in the narrow cylindrical space between the outer wall and the vacuolar membrane and are cylindrical. Bonner (1963, pp. 156-159) argues that the shape of such asexually produced colonies of *H. reticulatum* is determined by the shape of the mother cell, and his argument may be applied with only minor changes to *Pediastrum* and

Sorastrum. Thus the colony shape for these genera of the Hydrodictyaceae would seem to be determined by the shape of the mother cell or the vesicle at the time the colony is forming.

Literature Cited

Bold, H. C. 1957. Morphology of Plants. Harper and Brothers, New York. XVIII + 669 pp.

Bonner, J. T. 1963. Morphogenesis, An Essay on Development. Antheneum, New York. VI + 296 pp.

Bowen, W. R. 1962. The demonstration of mitotic figures in green algae. Proc. Iowa Acad. Sci. 70:138-142.

Davis, J. S. 1962. Resting cells of *Pediastrum*. Amer. J. Bot. 49:478-481.

———. 1963. The influence of the bicarbonate ion concentration on cell division and cell orientation in *Pediastrum*. Proc. Iowa Acad. Sci. 70:100-107.

———. 1964. Colony form in *Pediastrum*. Bot. Gaz. 125:129-131.

De-La-Rue, M. E. 1873. Sur le développement du *Sorastrum* Kg. Ann. Sci. Nat., Bot. ser. 5, 17:400-402.

Fritsch, F. E. 1935. The Structure and Reproduction of the Algae. Vol. 1. Cambridge Univ. Press, London. XVII + 791 pp.

Geitler, L. 1924. Die Entwicklungsgeschichte von *Sorastrum spinulosum* und die Phylogenie der Protococcales. Arch. Protistenk. 47:440-447.

Johansen, D. A. 1940. Plant Microtechnique. McGraw Hill, New York. XI + 523 pp.

Meyer, B. S., D. B. Anderson, and C. A. Swanson. 1955. Laboratory Plant Physiology. D. Van Nostrand Co. Inc., New York. VIII + 168 pp.

Monee, J. G. 1953. Morphogenesis in *Pediastrum*, including studies on developmental physiology and cell wall structure. Ph.D. Thesis, Princeton Univ.

Palik, P. 1936. Untersuchungen über die Entwicklung von *Sorastrum spinulosum* Näg. Beih. bot. Zbl. 55A:421-428.

Pocock, M. A. 1960. *Hydrodictyon*: A comparative biological study. J. S. African Bot. 26:167-313.

Pronst, T. 1916. Ueber die ungeschlechtliche Vermehrung von *Sorastrum spinulosum* Nägeli. Tätigk. ber. Naturf. Ges. Baselland 1911-1916: 174-177.

———. 1926. Ueber die Vermehrung von *Sorastrum* Nägeli, *Pediastrum* Meyen, und *Tetraedron* Kützing. Tätigk. ber. Naturf. Ges. Baselland. 7:29-36.

Sass, J. E. 1958. Botanical Microtechnique. Iowa State College Press, Ames, Iowa. XI + 228 pp.

West, G. S. 1916. Algae. Vol. 1. Cambridge Univ. Press, London. IX + 475 pp.

Manuscript received March 10, 1966.