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THE CYTOLOGY OF ZOOSPOROGENESIS IN THE FILAMENTOUS GREEN ALGAL GENUS *KLEBSORMIDIUM*

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Cain, J. R., Mattox, K. R. & Stewart, K. D. 1973. The cytology of zoosporogenesis in the filamentous green algal genus *Klebsormidium*. *Trans. Amer. Micros. Soc.*, 92: 398–404. In cells of the ulotrichalean alga *Klebsormidium*, vegetative protoplasts are transformed into single motile cells. Zoosporogenesis is signaled by the appearance of lateral papillae and is accompanied by the rearrangement of cell organelles. Numerous contractile vacuoles arise de novo and seem to "pump-out" the contents of the larger vacuoles. Mature zoospores have only contractile vacuoles and contain many lipid-body inclusions. Biflagellate zoospores escape through pores formed in the papillae. Zoosporogenesis in *K. sterile* is essentially like that of *K. flaccidum*. Attempts to induce zoosporogenesis in *K. marinum* have not been successful.

The genus *Klebsormidium* Silva, Mattox & Blackwell (1972) included five species, all of which are filamentous, produce no holdfasts, and have uninucleate cells that contain one parietal plastid with a single pyrenoid. Although fragmentation of the filaments is the most common means of reproduction, biflagellate zoospores are formed under certain conditions. Sexual reproduction has been described only by Wille (1912).

Since the turn of the century, when Klebs (1896) first reported on studies of zoosporogenesis in the genus, there has been little additional descriptive or experimental work on the phenomenon, although many writers (including Fott, 1959; Gerneck, 1907; Hazen, 1902; Heering, 1914; Mattox & Bold, 1962; Peterson, 1935; Prescott, 1968; and Ramanathan, 1964) have recorded the presence of zoospores in one or more species of the genus. Farooqui (1969) and Mattox (1971) have provided additional information on conditions which induce zoosporogenesis in *K. flaccidum*. For two species, *K. sterile* and *K. marinum*, zoosporogenesis has not been reported. In short, zoospores and the mechanisms by which they are produced have been incompletely described for three species of the genus and not recorded at all for the remaining two species.

In this paper we shall present Nomarski and electron microscope observations on the cytology of zoosporogenesis in *K. flaccidum*. We shall also record new information on the fine structural organization of *K. sterile* and *K. marinum* and report on our attempts to induce zoosporogenesis in these species. Further studies on the induction of zoosporogenesis in *K. flaccidum* will be reported in a later paper.

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MATERIALS AND METHODS

K. flaccidum (isolate F-41 of Mattox, 1971) was isolated from soil; *K. sterile* was obtained from the Indiana University Culture Collection of Algae (#978, Starr, 1964); and *K. marinum* was provided by T. R. Deason, the isolator. Stock cultures were routinely maintained on agar-solidified slants of BBM (Nichols & Bold, 1965) at 20 ± 2 C on a 12-12 lt-dk cycle under 0.91 cal/cm^2/hr of cool white fluorescent light.

For induction of zoosporogenesis, algae were transferred to BBM or to soil water extract in Fernbach lo-form culture flasks and kept at 20 C on a 16-8 lt-dk cycle under 0.54 cal/cm^2/hr of cool white fluorescent light. Cultures were bubbled 3–5 min daily with 5% CO_2 in air. From these flasks filaments were transferred to fresh BBM in 15 × 60 mm petri dishes and observed after 16 hr additional incubation, usually in darkness. Observations were made with Zeiss bright field and Zeiss Nomarski microscopes. Light intensities were measured with a YSI integrating pyranometer.

Rapidly growing cultures were prepared for electron microscopy with Karnovsky's fixative and post fixed in 2% aqueous OsO_4 (8–12 hr, 4 C). Following dehydration, the algae were embedded in Epon or in Spurr's low viscosity medium.

RESULTS

Observations on Klebsormidium flaccidum

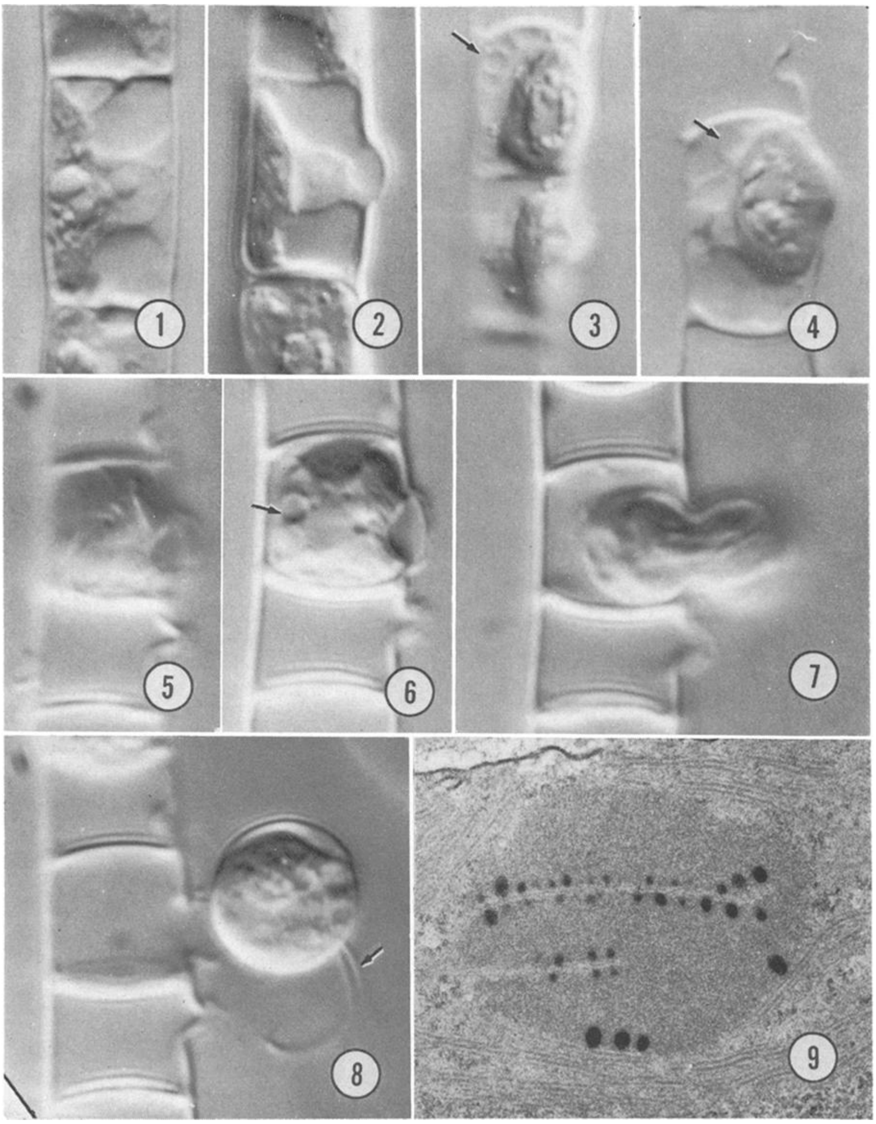
The consistent arrangement of organelles in the *K. flaccidum* interphase cell has been described recently by Floyd et al. (1972). Our subsequent observations on zoosporogenesis in *Klebsormidium* were begun using Nomarski optics with the intent of following and describing in some detail the sequence of events which takes place in the transformation of a vegetative cell protoplast (Fig. 1) into a zoospore.

The first indication of the initiation of zoosporogenesis in *K. flaccidum* is the appearance of a small vacuolate papilla (Fig. 2) on the lateral wall of one or more cells of a filament (Mattox, 1971). There is a marked increase in the number of refractive bodies, presumably lipid droplets, in those cells with papillae (Figs. 3, 10), and some rearrangement of organelles, including the appearance of mitochondria between the chloroplast and the wall (Fig. 10).

The papillae in the walls of cells in early zoosporogenesis may be located either opposite the nucleus and cytoplasmic bridge (Fig. 2) or on the side of the chloroplast (Fig. 4). A constant feature of zoosporogenesis is that the chloroplast is on the same side of the cell as the papilla at the time of release; therefore, a rotation of the chloroplast may be observed fairly early in development of those cells with the papillae initially on the nuclear side.

Either before or shortly after chloroplast rotation, numerous small contractile vacuoles appear in the cytoplasmic bridge and in the thin layer of peripheral cytoplasm (Figs. 3, 4). The contractile vacuoles appear to be involved in eliminating the contents of the two larger vacuoles, which are conspicuously absent in the zoospores.

As contractile vacuole activity continues, the nucleus becomes less conspicuous as it is obscured by the chloroplast. As the large vacuoles disappear, formation of the flagella occurs. Although stages of flagellar formation have not been observed, the flagella, when finally formed, are visible inside the parent cell just prior to release of the zoospore (Fig. 5). Contractile vacuole activity is intense at this stage, and a concave depression is clearly visible in that portion of the chloroplast contiguous with the papilla (Fig. 6). As a result, the papilla itself appears to be devoid of contents.



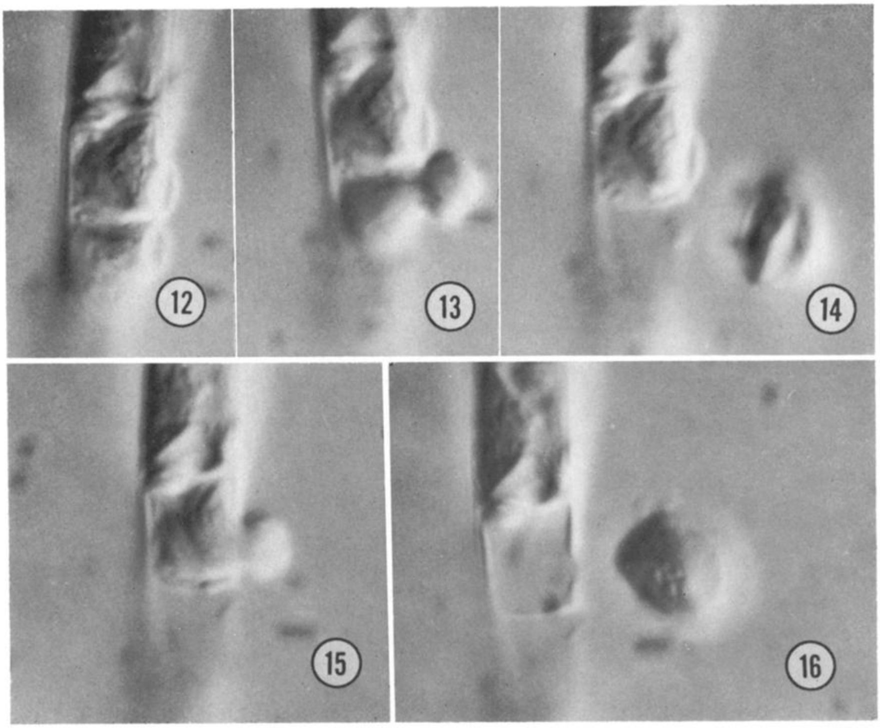
FIGS. 1–8. *Klebsormidium flaccidum.* Fig. 1. Vegetative interphase cell. Figs. 2–8. Stages in zoosporogenesis. Note contractile vacuoles (arrows) in Figures 3, 4, and 6 and flagella (arrow) in 8. × 2,700.

FIG. 9. *K. marinum.* Pyrenoid with osmiophilic globules associated with traversing membranes. × 55,000.

The zoospore squeezes out (Fig. 7) through a perforation in the papilla by an amoeboid type of movement, the flagella generally trailing behind the main body of the zoospore. Occasionally the released zoospore may remain attached to the mother cell wall by a thin cytoplasmic strand which must be broken before escape from the parent filament is accomplished. Pigmented stigmata are not present in the zoospores. The pyrenoid remains intact throughout the process and may be observed in the released zoospore.

FIG. 10. *K. flaccidum*. Cell in early zoosporogenesis; positions formerly occupied by large, terminal vacuoles are now occupied by smaller, contractile vacuoles and by dark-staining lipid droplets. × 24,500.

FIG. 11. *K. marinum*. Whole cell. × 18,000.



FIGS. 12–16. *K. sterile.* Successive release of zoospores from two cells. × 2,700.

Observations on Klebsormidium sterile

Although production of biflagellate zoospores is considered to be a generic attribute of *Klebsormidium*, two of the species included in the genus by Silva, Mattox & Blackwell (1972) lack documented observations of zoospores. These are *K. sterile* and *K. marinum* (Deason, 1968).

In the discussion accompanying the original description of *K. sterile* (Deason & Bold, 1960) the authors state that no motile cells were observed in this organism "despite the usual manipulations designed to evoke their formation."

To induce zoosporogenesis in *K. sterile*, filaments were taken from the surface of a two-week old agar slant culture, placed in liquid BBM, and incubated in the dark for two, three, or four day periods at 20 C. Zoospores were produced in all samples, though not in great numbers (Figs. 12–16).

Both cytological organization and the process of zoosporogenesis in *K. sterile* appear to be essentially similar to *K. flaccidum*, the chloroplast being on the same side of the cell as the papilla at the time of release and contractile vacuoles being present (cf. Figs. 1–8 with 12–16). A second isolate of *K. sterile*, isolated from soil, has been identified by comparison with the type, and it too has produced zoospores. On one occasion, a culture of the type isolate was placed in the dark for 64 hr, was removed and observed, and was left exposed to the ambient light and temperature conditions of the laboratory. Upon reexamination of the culture 24 hr later, many zoospores had been released and were motile in the culture vessel, indicating that this *Klebsormidium* species has capabilities to produce zoospores in quantity under certain conditions.

Observations on Klebsormidium marinum

Attempts to induce zoosporogenesis in *K. marinum* have been unsuccessful, although all techniques useful in other species have been tried repeatedly. The failure is perhaps understandable in light of the extent to which *K. marinum* differs cytologically from other investigated species including *K. sterile*, *K. flaccidum* (Floyd et al., 1972), and *K. subtilissimum* (J. D. Pickett-Heaps, personal communication).

Cells of *K. marinum* are conspicuously smaller than those of other species, about 3 μm maximum diameter, usually much less, and nearly always solitary. Arrangement of organelles is variable, in sharp contrast to *K. flaccidum*. In some cells the nucleus is median, but in others the nucleus and plastid occupy opposite ends. Osmiophilic globules are always associated with the traversing membranes of the pyrenoids (Fig. 9). Mitochondria and microbody-like organelles may be large and few or small and many. Vacuoles, when present, frequently seem to be invaginations of the plasmalemma (Fig. 11) and often are filled with concentrically swirled membranes. Cytokinesis is by furrowing, as in other species of the genus, but details of mitosis have not been observed.

DISCUSSION

The zoosporogenesis of *K. sterile*, here reported for the first time, is essentially similar to that of *K. flaccidum*. In both species there is a one for one transformation of vegetative cells into zoospores. The conspicuous vacuoles of the vegetative cells are apparently emptied by the numerous contractile vacuoles which appear early in zoospore development. At the time of their release, zoospores have only contractile vacuoles.

At least in *K. flaccidum*, the appearance of lipid droplets in the peripheral cytoplasm and the rearrangement of certain organelles coincides with the development of contractile vacuoles. Synthesis of the two anterior flagella completes the development of the zoospore.

Klebsormidium marinum differs from all other investigated species of the genus in several respects. Cells are conspicuously smaller in diameter and rarely associated into filaments of more than two or three cells. Organelles do not have the same precise arrangement and number in the cells of *K. marinum* as they have in other species. Finally, those circumstances and manipulations of culture conditions which evoke zoosporogenesis in other species fail to do so in *K. marinum*.

Transfer of *K. marinum* to another or to a new genus is probably warranted. However, we do not formally propose this at present because we have not yet observed all details of mitosis in *K. marinum* and because we have not finished our examination of the fine structure of other filamentous green algae which are likely to be similar to *K. marinum*.

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BIOTIC CHARACTER AS RELATED TO STREAM MINERAL CONTENT[1]

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NEEL, J. K., SR. 1973. Biotic character as related to stream mineral content. *Trans. Amer. Micros. Soc.*, 92: 404–415. Nine montane streams in or near deciduous forests in eastern Kentucky and western Virginia fell into two distinct classes with respect to hardness and alkalinity—five with these values below 30 mgl (Type B streams) and four with them exceeding 50 mgl (Type A streams). B streams contained as much or more phosphorus and nitrogen as the A's, but had no vegetation other than very sparse algal growth, and, with one exception, lacked molluscs. Type A streams had abundant flowering plants and/or algae at all seasons and well-developed mollusc populations. Benthic insects also showed definite A and B characteristics, although the two stream types had a number of forms in common. Neither degree of shading nor stream size seemed primarily involved in scarcity of plants in B streams. Allochthonous debris was more concentrated in B streams. In one stream system the biota changed from B to A in character with downstream mineral increase.

Macrobenthos populations noted in two streams in summer of 1964, led to a general reconnaissance of small streams in the Blue Ridge Mountains of Virginia in early 1965, and this in turn led to study of factors involved in level of autotrophism and benthos composition in streams of the generally mountainous and

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