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SUMMARY

Trichosarcina polymorpha gen. et sp. nov. is newly described as a member of the Ulvales, Schizomeridaceae. The life cycle includes a uniseriate, Hornidiurn-like phase; a pluriseriate stage, and, finally, a chain of sarcinoid packets which may dissociate. The cells are uninucleate, with a parietal chloroplast and single pyrenoid. Quadriflagellate zoospores are produced singly by cells of the pluriseriate and sarcinoid stages. Sexual reproduction was not observed.

The alga herein described as Trichosarcina polymorpha occurs in shallow, temporary pools in granitic rocks in Llano County, Texas. It was first encountered at Enchanted Rock in November, 1960, was collected again in October, 1961, from the same general area, near Balanced Rock, and has been collected several times since especially during the winter months. These pools are intermittently filled with rain water and harbor such other aquatics as Isoetes melampoda and several sedges. The pH of the water usually ranges between 6.3 and 6.9. Unialgal cultures, in which the plants corresponded in all respects to those studied from natural collections, were isolated by removing single, pluriseriate branches from the natural collections, rinsing them in distilled water and inoculating them into tubes of soil-water medium and Bold's Basal medium. The alga grows luxuriantly in the inorganic medium and less so in the same medium with agar and in media with soil-extracts. Unialgal and axenic cultures were achieved by the familiar techniques of streaking and/or plating, followed by selection of bacteria-free colonies. Repeated transfer of axenic culture in the basal medium (without agar) during several years has demonstrated the absence of special growth-factor requirements.

OBSERVATIONS

Inasmuch as Trichosarcina produces zoospores in natural habitats and in culture, it is convenient to describe ontogeny beginning with these agents of asexual reproduction. The individual quadriflagellate zoospores range from spherical to ovoid (Fig. 1, 19) and are approximately 6 x 4 μ. Each has 4 anterior contractile flagella. Upon germination, the zoospores produce a single anterior contractile flagellum which remains for the life of the filament. There is no indication of an anterior contractile way of differentiation. The single flagellum is not used in swimming. Figs. 1-17. Trichosarcina polymorpha gen. et sp. nov. Fig. 1. Motile zoospore, X 2666. Fig. 2. Quiescent zoospores, X 2166. Figs. 3-7. Successively older germlings from zoospores, X 1200. Fig. 8. Cellular organization in uniseriate filament, X 1715. Fig. 9. Apex of uniseriate filament, X 857. Fig. 10. Cellular detail in apical region of uniseriate filament, X 857. Fig. 11. Early sporogenesis in young pluriseriate filament; note appearance of stigma before zoospore release, X 1715. Fig. 12. Young pluriseriate filament, diagrammatic, X 1715. Figs. 13-15. Variation in pluriseriate holdfasts, X 857. Fig. 16. Pluriseriate filament; note early sarcinoid appearance, X 857. Fig. 17. Pluriseriate filament with loosely associated packets of cells, X 857.

To 940 ml of distilled water were added 10 ml of each of stock solution and 1.0 ml of each of the 4 stock, trace-element solutions prepared as follows:

1. 50 g EDTA and 31 g KOH dissolved in 1 liter H₂O.
2. 4.98 g FeSO₄·7H₂O dissolved in 1 liter acidified H₂O. (Acidified H₂O: 1.0 ml H₂SO₄ added to 999 ml distilled water.)
3. 11.42 g H₃BO₃ dissolved in 1 liter H₂O.
4. The following, in amounts indicated, all dissolved in 1 liter H₂O: ZnSO₄·7H₂O, 8.82 g; MnCl₂·4H₂O, 1.44 g; MoO₃, 0.71 g; CuSO₄·5H₂O, 1.57 g; Co(NO₃)₂·6H₂O, 0.49 g.
vacuoles and a parietal chloroplast with a single pyrenoid and prominent equatorial stigma. A single equatorial to slightly anterior, nucleus is embedded in the colorless cytoplasm. Motility of zoospores is brief and rarely exceeds 3 min from the time of emergence.

Upon quiescence, the zoospores withdraw their flagella, elongate slightly (Fig. 2, 20), and within an hour undergo a transverse division (Fig. 3, 4). The 2-celled germinating usually exhibits polarity in that the proximal cell elongates as a primary holdfast (Fig. 4-6, 21), while the other continues to divide transversely to form a distal uniseriate filament (Fig. 8, 22, 23), in which growth is generalized. The apical cell of each filament is bluntly rounded, never acuminate (Fig. 9, 22). The component cells are cylindrical with straight sides or markedly tumid (Fig. 2). In older filaments the primary holdfast grows into a simple or forked, pluriseriate structure (Fig. 1-15, 25). The proximate holdfast secretes copious colloidal material, as revealed by the India ink test, while the erect system is devoid of such a matrix. The filaments are unbranched throughout development.

Cellular organization of Trichosarcina polymorpha is Hormidium-like in the young filaments (Fig. 8, 10, 21-23). Each cell contains a prominent, incomplete, band-like chloroplast which, in turn, contains a single pyrenoid. The latter typically is surrounded by two starch segments (Fig. 8, 11). The plastid usually is less than cell length. A single nucleus lies in the colorless cytoplasm.

Although the uniseriate condition may continue for long periods in actively growing cultures in liquid media, the pluriseriate condition arises ultimately by longitudinal divisions in some or all of the cells of each filament (Fig. 11, 12, 26). Individuals are occasionally observed in which pluriseriate regions are separated by intercalary, uniseriate segments. Two longitudinal divisions in perpendicular planes result in delicate cylinders composed of 4 rows of cells (Fig. 11, 12).

Continuing longitudinal and transverse divisions of original cell of the primary filament and its derivatives transform the filament into a series of sarcinoid packets (Fig. 16-17, 24, 26) which ultimately may dissociate (Fig. 17, 27, 28). Dissociation occurs also in agar cultures. Upon completion of dissociation, all evidence of filamentous organization may be lost (Fig. 28).

The uniseriate filaments are about 7 μ, while the pluriseriate stages may exceed 200 μ in diameter. The filaments may become several hundred cells long. Filamentous plants may become detached from their holdfasts, apparently by dissolution of their colloidal matrices, or by fragmentation and become free-floating in the culture vessel and in the granitic pools where the plant was collected.

The sole methods of reproduction so far observed in Trichosarcina polymorpha are by zoospores and by fragmentation and dissociation of the pluriseriate filaments, especially in the sarcinoid stage. Only the pluriseriate filaments and sarcinoid packets have been observed to undergo zoosporogenesis. Cells of intercalary, uniseriate regions of mature plants disintegrate when the pluriseriate regions form zoospores.

Zoosporogenesis can be evoked readily by transfer of pluriseriate filaments or their sarcinoid derivatives to fresh culture media. They respond within 24 hr by forming zoospores (Fig. 11). Impending zoosporogenesis is manifested by the appearance of stigmata and contractile vacuoles in the vegetative cells. Each of the latter usually liberates a single zoospore, and the empty parenchymatous mother cell walls persist for sometime in axenic cultures. Zoospore release may be almost simultaneous in some instances.

Electron micrographs of certain stages in the life cycle have provided information regarding cellular organization, especially with respect to the cell walls (Fig. 18). From such micrographs, it is clear that in the uniseriate filament, each protoplast is surrounded completely by an inner wall layer. This, in turn, is covered by an outer layer continuous over the lateral cell surfaces but less-well developed between contiguous cells which have recently arisen by division. In the older, pluriseriate stages (Fig. 18), the "outer" wall layer (i.w.) also is deposited on the transverse (t.w.) and, subsequently on the internal longitudinal, cell faces. In older packets of the sarcinoid stages, limits between outer and inner wall layers become obliterated.

Special study was not made of the cellular organelles, but it may be mentioned that the uninucleate cells contain, as expected, (Fig. 18) a massive, lamellate chloroplast, (ch) with a single pyrenoid, mitochondria, (m) Golgi bodies, endoplasmic reticulum, vacuoles and additional unidentified particles. Both autochthonous (Fig. 18, 5) and pyrenoid starch occur. The pyrenoid is characteristically bisected by 2 double lamellae with intrude from the chloroplast.

DISCUSSION

After search of the literature, discussion with several phycologists, and appraisal of the attributes of the organism herein described, the authors were impelled to assign it to a new taxon, Trichosarcina. Pluriseriate, zoosporiferous Chlorophyceae are unknown, except in the Ulvales and Chaetophorales. One immediately recalls genera as Percursaria, the early stages of Ulva and Enteromorpha, and especially, Schizomeris and Fritschiella, as examples of the pluriseriate condition.

Trichosarcina is, perhaps, most like Schizomeris and the terrestrial Fritschiella but differs from the former in lacking ring-like wall thickenings, in having cells with a single pyrenoid, and in its characteristic Sarcina-like configuration and dissociation of the latter into packets, which suggest strikingly the chlorosphaeraceous genus, Chlorosarcinopsis (6, 1). It is unlike Fritschiella in habitat and in lack of branching and differentiation into prostrate and aerial branches. Furthermore, unlike other algae in Trichosarcina, termination of increase in plant length at the onset of the pluriseriate condition and restriction of zoosporogenesis to the sarcinoid phase emphasize that the latter is a stage in maturation and completion of ontogeny.

Three possibilities occurred to the writers regarding the affinities of the genus Trichosarcina: (1) It might be considered as a member of the Schizomeridaceae; (2) It might be assigned to the Ulvaceae; or, (3) It might be classified in a new family of its own.

The writers, at present, prefer the first alternative. The affinities of Trichosarcina are probably with the filamentous Ulotrichales and the Chlorosphaerales both of which undergo "true vegetative" (5, 6, 4) or par echymatous cell division which is lacking in the Volvales, Tetrasporales and Chlorococcales.

The new organism is described as follows: Trichosarcina polymorpha gen. et sp. nov.

Planta in tribus formis sequentialiter affinis reperita; primum filamento uniseriato Hormidio simili; deinde filamento pluriseriato; demum catena fascicularum. Planta conditionibus novioribus affecta, postea per fragmentationem libere fluitantes. Cellulae uninucleatae, chloroplasto unico pyrenoideo, unico nucleo et stigma equatoriale habentes.

Reproducitio sexualis non observata.


Plants occurring in three sequentially related forms: first a uniseriate Hormidium-like filament; second, a pluriseriate filament; and third, a chain of sarcinoid packets. Earlier stages attached, later free-floating by fragmentation. Cells uninucleate, with a parietal chloroplast containing a single pyrenoid.

Reproduction by fragmentation of sarcinoid packets and by zoospores produced in pluriseriate and sarcinoid stages; zoospores one per cell, spherical to ovoid (6 x 4 μ), quadriflagellate with 4 anterior contractile vacuoles, single pyrenoid and nucleus, and equatorial stigma.

Sexual reproduction not observed.


Axenic cultures of the organism have been deposited in the Culture Collection of Algae, Indiana University. Herbarium specimens have been deposited in the Chicago Natural History Museum.

REFERENCES