Protoplast Induction in *Micrasterias* and *Cosmarium*  
Brief Report

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Summary

Protoplasts of the desmids *Micrasterias angulosa*, *M. denticulata*, *M. thomasiana* and *Cosmarium turpinii* were obtained by incubating cells in Waris' liquid medium + 0.3 M mannitol + 2% Cellulysin for 1–3 hours. One osmotically fragile protoplast was formed at the isthmus from the joint contents of both semicells. The resultant protoplasts were bright green and remained so for more than 5 days in the osmotically protective medium. The protoplast yield was better than 80%. The empty cell walls were not digested by the Cellulysin or by autolytic enzymes.

1. Introduction

The control mechanisms and determinants of morphogenesis in constricted desmids such as *Micrasterias* and *Cosmarium* continue to fascinate cell biologists (TIPPIT and PICKETT-HEAPS 1974, KIERMAYER 1970, HACKSTEIN-ANDERS 1975). One tool which would greatly facilitate such studies, namely protoplasts, has not been generally available. Such protoplasts would be needed in large quantities and must remain viable for regeneration studies. No protoplasts have been obtained from *Micrasterias* although unsuccessful attempts to obtain them have been reported (TIPPIT and PICKETT-HEAPS 1974). We combined our own experience in obtaining protoplasts of yeasts (BERLINER and RECA 1970, BERLINER 1971) and of *Cosmarium* without the use of hydrolytic enzymes (BERLINER and WENC 1976), and that of others with green algae (CHARDARD 1972, BRAUN and AACH 1975) and higher plants (COCKING 1973, WAGNER and SIEGELMAN 1975), to devise methods to obtain large quantities of bright green protoplasts of 3 species of *Micrasterias* and 1 species of *Cosmarium* in 3 hours of incubation.
2. Materials and Methods

Cosmarium turpinii and Micrasterias thomasiana were obtained from Carolina Biological Supply Co., Burlington, N.C., and M. angulosa and M. denticulata were the gift of Dr. David H. Tippit. Stock cultures of Cosmarium were grown for 1–2 weeks in 50 ml of FWV medium (Lee and Loeblich 1971) in 250 ml flasks. Micrasterias stock cultures were grown for 3–4 weeks in Waris’ medium (Waris 1953). All glassware was rinsed in deionized glass-distilled water + 1 g EDTA per liter. All flasks were cotton-plugged. Incubation was at 40 °C with 15 hours of light and 9 hours of dark. Light was provided by two 15 Watt daylight fluorescent bulbs 20 cm from the cultures. One ml of a dense suspension of organisms harvested in the middle of the dark cycle, was put in each well of a 2-chamber slide (Lab-Tek); or 3 ml were put in 60 × 15 mm plastic petri dishes (Falcon). The d-mannitol was added to FWV or Waris solution in double the desired molarity and autoclaved. The double strength mannitol solutions were then added as either 1 ml to each slide culture, or 3 ml to each petri dish. The Cellulysin (Calbiochem) (Wagner and Siegelman 1975) was dissolved in the appropriate medium as a 20%/v W/V solution and added to the cultures + mannitol in appropriate amounts to achieve a final 2%/w/v concentration. The slides or plates were examined immediately after addition of the mannitol + enzyme on a Zeiss phase-contrast inverted microscope equipped with a 35 mm Zeiss camera and photographed either on Kodak Panatomic-X or SO-410 film. They were subsequently observed every 15 minutes for 4 hours, every 2 hours thereafter for 8 hours, and then at twice daily intervals for 5 days. Between microscopic examinations, the plates and slides were re-incubated as for stock cultures.

Figs. 1–9. Micrasterias species incubated for 4 hours in Waris’ medium + 0.3 M mannitol + 2%/v Cellulysin. The size bar in Fig. 9 is equivalent to 100 microns. Fig. 1. M. angulosa. Within 15 minutes the 2 semicells have separated at the isthmus but remain connected. The semicell contents remain lobed but have retracted from the walls. Fig. 2. M. angulosa. A vesicles has been formed between the 2 semicells. The membrane-bound contents (arrow) of each semicell enter into the vesicle which will become the protoplast. 30–45 minutes. Fig. 3. M. thomasiana. Later stage as in Fig. 2. 1 hour. Fig. 4. M. angulosa. Once the contents of one of the semicells has entered the protoplast the empty cell wall falls off. The separate contents of the 2 semicells merge and can no longer be distinguished. 90 minutes. Fig. 5. M. angulosa. Protoplast emerging almost completely from hole at the isthmus. 2 hours. Fig. 6. M. thomasiana. One very dense and bright green protoplast immediately after emerging from 1 of the semicells. 2–3 hours. Fig. 7. M. angulosa. 2 empty semicells which have remained close together and showing clearly the holes at the isthmus through which the cell contents passed into the protoplast. Fig. 8. M. angulosa. 2 empty semicells which have remained attached after release of the protoplast. The semicell on the left is the older one. Fig. 9. M. angulosa. Within 2 hours there are protoplasts, empty cell walls, and some cells which are beginning the protoplast forming process by having their contents retract away from the cell wall

Figs. 10–12. Cosmarium turpinii incubated in FWV + 0.4 M mannitol + 2%/v Cellulysin. Size bar in Fig. 10 is equivalent to 45 microns. Fig. 10. Pattern of protoplast formation similar to that in Micrasterias (Figs. 1–9). 1 hour. Fig. 11. Typical microscopic field after 3 hours of incubation showing an almost total conversion to protoplasts and empty cell walls. Fig. 12. Field similar to Fig. 11 after 5 days in the incubation medium. Protoplasts are not as dense, their limiting membranes are wrinkled, and they have faded to a pale yellow with conspicuous pyrenoids