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Growth of *Acetabularia calyculus* in Three Different Media

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ABSTRACT: *Acetabularia calyculus* (Dasycladaceae) has been cultivated in three different media: (1) enriched sea water; (2) artificially upwelled deep sea water; (3) tropical surface sea water. The alga is able to complete its life cycle only in the enriched sea water. Cultured *A. calyculus* cells are not calcified. They show several types of morphological anomalies which deserve further investigation. *A. calyculus*, like other *Acetabularia* species, may be useful for studies on morphogenesis regulation.

INTRODUCTION

Members of the giant unicellular marine alga *genus Acetabularia* are suitable objects not only for investigations on the subtle relationships between nucleus and cytoplasm but also for ecological and biological research (Puiseux-Dao, 1970; Arasaki and Shihira-Ishikawa, 1979; Cinelli, 1979). *A. calyculus* is widely distributed along the Brazilian coast (Joly and Cordeiro, 1962; Joly, 1965; Pinheiro-Vieira and Ferreira, 1968; Oliveira Filho, 1977) and has recently been found in hypersaline waters in the Araruama Lagoon associated with *A. schenckii* (Oliveira Filho, 1977). The life history of *A. calyculus* has been studied by Arasaki (1942). This paper deals with the growth of *A. calyculus* in three different culture media: (1) enriched sea water; (2) artificially upwelled deep-sea water; (3) tropical surface sea water.

MATERIALS AND METHODS

Cultures of *Acetabularia calyculus* Quoy et Gaimard were obtained from fertile plants collected in the Araruama Lagoon, Brazil. The mature reproductive caps containing the cysts (gametangia) were cut from the plants, washed 10 times with sterile sea water and then kept at 10 °C in the dark for 10 d. After this dark period, the caps were transferred into flasks containing 300 ml of culture medium. Sterilized bivalve shells were added to some flasks to investigate the eventual effect of a calcareous substrate on cell growth. The cultures were made under 12–12 h light dark cycle (fluorescent tubes) at 27 °–29 °C, in three different media: (1) enriched sea water (Erdschreiber medium; Lateur and Bonotto, 1973; Ukeles, 1976; (2) untreated deep water, artificially upwelled from a 50-m depth, south of Cabo Frio Island; (3) filtered sterile surface sea water from Enseada dos Anjos Bay. Their chemical composition is listed in Table 1.

Culture media were renewed once a week. Germanium dioxide was used to inhibit the growth of contaminant diatoms (Stein, 1973). Cell length was measured with millimeter paper beneath the container (Petri dish).

Table 1. Chemical composition of the 3 culture media used for growing *Acetabularia calyculus*

Compound	Erdschreiber medium	Upwelled sea water	Surface sea water
NO_3 (μg–at N l^{-1})	6.54	11.90	4.18
NO_2 (μg–at N l^{-1})	–	0.12	0.26
NH_3 (μg–at N l^{-1})	–	2.00	2.81
PO_4 (μg–at P l^{-1})	0.54	1.03	0.35
Soil extract (ml)	50	–	–

RESULTS

Effect of Culture Media on Development

The results obtained with three different media are shown in Figure 1. Erdschreiber medium with calcareous substrate gave the best growth. Surface sea water allowed only poor growth, the plants remaining at the vegetative stage (Stage 4 according to Bonotto and

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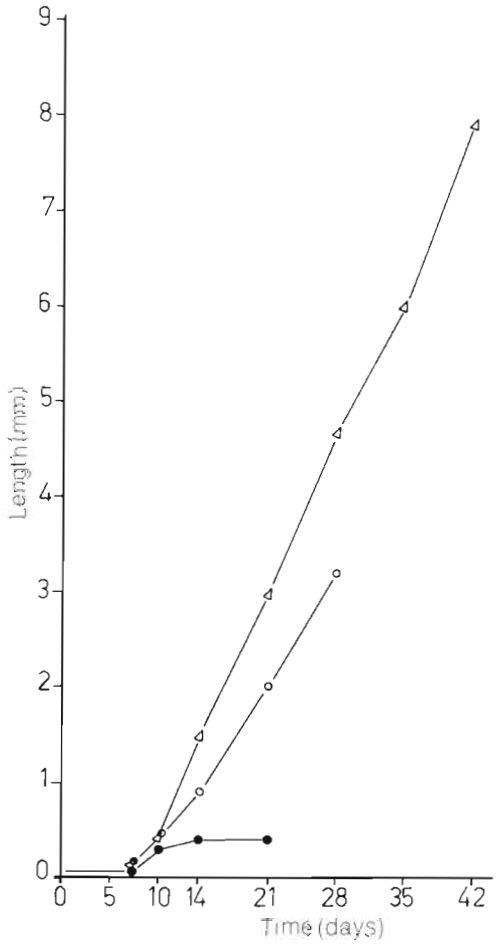

Fig. 1. *Acetabularia calyculus*. Growth in three different culture media: ▲ Erdschreiber medium; ○ artificially upwelled deep sea water; ● tropical surface sea water

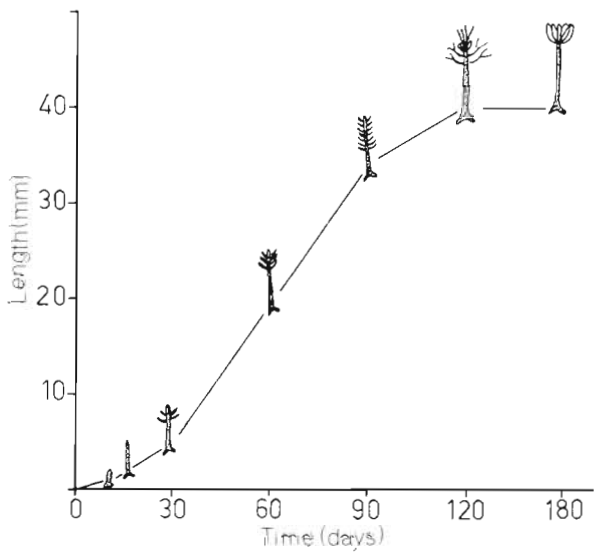

Fig. 2. *Acetabularia calyculus*. Growth in Erdschreiber medium plus calcareous substrate

Kirchmann, 1970). Upwelled sea water permitted better growth, but again the plants remained at the vegetative stage. Under optimal growth conditions, *Acetabularia calyculus* cells needed 6 months to reach maturity (reproductive phase). Cultured cells were not calcified.

Effect of Calcareous Substrate on Growth

The presence of sterilized bivalve shells in the culture flasks did not affect cell growth during the first stages of development. Later, however, plants attached to the calcareous shells showed more rapid growth, reaching maturity at 4-cm length (Fig. 2).

Morphology of *Acetabularia calyculus* Grown in the Laboratory

Young germlings of *Acetabularia calyculus* show a marked polarity; they develop a growing stalk (Fig. 3 A, B, C). The latter forms one or more sterile whorls of branched hairs (Fig. 3 D and 4 A) and then the reproductive cap, which comprises 25–35 rays (Fig. 4 C, D, E, F). Numerous spherical cysts (gametangia) form in the cap's rays (Fig. 4 G, H). The cysts of *A. calyculus*, like those of other *Acetabularia* species, possess a lid (Fig. 4 I) through which the gametes are released.

Cultured *Acetabularia calyculus* may develop morphological anomalies: branching of the stalk (Fig. 3 C), enlargement of first and second order articles of the sterile whorls (Fig. 4 B), or formation of abnormal caps (Fig. 4 C) with a reduced number of rays.

DISCUSSION

The results reported show that the best growth of *Acetabularia calyculus* is obtained with Erdschreiber medium. Although upwelled water is rich in nitrate and phosphate (Table 1), it is unable to sustain satisfactory growth. Surface sea water seems a rather poor culture medium for *A. calyculus*. Our findings suggest that the compounds present in the soil extract promote development. It is known that amino acids and ammonia are present in soil extracts (Bonotto, 1976). Possibly, soil extracts contain other organic substances required for the growth of *Acetabularia*. We have observed that the growth of phytoplankton is also poor in upwelled waters, in spite of the fact that the latter are rich in nutrient salts (Yoneshigue-Braga et al., 1979). Probably, important factors such as chelating substances (Barber et al., 1971) are lacking in the upwelled waters used in our laboratory. Cultured *A.*

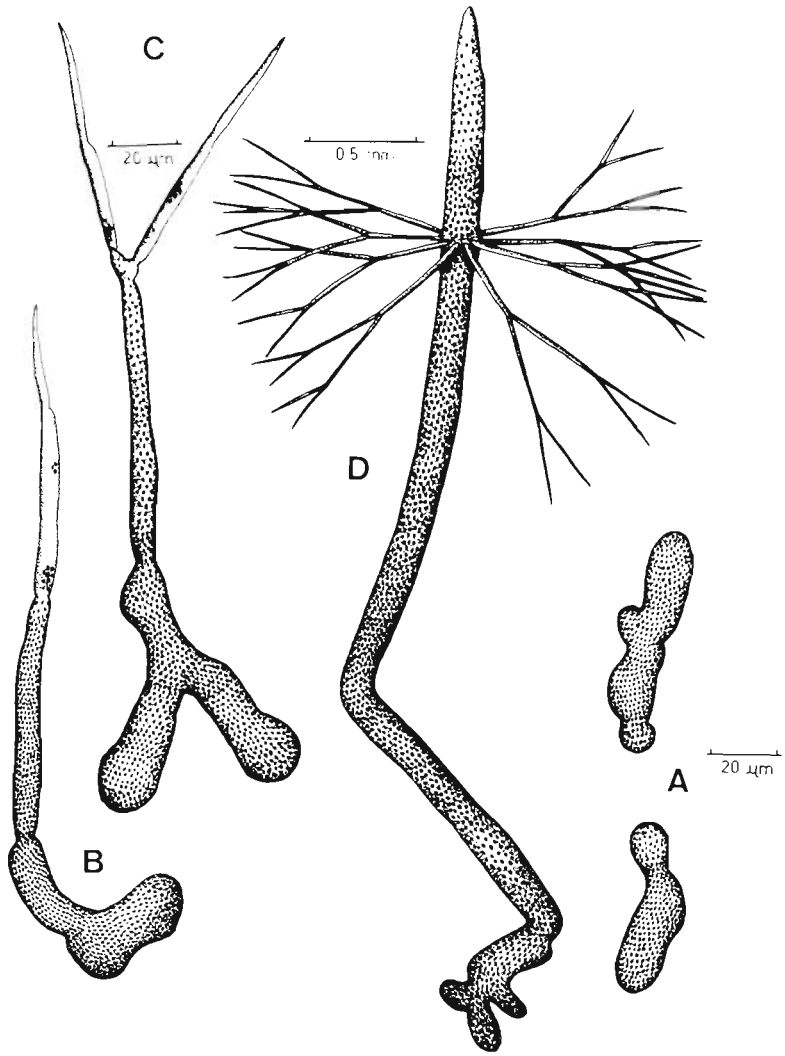

Fig. 3. *Acetabularia calyculus*. Young germlings. A: Germinating zygotes; B and C: growing stalks; D: formation of first sterile whorl of branched hairs

calyculus cells are not calcified. Observations with the optical microscope failed to detect the presence of a very thin layer of lime, revealed by a scanning electron microscope in *A. mediterranea* cells (Berger et al., 1974). That plants attached to shells show better growth is particularly interesting: in nature, *Acetabularia* is frequently found on a calcareous substrate (Patel and Francis, 1970); Arasaki and Shihira-Ishikawa (1979) observed that this alga is able to perforate and penetrate dead shell pieces. Biochemical investigations appear necessary in order to establish whether the alga is capable of utilizing shell substances.

The morphological anomalies observed in our cultures are difficult to explain. Similar anomalies were found in laboratory cultures of *Acetabularia mediterranea* (Bonotto, 1970). The artificial growth conditions provided do not seem to be the only factor responsible for heteromorphoses, since cells with abnormal morphological traits were also found in nature (Woronin, 1861; Bonotto, 1970). Our observations on *A. calyculus* are in agreement with those of Arasaki (1942) and underline the suitability of Erdschreiber medium for cultivating these algae in the laboratory.

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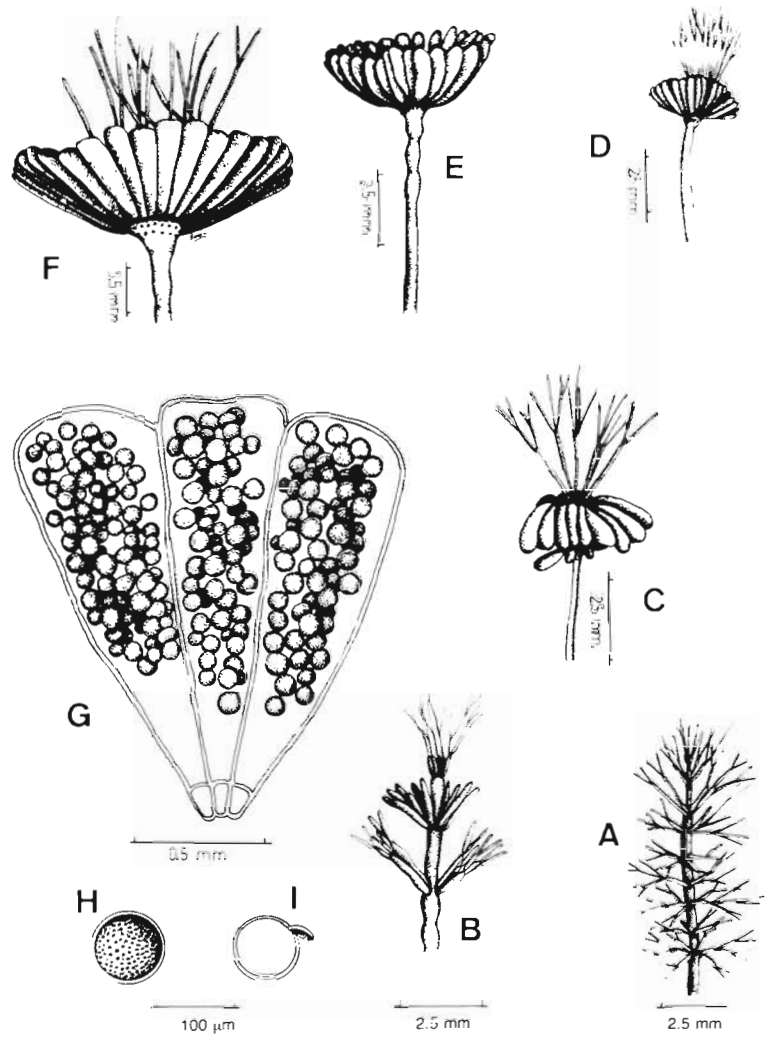

Fig. 4. *Acetabularia calyculus*. A: Vegetative cell bearing 8 whorls of branched hairs; B: abnormal enlargement of first and second articles of sterile whorls; C: abnormal cap with reduced number of rays; D and F: normal reproductive cap; E: mature cap without sterile hair whorls; G: rays with cysts (gametangia); H: free cyst; I: empty cyst showing the lid through which gametes were released

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