Callus-like Formations from Irish Moss

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ABSTRACT

Explants were obtained from callus-like bodies which had formed on the surface of segments excised from the narrow form strain of Irish Moss, *Chondrus crispus* Stackh. The segments were inoculated on solidified agar medium, TC-5, and in enriched seawater medium, D-5, at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$, 12:$\overline{12}$, and 15°C.

INTRODUCTION

There is a growing interest in application of modern tissue or somatic cell culture techniques applicable to multicellular marine algae (Fries, 1980; Gibor *et al.*, 1981, Zhao and Zhan, 1981; Saga *et al.*, 1978; Chen and Taylor, 1978). Such would certainly increase the scope of marine plant research. At present, commercially valuable plants such as kelp (*Laminaria*) and nori (*Porphyra*) are grown in the ocean in large quantities and must start from small sporelings. It may, therefore, be advantageous to employ modern culture techniques to propagate "seed material" using cell or tissue culture instead of spore germination.

Chondrus crispus, an important, commercially harvested red alga found in our regional coastal waters, is a valuable source of carrageenan. During the last few years, scientists have investigated its life history, carrageenan content, growth rate, and productivity in nature. Currently we are attempting to devise procedures which will facilitate the prepagation of this plant. Recently, we have isolated an axenic culture of medullary tissue explants from a femle gametophytic upright frond of a narrow form strain. Pieces of colorless medullary tissue, approximately 2 mm³, developed into multibranched fronds, exhibiting the normal morphology of the parent plant, in an enriched seawater medium (Chen and Taylor, 1978).

Unfortunately, the procedure for isolation was dependent upon the laborious and precise cutting of cortical tissue from the segment.

This paper outlines alternative procedures for tissue culture of this plant by obtaining callus-like formations on solidified medium.

MATERIALS AND METHODS

Healthy fronds were chosen from the narrow form strain of *Chondrus crispus* from our stock unialgal culture, number 750125-T (Chen *et al.*, 1982). Cylindrical, thick and unbranched fronds were excised and wiped quickly on paper soaked with 70% ethanol. After being rinsed thoroughly with sterilized seawater at least three times, they were inserted into 2% RX-100 solution in seawater for 2-5 minutes, depending upon the size, shape and age of the frond. After rinsing in sterilized seawater, the fragments were centrifuged at 1000 rpm for 2-3 minutes and after again being rinsed thouroughly in sterilized seawater, were immersed in a 1% Betadine solution (Gibor *et al.*, 1981) for 2 minutes. Excess solution was removed by rinsing a few times with sterilized seawater. Finally they were transferred to as antibiotic

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solution containing 500 μm ml^{-1} Penicillin G, 100 μg ml^{-1} streptomycin sulphate, 50 μg ml^{-1} Neomycin B, and 200 μg ml^{-1} Kanamycin (Chen and Taylor, 1978) for 72 hours at 150°C, 100 $\mu E \cdot m^{-2} \cdot s^{-1}$, and 16:$\bar{8}$ photperiod.

In order to allow recovery from the above treatments, and to promote more vigorous growth, the fragments were incubated for a week in D-5 medium (Table 1) without antibiotics, but with added kinetins, 2,4-D, and IAA, each at a concentration of 10^{-7} M (Chen and Taylor, 1978). Fragments with faded pigments were discarded, while those that had retained the pigmentation characteristic of *Chondrus crispus* were retained. Following the period of recovery, the fragments were trimmed in D-5 medium with a sharp knife into approximately one cm segments. Each of the segments was inoculated in an 80 ml sterile plastic

Table 1. Tissue culture medium TC-5 compared with algal growth medium D-5 (all weights per 1000 ml)

Compound	TC-5	D-5
$NaNO_3$	0.75 mM	0.5 mM
NH_4NO_3	0.25 mM	0.25 mM
$Na_2SiO_3 \cdot 9H_2O$	0.2 mM	0.2 mM
Na_2EDTA	30 μM	30 μM
$FeCl_3 \cdot 6H_2O$	2 μM	2 μM
$MgSO_4 \cdot 7H_2O$	2 mM	2 mM
$Na_2MoO_4 \cdot 2H_2O$	50 μM	10 μM
H_3BO_3	50 μM	50 μM
$NaH_2PO_4 \cdot 2H_2O$	75 μM	50 μM
TRIS	~5 mM	~5 mM
Seawater	up to 1000 ml	1000 ml
*Sucrose	20 g	—
*Galactose	5 g	—
+Kinetin	1 mM	—
+Naphthaleneacetic acid	1 mM	—
+Indole-3-acetic acid	2 mM	—
Agar	15 g	—
pH	7.3	7.9

Milipore filtration (0.3 μm) was used for sterilization of *carbohydralics and +hormones.

Table 1. (continued)

P1-5X	TC-5	D-5
$MnCl_2$	7 μM	7 μM
$ZnCl_2$	8×10^{-5} μM	8×10^{-5} μM
$CoCl_2$	2×10^{-2} μM	2×10^{-2} μM
$CuCl_2H_2O$	2×10^{-5} μM	2×10^{-5} μM
V-3		
Thiamine-HCl	0.5 mg	0.5 mg
Nicotinic acid	0.1 mg	0.1 mg
Ca-pantothenate	0.1 mg	0.1 mg
Biotin	1 μg	1 μg
Folic acid	2 μg	2 μg
Thymine	5 μg	5 μg
Cobalamine	1 μg	1 μg
Inositol	5 mg	5 mg
Cyãnocobalamin	1 μg	1 μg

Inorganic micronutrients (P1-5X), 2 ml of stock solution into 1000 ml—given required amounts. Organic micronutrients (V-3), 2 ml of stock solution into 1000 ml—given required amounts.

Captions for Figures

Fig. 1. Formation of a callus-like body on the surface of a segment cultured at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$, 15°C and 16:$\bar{8}$ for 4 weeks.

Fig. 2. Enlargement of Figure 1, showing proliferation on the tip of the callus-like body.

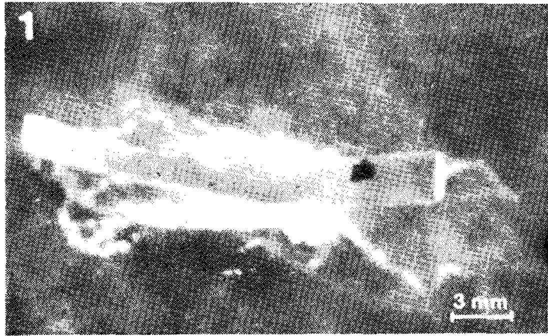
Fig. 3. After an additional 3 weeks in culture the callus-like body.

Fig. 3. After an additional 3 weeks in culture the callus-like body is slightly enlarged.

Fig. 4. Enlargement of Figure 3.

Fig. 5. Callus-like body along with segment in D-5 medium after at room temperature 4 months. Note that the callus-like body has become a massive growth of cells and the segment tissue has disintegrated.

Fig. 6. New plant development from callus-like body after approximately 13 months in D-5 medium.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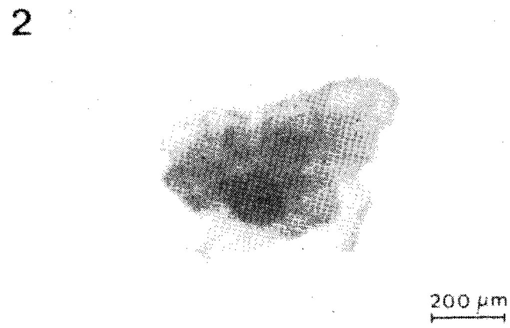
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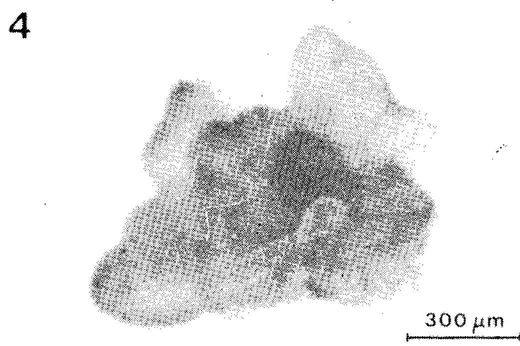
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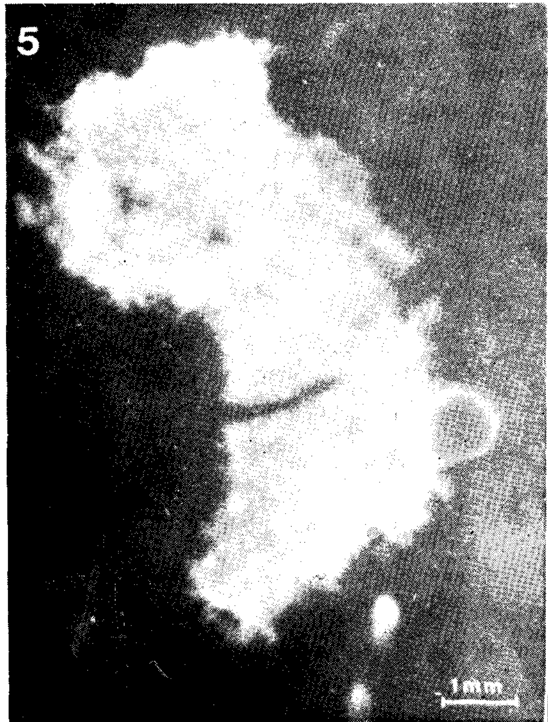
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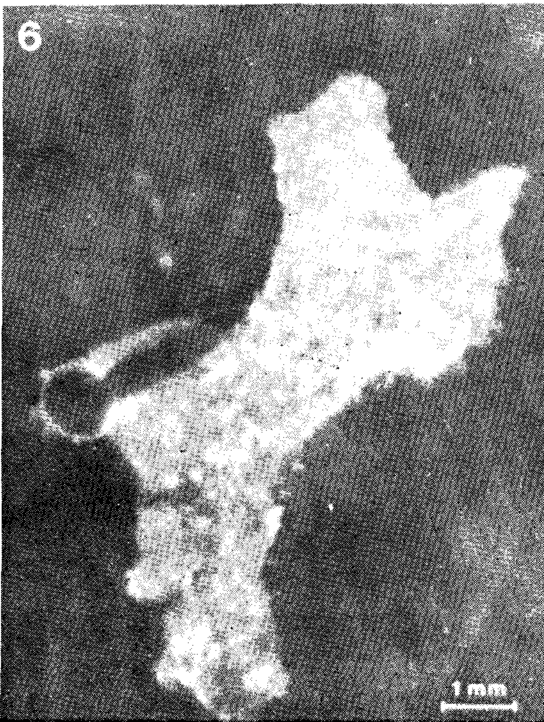
6
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jar containing about 30 ml of pre-prepared agar medium, TC-5 (Table 1). The cultures were incubated at 15°C, 16:8, and 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. All operations and procedures were performed in a laminar-flow hood in an attempt to minimize possible contamination from handling. Segments contaminated with bacteria or fungi can usually be detected within three days of inoculation. After this initial period, the segments were checked weekly under a dissecting microscope. The sterility of the segments into a nutrient broth solution enriched with glucose and yeast extract (Fries, 1963).

RESULTS AND DISCUSSION

After one week of incubation at 15°C, the segments appeared to be healthy and pigmented, but no growth could be detected. Some were discarded due to incomplete sterilization. These segments were commonly found with white or with black and blue clumps of fungi. Thirty percent of the segments which had recovered from the treatment appeared to be clean, although the original pigments faded and they gradually became yellowish. It appears that these segments were unable to absorb sufficient nutrition from the agar medium, since on transfer to normal D-5 medium, some recovered their color and resumed growth.

Under dissecting microscopy the surface of some segments showed distinct red spots. Four weeks later these red spots (Fig. 1) became proliferous, with multi-apical budding (Fig. 2), and appeared to be callus-like in nature. The color became more or less redish-brown, quite distinct from the yellowish color of the segment's surface. These callus-like structures (Figs. 3, 4) enlarged slightly after an additional two or three weeks' incubation. After being incubated for ten weeks, the tips of the buds became white and, gradually, the entire callus-like body turned white. Despite replacement of the agar medium, these callus-like bodies eventually faded completely and died. It is quite possible that the act of transferring the act of transferring the segments to fresh medium damaged them enough that they could not absorb sufficient nutrition.

Close examination of the sterilization procedures show that there was about a 40% survival rate for the segments after treatment with the various chemical solutions. Within this 40% however, many were still contaminated. The Betadine solution improved the sterilization although we noticed that differences in the age and size of segments within the same strain affected the required length of immersion in the solution for it to be effective. The results obtained here may not be comparable with which good results were obtained using the Betadine solution (Gibor *et al.*, 1981).

In higher plants suspended cell cultures derived from calli are very common. When these suspended cells are inoculated on solidified medium with the addition of hormones, they differentiate and form new plants resembling the parent. Sega *et al.* (1978) have obtained sporophytes from single cells of the brown alga *Laminaria japonica* in enriched seawater under non-axenic conditions. Furthermore, Fries (1980) was able to produce sporophytes from callus-like bodies of *L. digitata* and *L. hyperborea*. These results indicate that it may also be possible to apply the same procedures to cell suspended culture. However, when the callus-like bodies which were formed from *Chondrus* plants were transferred to liquid D-5 medium, they formed a single plant, (Figs. 5, 6) rather than the expected suspended cell culture. Microscopy confirmed that the cells were undifferentiated and resembled cortical rather than medullary cells. Perhaps the hormone levels and the composition of the medium may affect the results.

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皺 波 角 叉 菜 癒 傷 組 織 型 的 形 成

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摘 要

盛產膘膠海藻，皺波角叉菜 (*Chondrus crispus*) 的組織培養中，首次從固態培養基 (TC-5) 中誘導出了愈傷組織型，由而在特定的環境中長出了完整的新藻體。