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Masahiro NOTOYA and Yusho ARUGA: Tissue culture from the explant of *Ecklonia cava* KJELLMAN (Laminariales, Phaeophyta)

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There have been eight reports on tissue culture of Laminariales, of which three were concerned with development of callus (SAGA and SAKAI 1983, POLNE-FULLER *et al.* 1986, NOTOYA 1988) and five were concerned with formation of sporophyte or aposporous gametophytes from the callus (SAGA *et al.* 1978, FRIES 1980, FANG *et al.* 1983, YAN 1984, LEE 1985).

FRIES (1980) reproted on *Laminaria digitata* and *L. hyperborea,* and LEE (1985) reported on *L. saccharina.* They observed explants of tissue to form callus which developed to aposporous male and female gametophytes. From these gametophytes were formed sporophytes by fertilization. SAGA *et al.* (1978) reported in *L. angustata* that single cells from callus-like structure formed new sporophytes. FANG *et al.* (1983) and YAN (1984) reported in *L. japonica* and *Undaria pinnatifida* that the tissue pieces developed to callus, and the callus cells differentiated and developed to new young sporophytes.

In this paper we describe the tissue culture of *Ecklonia cava* KJELLMAN in which explants from the blade formed callus-like filamentous cells and the filamentous cells developed to sporophyte-like plantlets.

A sporophyte of *Ecklonia cava* was collected in Nabeta Bay, Shimoda, Shizuoka Prefecture, on July 21, 1988. The tissues were excised from the blade meristematic zone, stipe and holdfast, and their surface was cleaned up with paper towels. The sterilization procedures of explants for tissue cultures were the same as described in a previous report (NOTOYA 1988).

The liquid and solid culture media were prepared using artificial seawater "Jamarin S" (Jamarin Laboratory) enriched with PES. The culture media were solidified with 1.5% agar in 90 × 15 mm Petri dishes. The liquid medium was renewed at two-month intervals. All cultures were incubated at 20°C in 12 : 12 h light-dark cycle and illuminated by cool white fluorescent lamps at 500–1000 lux.

The explants of tissue from blade meristematic zone, stipe and holdfast were cultured on the solid media for about one month. Many callus-like filamentous cells were developed from all these tissues; mostly from medullary parts and little from the undersurface of inner cortex cells. These callus-like cells were unpigmented at the early stage of development. Mass of filamentous cells were gradually grown densely, and round cells were observed sparsely among the massive filaments.

The developmental process of callus-like filamentous cells on agar plates observed in the present experiment was basically the same as that previously reported with explants from the blade, stipe and holdfast of *Ecklonia stolonifera* (NOTOYA 1988). It was also similar to the results of other reports on *Laminaria digitata, L. hyperborea* (FRIES 1980) and *L. saccharina* (LEE 1985).

The explants grown to the filamentous callus-like tissues were transferred into the liquid media.

Two months after transferred into the liquid media, the growth was not observed in the callus-like tissue from stipe and holdfast, and color of the whole tissues became reduced.

The growth of filamentous callus-like tissue was not so fast in the explant from the blade, and color of the central part of the tissue

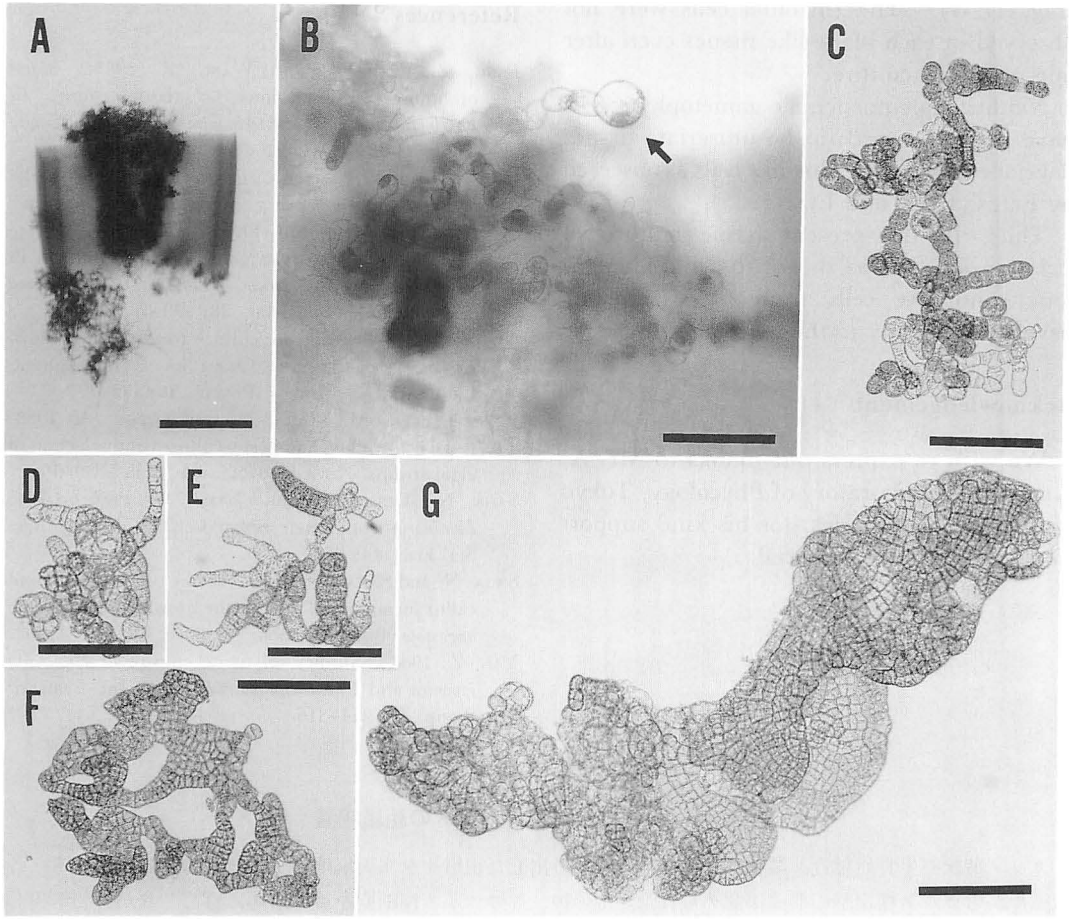

Fig. 1. Tissue culture from the blade explant of *Ecklonia cava* KJELLMAN. (A) Filamentous callus-like cells developed from the explant of blade in four months after transferred to the liquid culture medium. (B) Round cells (arrow) issued from the explant tissue. (C) Uniseriate cells developed from the detached round cells. (D) Clustered cells. (E) Early stage of blade-like plantlet developed from detached round cells. (F) Blade-like plantlet which was like net by irregular cell divisions. (G) More developed plantlet having partially one- or two-cell layers, spreading like sporophyte. Scale bar: (A) 2 mm, (B)–(G) 200 μm.

became brownish, while color of the cells in the peripheral tissue became reduced. The uniseriate round cells which developed from the inner part of tissue were colored or pigmented. These uniseriate round cells were partly detached easily when the culture flask was shaken strongly.

After four months in culture, all the tissues from stipe and holdfast explants were completely bleached and died, while in the tissues from the blade explant the filamentous or round cells were growing slowly and especially the round uniseriate cells grew well and pigmented (Fig. 1, A–B).

After six months in culture, round filaments (Fig. 1, C), cluster (Fig. 1, D) or one- or two-layered blade-like tissues were observed (Fig. 1, E–G) in the culture of detached cells. In the early stage of blade-like development the filamentous tissue was narrow due to the repeating transverse cell divisions (Fig. 1, E). After longitudinal or irregular cell divisions occurred, the tissues became irregular in shape comparable to the early stage of sporophyte produced from female gametophyte (Fig. 1, F & G). This, however, developed to tissues of partially one- or two-cell layers, spreading like sporophyte

(Fig. 1, G). The rhizoidal cells were not observed in each blade-like tissues even after one month in culture.

Neither male nor female gametophyte cells have been observed in the uniseriate tissues developed from the callus-like cells as observed by FRIES (1980) and LEE (1985).

Thus, in the present tissue culture of *Ecklonia cava*, it was shown that the filamentous callus-like cells from blade explants developed directly to the new sporophytes.

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能登谷正浩・有賀祐勝：カジメの組織培養

カジメ葉状部生長点付近，茎状部，仮根部から切り出した組織片を寒天培地で1か月間培養したところ，各組織片からカルス様細胞の形成が認められた。その後，このカルス様細胞を液体培地に移して6か月間培養した結果，葉状部生長点付近の組織からの培養では，カルス様細胞から発達した連続した球形細胞に色素体の形成が顕著に認められ，これらの細胞から葉状体への分化がみられた。(108 東京都港区港南4-5-7 東京水産大学藻類学研究室)