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Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of stipe of *Eisenia bicyclis* (Kjellman) Setchell (Laminariales, Phaeophyta)

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Masahiro Notoya and Yusho Aruga, Laboratory of Phycology, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan

There have been reports of tissue culture in eleven species of Laminariales (Saga *et al.* 1978, Fries 1980, Fang *et al.* 1983, Saga and Sakai 1983, Yan 1984, Lee 1985, Polne-Fuller *et al.* 1986, Polne-Fuller and Gibor 1987, Notoya 1988, Heather *et al.* 1989, Notoya and Aruga 1989) (cf. Table 1). Among them it is reported that in *Laminaria japonica, Undaria pinnatifida* (Fang *et al.* 1983, Yan 1984) and *Ecklonia cava* (Notoya and Aruga 1989) sporophytes developed directly from callus which was formed from excised tissues, whereas in three species of *Laminaria, L. digitata, L. hyperborea* (Fries 1980) and *L. sacchrina* (Lee 1985), explants formed callus and the callus differentiated into aposporous male and female gametophytes, from which were formed sporophytes by fertilization. In *L. angustata*, Saga *et al.* (1978) reported that a single cell of the callus-like structure formed from a long-term cultured sporophyte blade developed to a sporophyte.

In this paper, we describe the culture of tissue excised from the stipe of *Eisenia bicyclis* (Kjellman) Setchell.

A sporophyte of *Eisenia bicyclis* was collected at Enoshima, Kanagawa Prefecture, on July 23, 1989. Tissues were excised from the stipe. The surface was cleaned up with paper towels. The sterilization procedures of the explants for tissue cultures were the same as described in a previous report (Notoya 1988).

Solid and liquid culture media were prepared using artificial seawater "Jamarin S" (Jamarin Laboratory) enriched with PESI medium (Tatewaki 1966). For the solid medium was used 1.5% bacto-agar (Difco Laboratories) in 60×10 mm Petri dishes. The cultures were incubated at 20°C and 500–1000 lux or at 15°C and 10000–12000 lux. The illumination was supplied by cool white fluorescent lamps under a photoperiod of 14L:10D. The liquid medium was renewed at one week intervals.

The explants of tissue from the stipe were cultured on the solid medium for a month at 20°C and 500–1000 lux. Filamentous cells began to grow on some explants in 1–2 weeks, and within 3 weeks they were observed on most explants. Massive filamentous cells were formed mainly on the medullary part (Fig. 1, A). Their development was morphologically very similar to those from tissues of *Ecklonia stolonifera* (Notoya 1988) and *E. cava* (Notoya and Aruga 1989) (Fig. 1, B). These filamentous callus-like cells became gradually long and dense. Then, the tissues with the filamentous callus-like cells were transferred into the liquid medium and cultured at 15°C and 10000–12000 lux or 20°C and 500–1000 lux. Massive filamentous cells grew slowly at 15°C and 10000–12000 lux in the liquid medium. These cells had very few, small pigments (Fig. 1, C), and their color was white or pale yellow.

After four months in liquid medium, a part of these massive filamentous cells was cut off from the original tissue, and cultured further under the same conditions. After another week, color of some cells of the massive filaments changed to yellow or brownish yellow, and shape of such cells became globular. Next week, some of the globular cells became more brownish and blade-like structures were observed after transverse cell

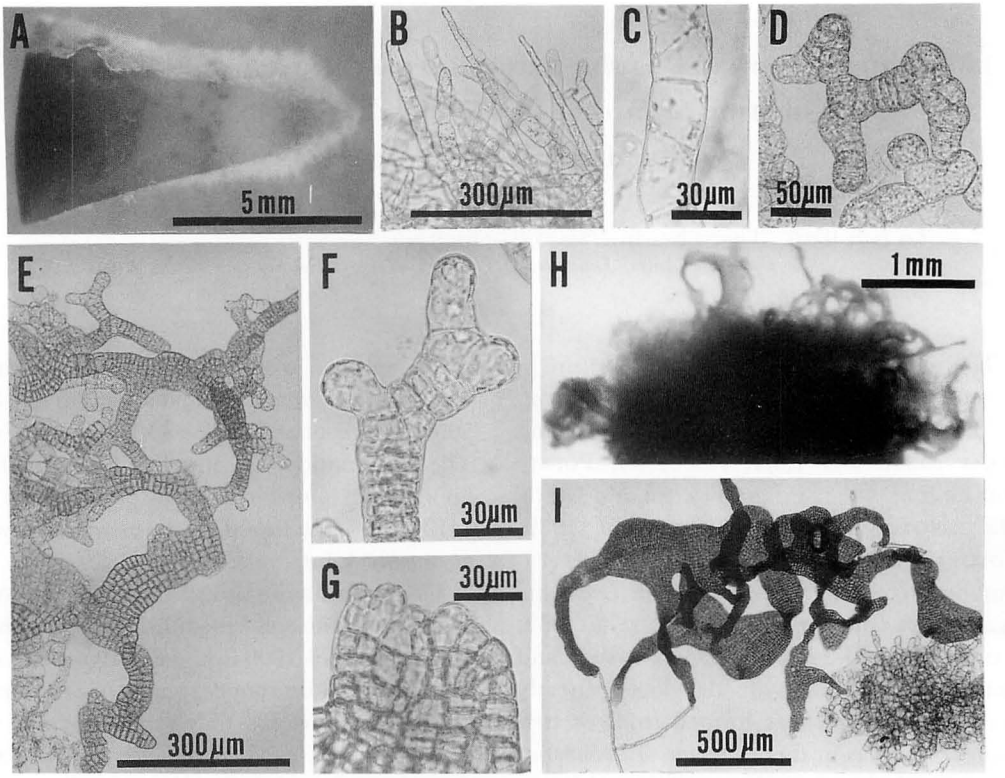

Fig. 1. Tissue culture from the stipe explant of *Eisenia bicyclis* (KJELLMAN) SETCHELL. (A) Filamentous callus-like cells formed on the explant mostly at medullary part one month after on the agar plate. (B) An enlarged part of the developed filamentous callus-like cells. (C) Pigments in developed filamentous callus-like cells. (D) Initial stage of blade-like plantlets developed from callus-like globular cells. (E) Blade-like plantlets formed by irregular cell divisions. (F) & (G) One cell or arranged cell lines on the apex of the blade-like plantlet. (H) A clump of callus with blade-like plantlets six months after in the liquid medium. (I) Formation of transparent rhizoidal cells on the tip of a blade-like plantlet.

divisions (Fig. 1, D). Thus, the initial stage of blade-like plantlets developed from callus-like cells was similar to that observed in *Ecklonia cava* (NOTOYA and ARUGA 1989). From the blade-like cell mass were formed irregularly shaped blade-like sporophytes by repeated transverse, longitudinal or irregular cell divisions (Fig. 1, E).

There were two types of cells at the apex of these plantlets, one cell or arranged lines of some cells (Fig. 1, F and G). These apex cells were distinguished from the blade-like cells by pigment content in the cell. They did not have so many pigments, and were more faint in color than the blade-like cells.

After the culture for six months in the liquid medium, the clump of callus with blade-like plantlets (sporophytes) grew to about 3 mm in diameter (Fig. 1, H). Transparent rhizoidal cells were observed at the tip of these irregular plantlets (Fig. 1, I).

The filamentous callus-like cells formed at 20°C and 500–1000 lux did not differentiate into blade-like plantlets within seven months of culture in the liquid medium.

From the above results, it seems that light intensity and/or temperature are very important factors for differentiation of callus into blade-like plantlet.

Blade tissues were used for the tissue culture of *E. cava* and most of other Laminariales species in which callus differentiated directly into blade-like plantlets (sporophytes) (FANG *et al.* 1983, SAGA *et al.* 1978, YAN 1984, NOTOYA and ARUGA 1989) (Table 1). In this study, however, stipe

Table 1. Results of tissue cultures in Laminariales seaweeds.

Species	Tissue	Result	Reference
Ecklonia stolonifera	Blade	Callus	NOTOYA 1988
E. stolonifera	Stipe	Callus	NOTOYA 1988
E. stolonifera	Haptera	Callus	NOTOYA 1988
E. cava	Blade	Callus→Sporophyte	NOTOYA & ARUGA 1989
E. radiata	Stipe	Callus	HEATHER *et al.* 1989
Egregia menziesii	Stipe	Callus	POLNE-FULLER & GIBOR 1987
Eisenia bicyclis	Stipe	Callus→Sporophyte	Present study
Laminaria angustata	Blade	*Callus→Sporophyte	SAGA *et al.* 1978
L. angustata	Stipe	Callus	SAGA & SAKAI 1983
L. digitata	Blade	Callus→♂ ♀ →Sporophyte	FRIES 1980
L. hyperborea	Blade	Callus→♂ ♀ →Sporophyte	FRIES 1980
L. japonica	Blade	Callus→Sporophyte	FANG *et al.* 1983
L. japonica	Blade	Callus→Sporophyte	YAN 1984
L. saccharina	Stipe	Callus→♂ ♀ →Sporophyte	LEE 1985
Macrocystis pyrifera	Stipe	Callus	POLNE-FULLER *et al.* 1986
Undaria pinnatifida	Blade	Callus→Sporophyte	FANG *et al.* 1983
U. pinnatifida	Blade	Callus→Sporophyte	YAN 1984

* Callus-like structure formed from cultured sporophyte blade.

tissues were used; from these tissues the filamentous callus was formed and the callus differentiated into plantlet like the sporophyte. This result suggests that cells of the stipe tissue possibly have totipotency in *Eisenia bicyclis*.

It was shown in this study that callus cells from the explant of *Eisenia bicyclis*, in a similar way as in *Ecklonia cava* (NOTOYA and ARUGA 1989), *Laminaria japonica* and *Undaria pinnatifida* (FANG *et al.* 1983, YAN 1984), directly differentiated into new sporophytes without forming aposporous gametophytes. This suggests the application of callus cells to micropropagation in these species.

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能登谷正浩・有賀祐勝：褐藻アラメの組織培養

アラメ *Eisenia bicyclis* (KJELLMAN) SETCHELL の茎状部を用いて組織培養を行なった。寒天培地上で 20°C·500–1000 lux (14L:10D) で約1か月間培養したところ，糸状のカルス様細胞の形成が認められた。これら組織片のついた糸状のカルス様細胞塊を液体培地に移し，15°C·10000–12000 lux (14L:10D) または 20°C·500–1000 lux (14L:10D) で培養したところ，前者の条件下では糸状のカルス様細胞は色素体の多い球形の細胞に発達し，それらの細胞から葉状体（胞子体）への分化が認められ，6か月後には仮根様細胞の形成まで認められた。しかし後者の条件下では葉状体への分化は認められなかった。(108 東京都港区港南4–5–7　東京水産大学藻類学研究室)