Studies on tissue culture of *Laminaria japonica* and *Undaria pinnatifida*

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Introduction

The totipotency of plant somatic cells was first demonstrated by Steward in carrot (Steward *et al.* 1964), and the tissue culture of higher plants has been widely practiced since the 1970's. So far, however, reports on tissue culture of multicellular marine algae have been infrequent. Chen & Taylor (1978) obtained initial success in tissue culture of *Chondrus crispus,* while Saga *et al.* (1978) discovered, by tissue culture of pieces of *Laminaria angustata* sporophytes, that bases of the fronds have regenerative ability. Our experiments began in 1980, on tissue pieces of the brown macroalgae *Laminaria japonica* and *Undaria pinnatifida* in suspension culture. A synthetic plant hormone, "C-751", was first used in tissue culture of marine algae. Depending on the concentration, this hormone could induce either callus formation or totipotency of the somatic cells of the two species. In certain experimental conditions, the callus cells differentiated and developed into young sporophytes.

Materials and methods

The materials used were healthy, young or immature adult sporophytes of *L. japonica* and *U. pinnatifida* collected from the sea-farm of Qingdao. Juvenile plants were selected to ensure that no reproductive cells were present. Sporophytes of *L. japonica* were about 150 cm long and 20 cm wide, while sporophytes of *U. pinnatifida* were about 90 cm long and 45 cm wide.

The algae were first cleaned with sterilized seawater, steeped in a 1.5% KI solution for ten minutes, sterilized with 70% alcohol, washed several times with sterilized seawater, and finally put into sterilized seawater for use. The basal, middle and terminal parts of the blade, and the rhizoids and stipes were cut lengthwise into pieces 3-4 mm long × 2-2.5 mm wide. Tissue pieces were cultivated singly in flasks containing the following culture media: (1) seawater plus 8 mg · l^{-1} NO_3^--N and 0.8 mg · l^{-1} PO_4^{3-}-P; (2) improved MS medium (Murashige & Skoog, 1962); (3) improved MS medium plus 0.5 mg · l^{-1} vitamin B_2; (4) improved MS medium plus 0.5 mg · l^{-1} vitamin B_2 plus 5 mg · l^{-1} synthetic plant hormone C-751 (sodium naphthenate). The experimental temperature was 10-15 °C, and the irradiation was 8:16 h (L:D), at 2,9 J · m^{-2} · s^{-1}. Callus formation was induced in medium (4) under these conditions. To establish somatic cell clones, calli were transferred to medium (1) from medium (4). Individual cells were separated and cultured separately in medium (1). The vegetative growth of these cells produced larger masses containing millions of cells.

Results

Of the basic medium, improved MS medium and improved MS medium added with vitamin B_2, none could induce totipotency of cells. Only improved MS medium with added vitamin B_2 and synthetic plant hormone C-751 could induce somatic cells to grow into young sporophytes (Fig. 1). The somatic cells first, however, had to pass through a callus stage. Formation of callus, a dark brown multicel-

Hydrobiologia 116/117, 314-316 (1984).

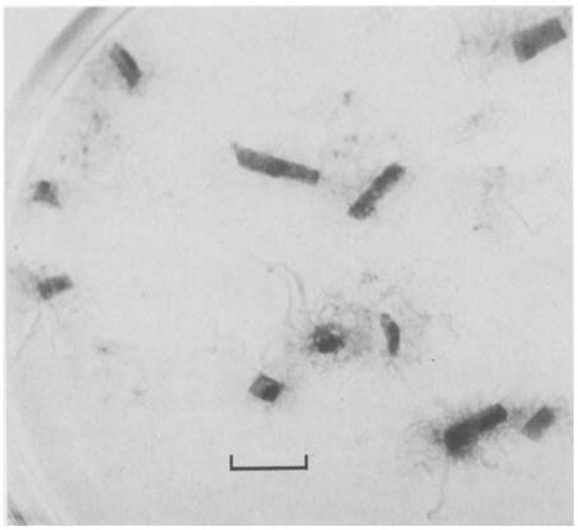

Fig. 1 Young sporophytes growing from the tissue pieces of *Laminaria japonica*. Scale bar = 3 mm.

lular mass, required at least 45 days. Only the cortex and pith could form callus. About a month later, the outer cells of the calli began to enlarge, and the color became yellowish. These outer cells divided transversely to give rise to filaments (Fig. 2), some cells of which carried on transverse division two or three times and then vertical division, and at last grew into young sporophytes (Fig. 3). Only culture medium (4) was effective in inducing formation of calli.

With the exception of those derived from the terminal part of the blade, all tissue pieces formed sporophytes. There were differences, however, in the ability of tissue pieces to differentiate, according to their origin on the thallus. Tissue from the basal and middle portions of the blade produced the most calli and sporophytes, while inocula from the stipes were intermediate, and those from the rhizoids showed the least ability to differentiate. Tissue from stipes and rhizoids had limited ability

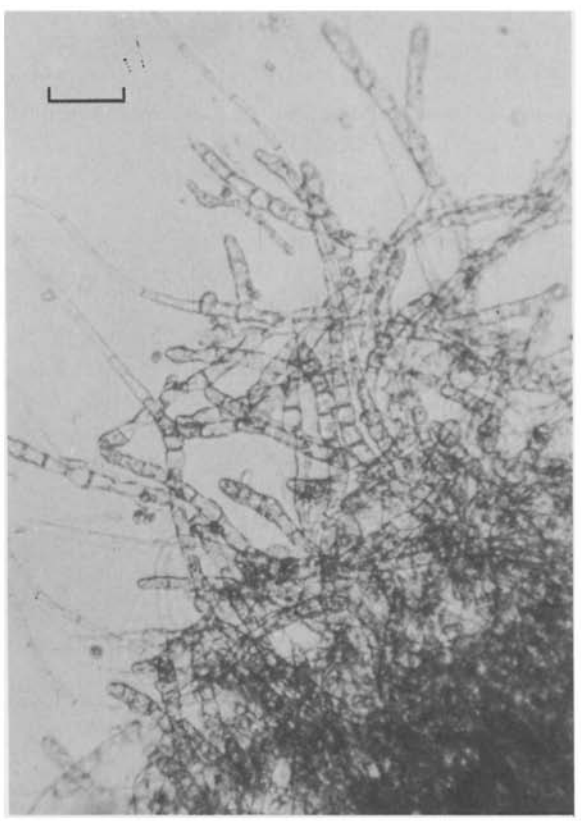

Fig. 2. Multicellular filaments formed from individual cells of *Laminaria japonica* callus. Scale bar = 200 μm.

Fig. 3. Young sporophytes formed from a somatic cell clone of *Undaria pinnatifida*. Scale bar = 300 μm.

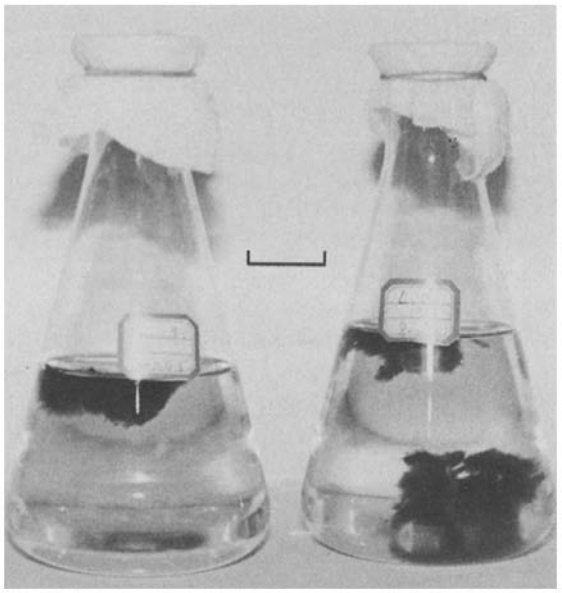

Fig. 4. Somatic cell clones of *Laminaria japonica*, cultured for more than three years. Scale bar = 2 cm.

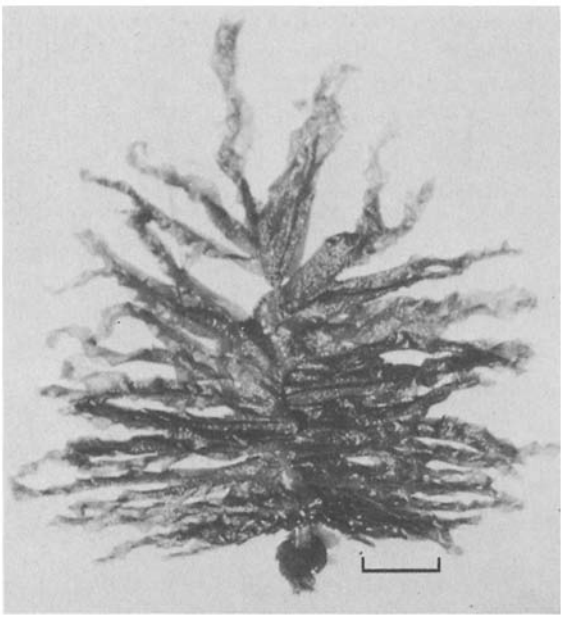

Fig. 5. Mature sporophyte of *Undaria pinnatifida* grown in the sea from a somatic cell clone. Scale bar = 15 cm.

to produce sporophytes, but comparable ability to form calli.

The vegetative growth of calli could result in formation of large masses consisting of millions of cells, each callus being a single-cell clone. These clones could be subcultured and developed into somatic cell clones (Fig. 4). Two main features of somatic cell clones were more rapid growth, and ability to endure higher temperatures for a longer period than the normal sporophytes. The somatic cell clones normally grew at 30 ° C, and had a long life-span; the oldest ones in our laboratory have lived for more than three years. All the somatic cell clones from *L. japonica* and *U. pinnatifida* developed normally into mature sporophytes in the sea (Fig. 5).

Discussion

These experimental results clearly demonstrate the formation of somatic cell clones in *Laminaria* and *Undaria* and their totipotency. As somatic cell clones can be subcultured, tissue culture can be used to breed varieties, fix heterosis, and preserve good genotypes. Therefore, the establishment of somatic cell clones may open up a new way to study basic genetics, breeding, physiology and biochemistry in the field of phycology.

In the process of the induction of totipotency in *L. japonica* and *U. pinnatifida*, there is a process of dedifferentiation. The formation of callus is very important and all the callus cells were much smaller than the cortical and medullary cells. The synthetic hormone C-751 seems to be more potent than kinetin in inducing totipotency in the somatic cells of these two species of brown algae.

References

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