Short Communication

Isolation of Protoplasts from Edible Seaweeds

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Protoplasts were isolated enzymatically from three species of Chlorophyta (Enteromorpha linza, Monostroma zostericola and Ulva pertusa) with high yield and viability. An enzyme solution appropriate for protoplast isolation from the marine green algae was the following: 2% Cellulase Onozuka R-10, 1.0 M mannitol, pH 6.0. Protoplasts could not be obtained from members of Phaeophyta or Rhodophyta.

Key words: Algae — Cellulase — Enteromorpha — Monostroma — Protoplast — Ulva.

According to the review of Adamich and Hemmingsen (1980), algal protoplast isolation has been achieved in 15 genera of eukaryotic algae, but most of them are fresh water algae and there is only one report of protoplast isolation from a multicellular marine alga, Enteromorpha intestinalis (Millner et al., 1979). The present paper deals with an advanced method for obtaining protoplasts from several edible seaweeds.

The materials used in this study were the following 5 species: Enteromorpha linza, Monostroma zostericola, Ulva pertusa (Chlorophyta); Porphyra yezoensis (Rhodophyta); Laminaria japonica (Phaeophyta). Young thalli of these species, several centimeters long, were collected at Muroran, Hokkaido, Japan, during various seasons in 1976-1980 except Laminaria. Sporophytes of L. japonica were prepared essentially according to the method described previously (Saga and Sakai, 1977). Marginal fertile parts of the thalli were removed in advance, and the remaining central or basal parts were cut into pieces of ca. 5 mm square, and 1 g (fresh weight) was maintained for 10 rain in 1.0 M mannitol solution. Then, they were incubated in 10 ml enzyme solution. The enzymes used were Cellulase Onozuka R-10 and Macerozyme R-10, obtained from Kinki Yakult Mfg. Co., Nishinomiya, Japan. All incubations were carried out at room temperature (20-25 C) and lasted for 10 min with reciprocal shaking (60 excursions/min) except as noted.

Isolated protoplasts were separated from tissue debris by filtration through a nylon mesh (30 μm opening). They were collected by centrifugation at 500 xg for 15 min and washed several times with 100 mM Tris buffer (pH 8.0) containing 1.0 M mannitol. The resulting cleaned pellet was resuspended in 1 ml seawater supple-

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mented with 0.5 M mannitol and adjusted to pH 8.0. Fragments of undigested cell walls were visualized by staining the preparation with 0.01% Calcofluor White M2R (American Cyanamid Co., Wayne, NJ, U.S.A.) dissolved in the hypertonic seawater (Nakazawa et al., 1969). The number of protoplasts was counted with a hemocytometer. Viability was assessed by examining the ability to exclude Evans Blue which indicates membrane semipermeability (Kanai and Edwards, 1973). Evans Blue (Sigma Chemical Co., St. Louis, MI, U.S.A.) was used as 1% solution in the hypertonic seawater.

To find an enzyme solution suitable for the isolation of algal protoplasts, various factors which influence enzyme activity and protoplast stability were examined using Monostroma zostericola. The enzymes appeared to be inactive in seawater (Table I-A). The yield of protoplast was not influenced by mannitol concentration over a range of 0.5–1.2 M, but higher concentrations of the osmoticum enhanced the viability of protoplasts (Table I-B). The yield of protoplasts decreased with increasing pH values over 6.0, and the enzymes were almost inactive at pH 10.0. On the other hand, the ratio of viable protoplasts decreased at pH 4.0 (Table I-C). Macerozyme R-10 had no effect on the isolation of protoplasts (Table I-D). The yield of protoplasts was low with the concentrations of cellulase below 1% (Table I-E).

From these results, an enzyme solution containing 2% (w/v) Cellulase Onozuka R-10 and 1.0 M mannitol in distilled water at pH 6.0 was adopted for further studies. Incubation for 10 min in this solution was sufficient for the isolation of protoplasts in significant amounts. The resulting protoplasts of Monostroma zostericola were green, spherical, and 11–25 μm in diameter (Fig. 1). The yield of protoplasts (ca. 5 × 10⁶ from 1 g fresh weight of tissue) was 20–30% of the initial cell number of the thalli and more than 80% of them were viable. The procedure was applied to other algae with an incubation time of 1 hr. Viable protoplasts were obtained from Enteromorpha (yield: ca. 10⁶ or more from 1 g fresh weight of tissue) and Ulva (yield: ca. 10⁴ or more from 1 g of fresh weight of tissue). However, protoplasts were not obtained from Laminaria and Porphyra. Even after prolonged incubation, there was no sign of improved yield of protoplasts from Laminaria and Porphyra.

The present method is characterized by quick isolation and high viability. It usually took less than 1 hr to obtain protoplasts by the present method. Millner et al. (1979) employed a long term incubation (15–17 hr) at a low temperature (10–12 C), and these were essential factors for the isolation of viable protoplasts. The percentage of viable protoplasts in their study was 85–90% just after isolation, but it decreased to about 10% within 24 hr, and they could not observe plant regeneration. Application of the low temperature incubation to the present method did not improve the protoplast viability and reduced the protoplast yield. A long term incubation caused a decrease in the ratio of viable protoplasts. On the other hand, the protoplasts obtained by the present author's method remained viable without cell wall regeneration for at least 2 days in the Tris buffer, and most of the viable protoplasts regenerated cell walls and complete thalli in Provasoli's enriched seawater medium (1968) as will be reported elsewhere. The author supposes that a quick isolation is essential for