Short Paper

Isolation of Protoplasts from *Laminaria* and *Porphyra*^{*1}

A few papers to date have been known about the protoplast isolation from the edible seaweeds.¹⁻³⁾ But they have been isolated chiefly from the green algae. The protoplast isolation from red or brown algae was difficult because their cell walls were resistant to cellulase or pectinase which was efficacious for higher plants.¹⁾ This is a preliminary paper which reports on a method to isolate protoplasts from red and brown algae by using sea urchin gut juice.

Materials used in the present study were sporophytes of *Laminaria japonica* (brown alga) and gametophytes of *Porphyra yezoensis* (red alga). The preparation of materials and the procedures of protoplast isolation were employed the similar one described before except enzyme solution.³⁾ The crude enzyme solution was prepared from the sea urchin *Strongylocentrotus intermedius*. Ten grams of guts of the sea urchin in 50 ml 0.1 M Tris buffer (pH 7.0) was homogenized with a glass homogenizer, stirred with a magnetic stirrer for over night and centrifuged at $10,000 \times g$ for 20 min. The supernatant was dialyzed with a cellulose visking tube against 0.1 M Tris buffer (pH 7.0) for 5 h, added 1.2 M mannitol and finally pH was adjusted at 7.0. All

the procedures have been done under cool ($<5^{\circ}\text{C}$) condition.

After several hours' incubation of the algae in the enzyme solution, protoplasts were isolated from them. The resulting protoplasts of *Laminaria* were brown, spherical and 12–28 μm in diameter (Figs. 1, 2), and those of *Porphyra* were red, spherical and 7–18 μm in diameter (Fig. 3). The yields of them were usually less than $10^3/\text{ml}$ and the viabilities of them were less than 10%. Efforts to culture of these protoplasts have been attempted using the several media, but they could not regenerate.

Some marine invertebrates whose feeds were benthic algae have various digestive enzymes for algal polysaccharides.^{4,5)} It is considered that the crude enzyme solution used in this study contained several enzymes which had the ability of cell wall digestion of the organisms, e. g. alginase, agarase, etc.

Though the yield and viability of protoplasts obtained in this study were insufficient, the protoplasts from red and brown algae were isolated for the first time. There are some problems to be solved in future, e. g. improvement of enzyme source, yield and viability of protoplasts and regeneration of protoplasts, etc. The present successful result will open a new approach to breeding and cultivation of economically valuable seaweeds.

This work was partly supported by the grants to Y. SAKAI from the Ministry of Education, Science and Culture (No. 00554217), and from the Ministry of Agriculture, Forestry and Fisheries (BCP 84-II-1-1). N. SAGA is indebted to the Japan Society for the Promotion for a postdoctoral fellowship granted during this study.

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Fig. 1. Protoplast of cortical tissue freshly isolated from *Laminaria japonica*.

Fig. 2. Protoplast of medullary tissue freshly isolated from *L. japonica*.

Fig. 3. Protoplast freshly isolated from *Porphyra yezoensis*. All scales show 10 μm .

(Accepted October 4, 1983)

^{*1} コンブとアマノリからのプロトプラストの単離.

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