海産緑藻アナアオサの固定化生細胞の調製

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Short Paper

Preparation of Immobilized Living Cells from a Marine Green Alga *Ulva pertusa*

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Recently the immobilized higher plant cells have developed rapidly. These systems have been applied to the production of useful fine chemicals.[1] In particular, these studies have been attempted for the production and biotransformation of secondary metabolites; alkaloids, pigments, terpenoids, steroids, oils and fats.[2,3] While none have been published on the study for immobilization of marine algae, in this paper, we describe the immobilization of cultured cells regenerated from protoplasts of a marine green alga, *Ulva pertusa*.

U. pertusa was collected from Aio coast of Yamaguchi, Japan in 1988. Isolation and culture of protoplasts from the vegetative thalli of *U. pertusa* were performed according to the method reported previously.[4] The 3 week old cell-cultures entrapped with calcium alginate as follows. The cultured cell aggregates were centrifuged at 1,500×g for 5 min. Then the collected cells (2 g of fresh weight) were resuspended in a small amount of Provasoli's enriched sea water (PES)[5] medium. This suspension was mixed with 20 ml of 4% (W/V) sodium alginate (Wako Pure Chemical Industries Ltd., Osaka, Japan). The resulting mixture was dropped slowly into 100 mM $CaCl_2$ solution. The formed beads were held for 1–2 h at room temperature in this solution to diffuse the cells into their center. The beads were then collected with a stainless mesh (149 μm opening) and washed three times with PES medium containing 5 mM $CaCl_2$, which was required to keep the beads intact. Intact cells of *U. pertusa* were immobilized in the calcium alginate beads and the formed bead size may mean 3.0–5.0 mm in diameter as shown in Fig. 1.

Subsequently, the immobilized cells obtained were transferred to 100×20 mm sterile disposable plastic dishes with 15 m*l* of PES medium containing 5 mM $CaCl_2$. The cultures were incubated at 16–18°C under white fluorescent light (4,000 lx, 14 h light–10 h dark cycle). After 1 week, the cells were transferred to 300 m*l* Erlenmeyer flasks containing 150 m*l* of PES medium and were cultured under white fluorescent light, 6,000 lx, (14 h light–10 h dark cycle) with aeration sterilized by passage through continuous cartridge filters (3.0 μm, 0.45 μm). All cultures were subcultured at intervals of 7–10 days. All these procedures were carried out under sterile conditions. The immobilized cells continued to live and to increase chlorophyll of the cells in the beads. Then, the immobilized cells were developed for 50 days after immobilization as shown in Fig. 2. The cells were capable of plasmolysis in the variation of osmotic pressure by mannitol. At the same time, from these results of the stain tests by a 0.1% solution of neutral red, Evans blue, phenosafranin, 75–80% of cell numbers were found to be still alive in the alginate beads. Thus, the cells were confirmed to be immobilized with calcium alginate as living cells. However, the cells were started to extrude themselves from the beads into the medium after 2 months of immobilization.

This immobilization of cultured cells regenerated from protoplasts of *U. pertusa* may be a promising application for the preparation of cells, which can control the growth, maintain the high productivity of the valuable substances, particularly, long chain aldehydes,[6] and for continuous long term use.

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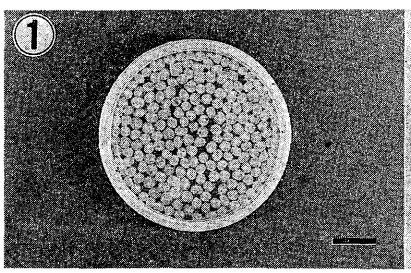

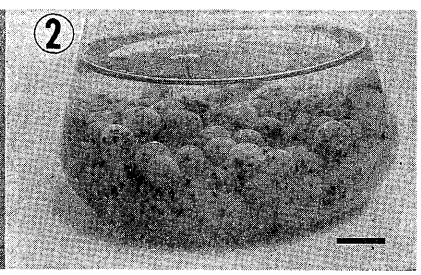

Fig. 1. Immobilized cells regenerated from protoplasts of *U. pertusa*. Scale bar=2.0 cm.
Fig. 2. Immobilized cells developed for 50 days after immobilization. Scale bar=1.0 cm.

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