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Production and Characteristics of Protoplasts in Green Sea Algae *Capsosiphon fulvescens*

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Introduction

The potential application of protoplasts useful for studies such as physiology, morphology, genetic engineering, etc has led to the development of suitable methologies for isolation and manupilation of protoplasts from a wide variety of algae (Waaland et al., 1990; Reddy and Fujita, 1991; Chen and Chiang, 1994). Protoplasts technology to seaweeds depends large on the ability to produce viable cells capable of regenerating into whole plantlets (Wakabayashi et al., 1999). Though *Capsosiphon fulvescens* is one of the important economic seaweeds culturing in Korea, surprisingly protoplasts approach on this species has not been reported so far. Consequently we investigated the various aspects related to the protoplasts of *Capsosiphon fulvescens* in this study.

Materials and Methods

Thalli of *Capsosiphon fulvescens* were collected from the culture net of Janghung during culture season of 1998~2000. They were throughly cleaned in filtered seawater, surface sterilized with 1% KI-I_2 solution for 2min, then rinsed three times with autoclaved seawater. Approximately 200㎎ of thalli sliced into small pieces was incubated in ϕ5cm petri dish containing 5ml filter sterilized enzyme solution for about 3~10 hrs at 20℃. 15 different enzyme combinations using 6 commercial enzymes and 2 crude enzymes were tested for their ability to produce protoplasts. Freshly isolated protoplasts were sieved through 30㎛ pore diameter nylon mesh and washed by centrifugation at 1000 rpm for 5 min. The procedure was repeated 3 times with 0.0M f/2 medium to dilute the media osmoticum finally at 0.2M mannitol concentration. Purified protoplasts were counted, characterized, and cultured in vitro.

Results and Summary

Protoplast yields varied with enzyme mixture and tissue condition. Abalone acetone powder, driselase, and the buccal juice of sea hare were effective among 8 enzymes applied. The suitable enzyme combination was 5% abalone acetone powder, 2.5% Cellulase R-10, 2.5% driselase, and 1㎖ buccal juice of sea hare in 50mM MES buffer(pH 6.0) containing 0.6M mannitol, and the yield was aproximately 300×10^4 cells/g fresh thalli. The addition of buccal juice of sea hare containing high activity of alginate lyase increased the protoplast yield about two times (Wakabayashi et al., 1999). Freshly isolated protoplasts were generally spherical in shape showing yellowish green color and ranged between 8~15㎛ in diameter. Most of protoplasts cultured in f/2 media regenerated cell walls within 7 days. The addition of antibiotic mixtures below 10% concentration did not inhibited microbial growth in culture.

References

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