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Effect of Seaweed Extracts on Viability of the Crustose Coralline *Lithophyllum yessoense*

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Introduction

Crustose coralline algae, which are non-articulated calcareous algae, grow and cover the rock surface with pink or white-colored crusts. One of the most dominants causing algal whitening in Korea and Japan is *Lithophyllum yessoense* Foslie (Tokuda et al., 1994; Kim,2000). Field observation (Kim, 2002) has shown that in the general region of an algal whitening area, there are no macroalgal epiphytes on the pink crustose algal surface, whereas a few seaweeds do grow on the white-patch crustose surfaces. Seaweed restoration may be tried by transplanting these neighbor species. Before transplanting seaweeds in the field, we need test in vitro inhibition activity of several prevalent seaweeds against *L. yessoense* to find suitable species for seaweed restoration in the algal whitening area. Tissue viability of the crustose coralline *L. yessoense* was quantitatively measured by triphenyltetrazolium chloride assay.

Materials and Methods

Lithophyllum yessoense Pink crustose coralline tissues of *Lithophyllum yessoense* Foslie, were collected from the algal whitening area of Pohang, where the species is one of the dominants. The tissue was brushed and cleaned with 5% Tween 80 and sonication and was then scraped off the stones using the saw.

Seaweed extracts For seaweed extracts, thalli of prevalent seaweed were collected from the east coast of Korea. For each 20 g seaweed powder, one L of methanol was added to extract the methanol-soluble fraction at room temperature for one day. The extraction was repeated three times and combined. For a stock solution of the methanol extract, one mL of methanol was added for each 40 mg of dried extract. The most inhibitive seaweed C. *fragile* has been fractionated into five main classes of constituent; saccharides,

lipids, phenolics, alkaloids and nitrogen compounds according to polarity (Harborne, 1998).

Viability of the *L. yessoense* To measure viability of the *L. yessoense* tissue, one mg (25 uL) of each seaweed extract was added to 5 mL PES medium (Provasoli, 1968) containing 0.1 g of *L. yessoense*, and cultured on rotator for 5 d at 18 ℃ with 20 rpm rotation under 40 mmol/m^2/s photon flux density (fluorescent light) on a 12L:12D cycle. Reference culture was prepared in the medium with 25 uL methanol. After harvesting the tissues by centrifugation at 3000 × g for 30 sec, the viability was measured by the TTC assay at 545nm(Nam et al., 1998).

Results

1. The extracts from *Codium fragile* and *Enteromorpha linza* demonstrated viability inhibition of 72% and 82%, respectively, while an extract from *Hizikia fusiformis* showed viability activation of 113%.
2. The methanol extract of *C. fragile* was tested at different concentrations in the culture medium. At 1 mg/mL, the methanol extract demonstrated a viability inhibition down to 61% relative to the reference culture. Furthermore, at 2 mg/mL, the extract strongly inhibited viability down to 52% relative to the reference culture.
3. Among five different classes of constituents from *C. fragile*, the neutral extract of lipid compound showed the main inhibition activity.

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