

A Simple Screening Method of Anti-attachment Compounds using *Porphyra yezoensis* Monospore

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Introduction

A marine red alga, *Porphyra yezoensis* has received much attention as an experimental model for fundamental and applied research of marine plants. Especially, monospores (blade archeospore) from juvenile blades can be easily produced by the addition of allantoin (Mizuta et al., 2003) or by the culture condition (Hwang et al., 1997).

In this paper, a preliminary screening system has been developed for the detection of allelopathic or antifouling compounds in the marine environment using the *P. yezoensis* monospore.

Materials and Methods

Monospore culture Juvenile blades of *Porphyra yezoensis* Ueda were collected from the rocky shore of Pohang, Korea. For monospore liberation, the blades were cultured in PES medium (Provasoli, 1968) under 40 mmol/m²/s light intensity (10L:14D) at 20 °C.

Seaweed extracts Seaweed thalli for methanol and water extractions were collected from the coast of Korea from October 2002 to January 2005. Seaweed extracts were prepared according to Jin et al.(1997a).

Constituent separation and test compounds The most active seaweed *Sargassum sagamianum* has been fractionated into five main classes of constituents; saccharides, lipids, phenolics, alkaloids and nitrogen compounds according to polarity (Harborne, 1998). The activities of ten known allelochemical and algicidal compounds chitosan, cupric sulfate, di-n-octylphthalate, ferulic acid, salicylic acid, umbelliferone, Irgarol 1051, pyroglutamic acid, triethyl citrate and tributyltin, were also tested for the anti-attachment activity.

Anti-attachment activity For anti-attachment bioassay using *P. yezoensis* monospores, one μL of seaweed extracts (40 mg/mL), 4 μL PES stock and approximately 200 spores were added in a well that was cut out from 96-well plate and then the final volume made immediately to 200 μL . The resulting spore suspensions were placed in the dark for 1 d at 20 °C to allow for even attachment on the bottom. Non-attached spores were removed from the well bottom by centrifugation at 1500 x g for 15 min (Wagner et al., 1992) and the number of attached spores remained on the bottom was counted under an inverted microscope ($\times 100$). Spore attachment was expressed as the percentage of the reference value.

Results

Anti-attachment activity of allelochemical or antifouling substances was measured using monospores from *Porphyra yezoensis* as an assay organism. When 20 mg/mL of each methanol or aqueous extract from 32 seaweeds were added to the monospore suspension, the methanol extracts from *Corallina pilulifera*, *Ishige sinicola*, *Sargassum horneri* and *Sargassum sagamianum* showed significant attachment inhibition of more than 10% against reference. Phenolic compounds fractionated from *S. sagamianum* caused potent inhibition the most. Responses of the *P. yezoensis* monospores to known allelochemical and algicidal compounds also showed good sensitivity.

References

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