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Partial purification of antifouling substances from the marine brown alga *Scytosiphon lomentaria*

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Introduction

Marine algae and invertebrates are major fouling organisms causing considerable economic damage to man-made structures such as ship hulls, aquaculture nets and pilings. Until recently most antifouling techniques have relied on organotin(tributyltin) or heavy metals(copper, zinc) based paints that act as broad spectrum toxins to target and non-target marine organisms, but recently their use is restricted due to their environmental damage. One of the most promising alternative techniques to tributyltin is the development of naturally occurring antifouling compounds from marine organisms. While some seaweeds are heavily fouled, other species in the same habitat are rarely epiphytized, indicating the presence of antifouling or allelopathy mechanism. We have screened a wide number of seaweeds for antifouling activity and found that the *Scytosiphon lomentaria* had strong antifouling activity among the seaweeds

Materials and methods

Scytosiphon lomentaria extract

Tissues of *S. lomentaria* were collected from Busan and Pohang. Seaweed tissues were dried completely for 7 d at room temperature and then ground to a powder for 5 min using a coffee grinder. The powder of *S. lomentaria* (1720 g) was repeatedly extracted with 30L of methanol-water (4:1) three times, and then combined. After filtration, the crude extract was evaporated under vacuum to give a dark brown tar-like gummy residue. The filtered extract was successively fractioned into different classes according to polarity as shown in Harborne (1998).

Silica gel chromatography

5ml(2g) of fraction III were loaded on silica gel (70-230 mesh) column, and then eluted with each n-hexane, chloroform, ethylacetate, ethylacetate:methonal=4:6 and methanol as eluants.

Sephadex LH-20 column chromatography

Using 100% methanol as eluant, Each two mL fraction was collected at a flow

rate of 0.5 mL min^{-1}.

Mussel bioassay

The silica gel and sephadex column fractions were tested against the M. edulis, a representative fouling invertebrate.

The shells were opened and then 10 μL fraction was dripped on the foot. The number of mussel feet which had contracted within the first few seconds was counted(Cho et al., 2001).

Algal spore bioassay

The silica gel fraction was examined using spores of the chlorophyta *E. prolifera* as a representative fouling seaweed. For algal spore settlement, 1 μL of fraction was added to the 200 μL PES medium in 96-well plate immediately after the addition of spores. After culturing at 18℃ on a 12L:12D cycle for 1 day, number of settled spores was counted.

Results and discussion

Mussel bioassay

1. Solvent extraction: The class that was acidified to pH 2 with sulfuric acid and extracted three times with chloroform(fraction III, 18.43 g) showed strong activity for foot concentration of M. edulis.
2. Silica gel chromatography: Choroform and Ethyl acetate:Methanol= 4:6 fraction showed 80% activity in each for foot contraction of M. edulis.
3. Sephadex LH-20 column chromatography: From 120 fractions, number 12-23 fractions showed strong activity for foot contraction of M. edulis.

Algal spore bioassay

Silica gel chromatography: MeOH fraction showed the highest activity up to 86% inhibition

Reference

Harborne J B (1998) A guide to modern techniques of plant anlysis. Phytochemical Methods

Cho JY, Kwon EH, Choi JS, Hong SY, Shin HW & Hong YK (2001) Antifouling activity of seaweed extracts on the green alga Enteromorpha prolifera and the mussel Mytilus edulis. J. of appl. phycol., 12, 117-125