J. Fish. Sci. Technol. 8(4), 243-246, 2005

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Notes

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

Se-Eun Kang, Sun-Mee Park, Jae-Suk Choi, Dong-Hyun Ahn[1], Young-Dae Kim[2] and Yong-Ki Hong*

Department of Biotechnology, Pukyong National University, Busan 608-737, Korea
[1]Department of Food Science and Technology, Pukyong National University, Namku, Busan 608-737, Korea
[2]Aquaculture Division, East Sea Fisheries Research Institute, Gangneung 210-860, Korea

The addition of seaweed extracts was found to regulate the viability of cultures of the crustose coralline alga *Lithophyllum yessoense*. The viability was quantitated using a triphenyltetrazolium chloride assay, and the methanol-soluble extracts from 18 prevalent seaweed species were tested. Extracts from *Codium fragile* and *Enteromorpha linza* inhibited viability, and a *Hizikia fusiformis* extract slightly increased viability. The methanol extract of *C. fragile*, which had the strongest inhibitory activity, decreased viability to 72 or 52% that of the control following addition of 0.2 or 2 mg/mL of extract to the culture, respectively. The main active compound in the *C. fragile* was lipid. This information is a preliminary result related to the exploration of seaweed restoration in the algal whitening area.

Key words: Algal whitening, *Codium fragile*, Coralline alga, *Lithophyllum yessoense*, Seaweed extract, Viability

Introduction

Crustose coralline algae, which are non-articulated calcareous algae, cover rock surfaces with pink or white crusts. The phenomenon known as algal whitening and the resulting coastal devastation, which is related to the disappearance of seaweed flora in some rocky areas, is associated with certain crustose algae species. One dominant species that causes algal whitening in Korea and Japan is *Lithophyllum yessoense* Foslie (Tokuda et al., 1994; Kim, 2000). Since 1990, the algal whitening area has expanded from south Cheju Island to the middle East Sea (Chung et al., 1998). In these areas, most fleshy seaweed has disappeared from rocks because of algal whitening, diminishing and devastating the food sources and spawning places of fish and shellfish. Algal whitening is now recognized as a natural hazard that adversely affects marine ecosystems and damages commercial fisheries (Kim, 2002). Algal whitening is partially dependent on reduced bio-availability of iron (Suzuki et al., 1995), sloughing of the coralline epithallium (Masaki et al., 1984), herbivore grazing (Watanabe and Harrold, 1991), and the presence of allelopathic substances (Kim et al., 2004). Field observations (Kim, 2002) have shown that no macroalgal epiphytes are present on pink crustose algal surfaces, whereas some seaweed does grow on white-patch crustose surfaces. One possible approach toward the restoration of seaweed colonization in these areas is the transplantation of these related species. Before attempting to transplant seaweed in the field, it was necessary to test the *in vitro* inhibitory activity of several prevalent seaweed species against *L. yessoense* to find a species suitable for seaweed restoration in algal whitening areas. The tissue viability of the crustose coralline alga *L. yessoense* was quantitatively measured using a triphenyltetrazolium chloride assay.

*Corresponding author: ykhong@pknu.ac.kr

Materials and Methods

Pink crustose coralline tissues of *L. yessoense* were

collected from the algal whitening area of Pohang, Korea, where *L. yessoense* is one of the dominant species. White or gray-pink patches of crustose surfaces on stones were removed using a grinding drill and steel saw. Then, stones that were covered with healthy looking pink tissue were selected. The pink tissue was brushed and cleaned three times with 1-min pulses in an ultrasonic water bath (low intensity with a frequency of 90 kc per sec). To remove microalgae and bacteria, 5% Tween 80 was added to the seawater before sonication. The tissue was then scraped off the stones using the saw. The tissue was thoroughly washed at least six times by centrifugation at 1000×*g* for 30 sec. After the pretreatment of the tissue, the numbers of microalgal and bacterial cells were reduced to less than 1% of the numbers on the original tissue.

For seaweed extracts, thalli of prevalent seaweed species were collected from the east coast of Korea. Epiphytes and salts were removed from the seaweed thalli by washing in tap water and distilled water. The thalli were dried completely for 1 week at room temperature and then ground to a powder in a coffee grinder for 5 min. To extract the methanol-soluble fraction, 20 g of each seaweed powder were mixed with 1 L of methanol, and the mixture was left at room temperature for 1 day. The extraction was repeated three times, and the extracts were combined and then dried. For a stock solution of the methanol extracts, 1 mL of methanol was added to 40 mg of each dried extract. After dissolving the extract, the solution was passed through a 0.22-μm syringe filter before use. The most inhibitive seaweed extract, that of *C. fragile*, was fractionated into five main classes of constituents according to their polarity: saccharides, lipids, phenolics, alkaloids, and nitrogen compounds (Harborne, 1998).

The triphenyltetrazolium chloride assay of Nam et al. (1998) was used. Four mL of 0.8% 2,3,5-triphenyltetrazolium chloride (TTC) in seawater containing 50 mM Tris-HCl buffer (pH 7.6) were added to 0.2 g of tissue in a 15-mL capped tube and incubated in darkness for 1 h at 20°C under two drops of mineral oil (M-3516; Sigma Chemical, St. Louis, MO, USA). The tissue was then rinsed three times with sterilized seawater followed by centrifugation at 3000×*g* for 30 sec. The triphenylformazan (TPF) that formed in the tissues was extracted with 2 mL of 0.2 N KOH in 25% ethanol by heating for 15 min at 60°C. Next, the TPF was partitioned away by the prompt addition of 2-mL hexane followed by vortexing. After centrifugation for 1 min, the amount of TPF in the top phase was quantified by measuring the absorbance at 545 nm.

To measure the viability of the *L. yessoense* tissue, 1 mg (25 μL) of each seaweed extract was added to 5-mL PES medium (Provasoli, 1968) containing 0.1 g of *L. yessoense*, and the mix was cultured for 5 d at 18°C with rotation at 20 rpm, under a photon flux density (fluorescent light) of 40 μmol/m^2/sec, and on a light cycle of 12-h light/12-h dark. A reference culture was prepared by mixing 25 μL methanol in the same medium. After harvesting the tissues by centrifugation at 3000×*g* for 30 sec, the viability was measured using the TTC assay. The relative viability (%) was calculated as: (S/C) x 100, where S equals the absorbance of *L. yessoense* with seaweed extract and C equals the absorbance of the reference culture.

For each seaweed extract and control sample, the experiments were repeated at least three times for each independent assay. The mean values of the index were compared to the control using Student's *t*-test.

Results and Discussion

The coralline *L. yessoense* tissue was cultured in PES medium at 18°C over 30 d to determine the optimal culture period under laboratory conditions. Viability was maintained until 10 d, but declined to 33% after 30 d (data not shown). Therefore, the effects of the seaweed extracts on the viability of *L. yessoense* were determined using the TTC assay after 5 d of culture. A reference culture lacking seaweed extracts reached an absorbance of 0.21±0.01. To estimate the effects of the inhibitors on the viability of the coralline alga, a methanol-soluble fraction was isolated from each of 18 seaweed samples. The methanol extracts were added to *L. yessoense* culture medium to 200 μg/mL. *Codium fragile* and *Enteromorpha linza* extracts inhibited viability to 72 and 82% that of the control, respectively, whereas an extract from *Hizikia fusiformis* increased the viability to 113% (Table 1). The *C. fragile* methanol extract suppressed *L. yessoense* viability the most, and was used in all further experiments.

The solvent methanol showed minimal inhibition of *L. yessoense* viability when added to 0.5%. Methanol-soluble components were therefore always added to the medium such that the final percentage of methanol was less than 0.5%. Different concentrations of the *C. fragile* methanol extract were tested in the culture medium (Fig. 1). At 1 mg/mL, the extract inhibited viability to 61% that of the reference culture, and increasing the extract concentration to 2 mg/mL

Table 1. Effect of seaweed extracts on viability of the coralline *L. yessoense*[1]

	Absorbance at 475 nm	Relative viability (%)
Carpopeltis affinis	0.21 ± 0.01	100
Chondrus ocellatus	0.20 ± 0.01	95
Codium fragile	0.15 ± 0.00***	71
Colpomenia bullosa	0.20 ± 0.02	95
Colpomenia sinuosa	0.22 ± 0.01	105
Endrachne binghamiae	0.20 ± 0.02	95
Enteromorpha linza	0.17 ± 0.01**	81
Hizikia fusiformis	0.24 ± 0.02*	114
Ishige okamurae	0.23 ± 0.01	110
Monostroma nitidum	0.21 ± 0.02	100
Sargassum confusum	0.20 ± 0.01	95
Sargassum horneri	0.21 ± 0.00	100
Sargassum sagaminanum	0.20 ± 0.01	95
Sargassum thunbergii	0.20 ± 0.01	95
Scytosiphon lomentaria	0.20 ± 0.00	95
Ulva pertusa	0.21 ± 0.03	100
Undaria pinnatifida	0.21 ± 0.01	100
Laminaria japonica	0.22 ± 0.00	105

[1]Reference culture without any seaweed extract reached absorbance of 0.21 ± 0.01. Data are the mean ± -SD (n>3). *P<0.1, **P<0.01 and ***P<0.001.

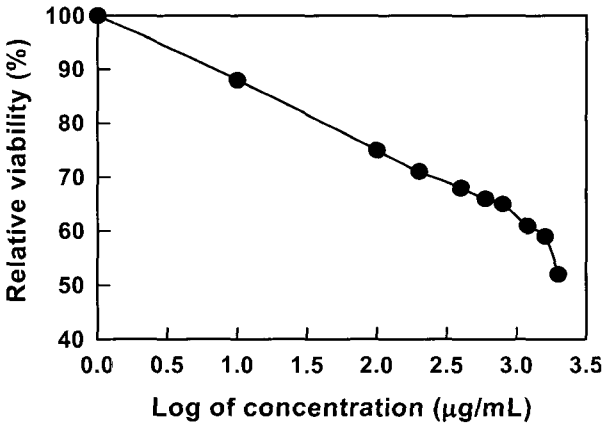

Fig. 1. Inhibitory effect of *C. fragile* extract on viability of the coralline *L. yessoense* tissue.

inhibited viability even more strongly, to 52% that of the reference culture.

To determine the main active compound in the *C. fragile* extract, *C. fragile* powder (10 g) was successively fractionated into five main classes of constituents according to their polarity: saccharides, lipids, phenolics, alkaloids, and nitrogen compounds (Table 2). After three extractions with 1 L of methanol-water (4:1), the remaining residue (8.6 g) was extracted five times with 500-mL ethyl acetate and evaporated to give an oily greenish residue (0.06 g). This neutral extract, which consisted of a lipid compound mostly, showed the bulk of the inhibitory activity.

Table 2. Comparison of different compound fractions from *C. fragile* on viability of the coralline *L. yessoense*[1]

	Yield (g/10 g powder)	Absorbance at 475 nm	Relative Viability (%)
Fiber	4.76	ND	ND
Saccharides	3.77	0.20 ± 0.01	95
Lipids	0.06	0.14 ± 0.01	67
Phenolics	0.46	0.21 ± 0.00	100
Alkaloids	0.05	0.20 ± 0.01	95
Nitrogen compounds	0.90	0.21 ± 0.00	100

[1]Data are the mean ± SD (n>3). ND; not determined.

This information is a preliminary result related to a project for the restoration of seaweed in the algal whitening area. Although conclusive evidence must be obtained, *C. fragile* likely has inhibitory activity against *L. yessoense*. Thus, one possible approach toward seaweed restoration in the algal whitening area would be the transplantation of *C. fragile*, followed by attempts to induce the recruitment of other seaweed species. It has been reported that several extracts from pink *L. yessoense* inhibit the spore settlement and germination of most seaweeds (Kim et al., 2004). Further experiments on the survival of *C. fragile* after *in situ* transplantation and the release of the putative inhibitor are needed to elucidate the most appropriate method for seaweed restoration.

Acknowledgments

This work was supported by the Ministry of Marine Affairs and Fisheries-R&D Project in Korea, and the Brain Busan 21 Project in 2005.

References

Chung, H.S., K.W. Cho, K.H. Chung, J.H. Kim, J.H. Shin, Y.W. Seo, J.S. Kang and I.K. Lee. 1998. Ecological characteristics of algal whitening in coastal zone of Seogwipo area, Cheju Island. Algae, 13, 361-374.

Kim, J.H. 2000. Taxonomy of the Corallinales, Rhodophyta in Korea. Ph.D. dissertation, Seoul National University, Korea, pp. 349.

Kim, H.G. 2002. Ecological studies of seaweed in artificial seaweed bed. In: Construction of Artificial Seaweed Bed for the Production Improvement in East Coast. East Sea Fisheries Research Institute, Korea, 31-41.

Kim, M.J., J.S. Choi, S.E. Kang, J.Y. Cho, H.J. Jin, B.S.

Chun and Y.K. Hong. 2004. Multiple allelopathic activity of the crustose coralline alga *Lithophyllum yessoense* against settlement and germination of seaweed spores. J. Appl. Phycol., 16, 175-179.

Harborne J.B. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall, London, pp. 302.

Masaki, T., D. Fujita and N.T. Hagen. 1984. The surface ultrastructure and epithallium shedding of crustose coralline algae in an Isoyake area of southwestern Hokkaido, Japan. Hydrobiologia,116/117, 218-223.

Nam, B.H., H.J. Jin, S.K. Kim and Y.K. Hong. 1998. Quantitative viability of seaweeds tissues assessed with 2,3,5-triphenyltetrazolium chloride. J. Appl. Phycol., 10, 30-36.

Provasoli, L. 1968. Media and prospects for cultivation of marine algae. In: Cultures and Collections of Algae, Watanabe A. and A. Hattori eds. Jap. Soc. Plant Physiol., Tokyo, 63-75.

Suzuki, Y., T. Takabayashi, T. Kawaguchi and K. Matsunaga. 1998. Isolation of an allelopathic substance from the crustose coralline algae, *Lithophyllum* spp., and its effect on the brown alga, *Laminaria religiosa* Miyabe (Phaeophyta). J. Exp. Mar. Biol. Ecol., 225, 69-77.

Tokuda, H., S. Kawashima, M. Ohno and H. Ogawa. 1994. Seaweeds of Japan. Midori Shobo Co. Ltd., Tokyo, pp 194.

Watanabe, J.M. and C. Harrold. 1991. Destructive grazing by sea urchins *Strongylocentrotus* spp. in a central California kelp forest: potential roles of recruitment, depth and predation. Mar. Ecol. Prog. Ser., 71, 125-141.

(Received October 2005, Accepted December 2005)