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Seaweed Biotechnology: Sustainable Development of Diverse Biologically Active Substances from Seaweeds

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Seaweed biotechnology is a multidisciplinary subject to produce food, pharmaceuticals, chemicals, and environmental remediation materials from seaweed resources. It uses various techniques of cell culture, enzyme reaction and genetic manipulation to increase the production efficiency of useful seaweeds or their products. Firstly, an overview of key topics will be introduced in the fields of seaweed tissue culture, strain improvement, genetic analysis briefly as basic techniques. Secondly, some biologically active substances such as anti-inflammatory and antifouling substances that have been screened in my laboratory will be focused.

Key words : antifouling, anti-inflammation, biologically active substance, seaweed biotechnology

Introduction

One quarter of the worlds drugs come from natural sources, primarily from microorganisms and plants. As terrestrial resources become over explored, attention has turned to the marine environment as an alternative source of novel bioactive metabolites. Compounds isolated from marine organisms have shown potent anticancer, antiviral, antibacterial, anti-inflammatory or painkilling activity[1]. Obstacle to the drug development of marine natural products is the lack of sufficient material for comprehensive pharmacological evaluation[2]. More often than not, compounds are isolated in mg quantities, and their structures are elucidated and published. For any pharmaceutical lead from a marine source, supply issues will always be a problem. Unless supply can be addressed in an economically feasible fashion, the dream of new effective drugs from the sea will falter. Thus, one of candidates for pharmaceutical source would be seaweed that is abundant or easily aquaculturable. The research in this laboratory is exploring abundant seaweeds that produce biologically active substances. In particular, our survey efforts in this area have led to two main themes in studies: (1) screening, isolation and biological analysis of biologically active substances, such as phospholipase A_2 inhibitor, antifouling agent, and microalgal growth enhancer; (2) tissue culture, mutant selection, and genetic analysis to overcome supply issues of useful substances. These studies are highly interdisciplinary in nature and draw on diverse methodologies in marine biotechnology.

Basic biotechniques for seaweed

Tissue culture

For the tissue culture of seaweed, callus and blade formation depended on the gelling agents used under axenic culture conditions[3]. Procedures were developed for the axenic isolation of chonchocelis and monospores from the red seaweed *Porphyra yezoensis*[4]. A spectrophotometric quantification method was optimized to evaluate its utility in seaweed tissue viability tests using the enzymatic reduction of colorless 2,3,5-triphenyltetrazolium chloride to a colored triphenylformazan[5].

Strain improvement

Putative transgenic *P. yezoensis* was obtained under the

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conditions optimized by the particle bombardment. To make new varieties of *P. yezoensis*, monospores are the most useful cells in maintaining and culturing[6]. Making mutants for overproduction of essential amino acids are in progress.

Genetic analysis

A rapid and economical method of DNA and RNA extraction from seaweed was developed by the use of lithium chloride[7]. Complete sequence of the 18S rDNA was amplified and sequenced from species of the aquaculturable Porphyra in Korea. A pollutant (pine needle ash)-responding gene for glutaredoxin was isolated from the seaweed *P. yezoensis* using differential display technique[8].

Biologically active substances

Anti-inflammatory substance

A number of seaweed species are used as traditional medicine and health care belief as well as food in various regions of the world. Almost all Korean women, even immigrated to foreign countries, eat the brown seaweed *Undaria pinnatifida* (known as Miyok) soup after childbirth for a month because of the belief that it cleans the blood and increases milk production[9]. Use of the *U. pinnatifida* for curing fever, urination, lump or swelling is recorded in an oriental medical textbook Donguibogam published in 1613[10]. It is also known to contain ingredients that contract the uterus after childbirth[11]. For herbal medicine in China, it is used to treat urinary diseases and dropsy[12]. All of these symptoms are related to anti-inflammation reaction. Thus, to evaluate medicinal activities of the *U. pinnatifida*, we have measured anti-inflammatory activities of methanol extract against mouse ear edema, analgesic, antipyretic, and toxicity[13]. The methanol extract showed IC_{50} value of 10.3 mg/mL against mouse ear edema induced by phorbol myristate acetate. Also in analgesic test, the extract showed inhibitory effect on acetic acid-induced writhing response with IC_{50} of 0.48 g per kg-body weight. The extract elicited antipyretic action when tested in yeast-induced hyperthermia. Of the common 37 seaweed compared, *U. pinnatifida* showed the strongest suppression (Table 1).

Table 1. Biological activities in the methanol-soluble extract from seaweeds*

Species	1	2	3	4	5
CHLOROPHYTA					
Codium fragile	-	-	ND	-	-
Enteromorpha compressa	ND	ND	+	-	ND
Enteromorpha linza	-	-	+	+	-
Monostroma nitidum	-	-	ND	ND	-
Ulva pertusa	+	-	-	-	-
PHAEOPHYTA					
Colpomenia bullosa	-	+	ND	-	-
Colpomenia sinuosa	-	-	ND	+	-
Costaria costata	ND	+	ND	-	ND
Ecklonia cava	-	+	ND	-	-
Endrachne binghamiae	-	-	ND	ND	-
Hizikia fusiformis	-	-	ND	-	-
Ishige okamurae	ND	ND	ND	-	+
Ishige sinicola	+	+	ND	-	ND
Kjellmaniella crassifolia	-	-	ND	ND	-
Sargassum confusum	-	-	ND	-	-
Sargassum horneri	+	-	+	-	-
Sargassum sagamianum	-	-	ND	-	+
Sargassum thunbergii	-	-	ND	+	-
Scytosiphon lomentaria	-	+	ND	ND	-
Undaria pinnatifida	-	-	-	+	-
RHODOPHYTA					
Carpopeltis affinis	-	-	-	ND	-
Carpopeltis cornea	ND	ND	+	-	ND
Chondrus ocellatus	+	-	+	-	-
Corallina pilulifera	+	-	ND	-	-
Gigartina intermedia	-	-	ND	ND	-
Gracilaria verrucosa	ND	ND	-	+	ND
Grateloupia prolongata	-	-	ND	ND	-
Grateloupia turuturu	-	-	-	ND	-
Hypnea charoides	-	-	ND	-	-
Pachymeniopsis elliptica	+	-	+	+	-
Porphyra yezoensis	-	-	ND	-	-
Symphyocladia latiuscula	-	-	ND	-	-

*(1) A "+" symbol indicates attachment inhibition of spores from the fouling seaweed *Enteromorpha prolifera* with 5 μL extract (200μg) in 1 mL seawater. (2) A "+" symbol indicates inhibition of foot repulsive reaction of the mussel *Mytilus edulis* with 10μL extract (40μg). (3) A "+" symbol indicates inhibition of seaweed spore settlement with 1 μL extract (4 mg) from the crustose coralline *Lithophyllum yessoense* in 200μL seawater. (4) A "+" symbol indicates anti-inflammatory activity with 10μL (400μg) against mouse ear edema induced by 0.2μg phorbol myristate acetate. (5) A "+" symbol indicates inhibition of bacterial phospholipase A_2 with 15μL (600μg) in 3.1 mL enzyme reaction mixture. ND, not determined.

The brown seaweed *Ishige okamurae* showed a potent inhibitory activity against bacterial phospholipase A_2 from *Vibrio mimicus*[14]. The purified compound inhibited the PLA_2 with IC_{50} of 1.86μg/mL and KI of 3.89μg/mL competitively. It showed more than twice stronger than a standard rutin compound (Fig. 1). Topical application reduced mouse ear edema with IC_{50} of 3.57 mg/mL (Table 1).

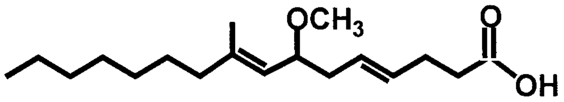

Fig. 1. Structure of 7-methoxy-9-methylhexadeca-4,8-dienoic acid (MMHDA) isolated from the brown seaweed *Ishige okamurae*. The compound strongly inhibits PLA_2 activity *in vitro* and has potent anti-inflammatory activity *in vivo*.

Antifouling substances

Three antifouling active compounds of L-pyroglutamic acid (PGA), triethyl citrate (TEC), and di-*n*-octylphthalate (DNOP) were isolated from the brown seaweed *Ishige okamurae* (Fig. 2). Approximately 2.8 mg PGA, 1.7 mg TEC, and 2.0 mg DNOP were isolated from 600 g of *I. okamurae* powder. The concentrations of PGA, TEC, and DNOP required to cause foot repulsion in 50% of mussels (RD_{50}) were 9, 26, and 0.08 mM, respectively[15]. The PGA, TEC, and DNOP concentrations required to inhibit 50% attachment of algal spores (ID_{50}) were 24, 50, and 0.1 mM, respectively. These compounds showed stable antifouling activities against mussel and algal spore attachment (Table 1).

A study was made to investigate possible formation by the crustose coralline alga *Lithophyllum yessoense* of multiple allelopathic-related substances against the settlement and germination of spores of various seaweeds[16]. Spore settlement of 14 species was inhibited over 90% by solvent extracts and conditioned seawater. The germination of spores from 13 species was also inhibited by the extracts and conditioned seawater (Table 1). Isolation of the main active compound is in progress.

Microalgal growth enhancer

Cell growth of the marine microalga *Isochrysis galbana* was regulated by the addition of seaweed extracts in the culture medium[17]. Methanol-soluble extracts from 27 species of seaweed showed growth activation only from *Enteromorpha linza*, and growth inhibition from *Ishige foliacea and Sargassum sagamianum* (Table 1). Water-soluble extracts from *Grateloupia turuturu and Monostrom nitidum* showed growth activation, while none of the seaweed showed growth inhibition. From results of growth activation of extracts on *I. galbana*, the water extract of *M. nitidum* was the most effective up to two-fold increase in cell density with the addition of 1 mg mL^{-1} of extract to the medium. The cell growth rate was increased from 0.52 to 0.65 d^{-1}. Cell size, gross biochemical compositions, fatty acid compositions, and digestion efficiency by shellfish differed marginally between cultures of *I. galbana* grown with and without the *M. nitidum* aqueous extract. This extract has

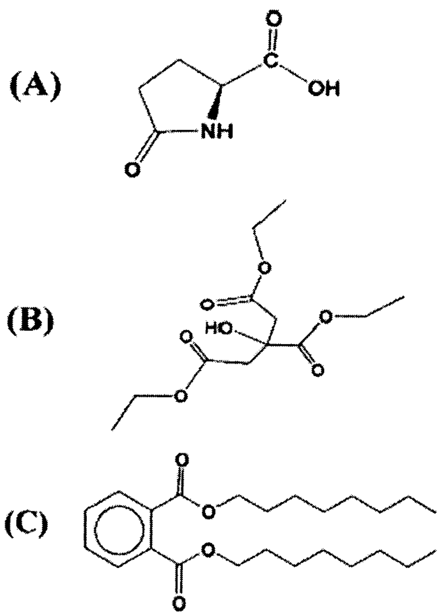

Fig. 2. Structures of L-pyroglutamic acid (A), triethyl citrate (B), di-*n*-octylphthalate (C) isolated from the brown seaweed *Ishige okamurae*. These compounds showed potent antifouling activities against mussel and algal spore attachment.

also enhanced the growth of other feed microalgae tested, including *Dunaliella salina*.

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References

1) Smit AJ: Medicinal and pharmaceutical uses of seaweed natural products: A review. *J Appl Phycol*, 16, 245-262 (2004)

2) Jensen PR, Fenical W: Marine microorganisms and drug discovery: Current status and future potential. In: Fusetani N. (ed), Drugs from the Sea, Karger, Basel, 6-29 (2000)

3) Jin HJ, Seo GM, Cho YC, Hwang EK, Sohn CH, Hong YK: Gelling agents for tissue culture of the seaweed *Hizikia fusiformis*. *J Appl Phycol*, 9, 489-493 (1997)

4) Choi JS, Cho JY, Jin LG, Jin HJ, Hong YK: Procedures for the axenic isolation of chonchocelis and monospores from the red seaweed *Porphyra yezoensis*. *J Appl Phycol*, 14, 115-121 (2002)

5) Nam BH, Jin HJ, Kim SK, Hong YK: Quantitative viability of seaweed tissues assessed with 2,3,5-triphenyltetrazolium chloride. *J Appl Phycol*, 10, 31-36 (1998)

6) Mizuta H, Yasui H, Saga N: A simple method to mass produce monospores in the thallus of *Porphyra yezoensis* Ueda. *J Appl Phycol*, 15, 345-349 (2003)

7) Hong YK, Kim SD, Polne-Fuller M, Gibor A: DNA extraction conditions from *Porphyra perforata* using LiCl. *J Appl Phycol*, 7, 101-107 (1995)

8) Jin LG., Choi JS, Jin, HJ, Shin HW, Hong YK: 2002. Isolation of pollutant (pine needle ash)-responding genes from the seaweed *Porphyra yezoensis* tissue. *Fish Sci*, 68, S1044-S1047 (2002)

9) Park KJ, Peterson LM: Beliefs, practices, and experiences of Korean women in relation to childbirth. *Health Care Women Int*, 12, 261-269 (1991)

10) Donguibogam committee: Translated Donguibogam. Bubinmunwha Publisher, Seoul, 2198 (1999)

11) Huh K, Song JW, Choi JW: Studies on uterus contraction of the components of *Undaria pinnatifida*. *Kor J Pharmacogn*, 23, 146-152 (1992)

12) Tseng CK, Chang CF: Chinese seaweeds in herbal medicine. *Hydrobiologia*, 116/117, 152-154 (1984)

13) Khan MNA, Gyawali YP, Yoon SJ, Choi JS, Kang SE, Hong YK: Anti-inflammatory activities of the methanol extract from the brown seaweed *Undaria pinnatifida*. *Proc Kor Soc Phycol*, 2005, 47 (2005)

14) Gyawali YP, Choi JS, Khan MNA, Ahn SH, Kong IS, Hong YK: Isolation of PLA_2 inhibitor from the brown seaweed *Ishige okamurae*. *Proc Kor Soc Phycol*, 2005, 48 (2005)

15) Cho JY, Choi JS, Kang SE, Kim JK, Shin HW, Hong YK: Isolation of antifouling active pyroglutamic acid, triethyl citrate, and di-n-octylphthalate from the brown seaweed *Ishige okamurae*. *J Appl Phycol*, 17, 431-435 (2005)

16) Kim MJ, Choi JS, Kang SE, Cho JY, Jin HJ, Chun BS, Hong YK: 2004. Multiple allelopathic activity of the crustose coralline alga *Lithophylum yessoense* against settlement and germination of seaweed spores. *J Appl Phycol*, 16, 175-179 (2004)

17) Cho JY, Jin HJ, Lim HJ, Whyte JNC, Hong YK: Growth activation of the microalga *Ishochrysis galbana* by the aqueous extract of the seaweed *Monostroma nitidum*. *J Appl Phycol*, 10, 561-567 (1999)