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Seaweed Biotechnology: Current Status and Future Potential

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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, genetic manipulation and chemical analysis 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.

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 (Smit, 2004). Obstacle to the drug development of marine natural products is the lack of sufficient material supply for comprehensive pharmacological evaluation (Jensen and Fenical, 2000). 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 focusing to explore abundant seaweeds that produce biologically active substances. In particular, our survey efforts in this area have led to two main themes: (1) screening, isolation and biological analysis of biologically active substances, such as anti-inflammatory agent, phospholipase A2 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 and draw diverse methodologies of marine biotechnology.

1. Basic bio-techniques for seaweed

1-1. Tissue culture

For the tissue culture of seaweed, callus and blade formation depended on the gelling agents used under axenic culture conditions (Jin et al., 1997). Procedures were developed for the axenic isolation of chonchocelis and monospores from the red seaweed *Porphyra yezoensis* (Choi et al., 2002). 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 (Nam et al., 1998).

1-2. Strain improvement

Putative transgenic *P. yezoensis* was obtained under the conditions optimized by the particle bombardment. To make new varieties of *P. yezoensis*, monospores are the most useful cells in maintaining and culturing (Mizuta et al., 2003). Making mutants for overproduction of essential amino acids are in progress.

1-3. Genetic analysis

A rapid and economical method of DNA and RNA extraction from seaweed was developed by the use of lithium chloride (Hong et al., 1995). 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 (Jin et al., 2002).

2. Biologically active substances

2-1. Anti-inflammatory substance

Anti-inflammatory activities of a methanol extract of the brown seaweed *Undaria pinnatifida* Suringar were measured. *U. pinnatifida* has been used as a traditional medicine in the treatment of fever, urination problems, lumps and swelling, and as a health-care diet for women after childbirth. Methanol extract showed an IC_{50} value of 10.3 mg/ml against mouse ear edema induced by phorbol myristate acetate (PMA). A 40-mg/ml methanol extract maintained a 50% or higher suppression when applied either 3 h before or 2 h after PMA application. With the extract application, edema diminished to half maximal levels after 23 h compared to 31 h with vehicle. The blade part of the thallus showed the highest activity, while the northern forma of *U. pinnatifida* had a slightly higher activity. Compared with 37 common seaweeds, *U. pinnatifida* showed the greatest suppression. In the analgesic test, the methanol extract showed a suppressive effect on acetic acid-induced writhing response, with an IC_{50} of 0.48 g/kg body

weight. The extract also showed antipyretic activity when tested in yeast-induced hyperthermic mice. The methanol extract showed no toxicity to mice after an oral administration of 10 g/kg.

A methoxylated fatty acid that inhibits phospholipase A_2 (PLA_2; EC 3.1.1.4) was purified from the brown seaweed *Ishige okamurae*. Approximately 8.1 mg of the inhibitory compound, 7-methoxy-9-ethylhexadeca-4,8-dienoic acid, were isolated from 1 kg of *I. okamurae* powder. Recombinant PLA_2 derived from the pathogenic bacterium *Vibrio mimicus* was used as the target enzyme. The methoxylated fatty acid compound competitively inhibited PLA_2 with a K_i value of 3.9 μg mL^{-1}. The concentrations required for 50% inhibition of PLA_2, edema, and erythema were 1.0 μg mL^{-1}, 3.6 mg mL^{-1}, and 4.6 mg mL^{-1}, respectively. The compound strongly inhibits PLA_2 activity in vitro and has potent anti-inflammatory activity in vivo.

2-2. 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* (Cho et al., 2005). 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. 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.

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 (Kim et al., 2004). 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. Isolation of the main active compound is in progress.

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