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Isolation of an Algal Growth-enhancer Polysaccharide from the Chlorophyta *Monostroma nitidum*

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A microalgal growth-enhancing polysaccharide compound was isolated from the green alga *Monostroma nitidum* using water extraction, molecular fractionation, a DEAE-cellulose column, and fast protein liquid chromatography using a Superose-12 column. The yield of the compound from the seaweed powder was 8.3×10^{-3}%. At 2 mg/mL concentration, the polysaccharide enhanced *Tetraselmis suecica* cell growth in f/2 medium by approximately 160%.

Key words: *Monostroma nitidum*, Chlorophyta, Microalgal growth enhancer, Polysaccharide

Introduction

Microalgal feeds are especially valuable for the early stages of marine fish and for shellfish species with fastidious dietary requirements that cannot be met by formulations of traditional agricultural commodity products. Microalgal diets commonly contain high nutritional quality and are in great demand because of their composition of protein, vitamins, pigments, and large amounts of docosahexaenoic acid (22:6 [n-3]) and eicosapentaenoic acid (20:5 [n-3]) (Mansour et al., 2005). Some microalgae have not been used extensively as nutritional diets because it is difficult to achieve high-density cultures, leading to unreliable production (Wikfors and Ohno, 2001). Much attention has been focused on the development of new biofermenter designs (Pulz, 2001), storage methods after harvest (Lim and Park, 1998), and the improvement of nutritional value (Lopez Alonso et al., 1992). Alternatively, cell growth-enhancing substances can be developed to increase cell biomass and the economic feasibility of small-scale production. Previous screening of seaweed extracts in our laboratory (Cho et al., 1999) showed that the aqueous extract from the green alga *Monostroma nitidum* Wittrock activates cell growth in several microalgae. We isolated a polysaccharide compound of high molecular weight (MW) with a yield of 8.3×10^{-3}% to help identify the microalgal growth enhancer.

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Materials and Methods

Seaweed preparation

Thalli of the green alga *M. nitidum* Wittrock (Monostromaceae, Ulvales) were collected in March 2004 and March 2005 from the upper intertidal zone of calm inlets at Imwhe (34°22'23"N, 126°15'16"E), Jindo Island, Korea. Seaweed tissue samples were dried completely for 1 week at room temperature and then ground to a powder for 5 min using a coffee grinder. The powder was stored at -20°C until use.

Isolation procedure

To isolate the active compound, *M. nitidum* powder (100 g) was extracted three times with 5 L of 100% methanol. After removal of the methanol extract, the remaining powder was dried, mixed with 5 L of distilled water (DW), and left for 1 day to extract the water-soluble fraction. The aqueous extract was evaporated under a vacuum. The extract was then dissolved in 0.1 M Tris-HCl buffer (pH 8.0) and separated into four fractions by molecular fractionation using diafilter membranes (Amicon Co., Danvers, MA, USA). An active fraction larger than MW 10,000 was loaded onto a DEAE-cellulose column (50 g; 30×6 cm; Sigma, St. Louis, MO, USA) equilibrated with the same Tris-HCl buffer. Elution was performed with each 500 mL of equilibration buffer, followed by 0.1 M NaCl stepwise in the same buffer. The active 0.5 M NaCl fraction was separated again using fast protein liquid chromatography (FPLC; Pharmacia, Uppsala, Sweden) using a Superose-12

gel filtration column (1.0 cm ID×30 cm). Each sample (3 mg/100 μL, prepared in distilled water) was eluted with DW for 25 min at a flow rate of 0.5 mL/min and collected as 0.4-mL aliquots. The UV absorbance was monitored at 254 nm.

Analysis of the compound

The compound was identified using biochemical detection reagents such as bromocresol green, ninhydrin, silver nitrate-sodium hydroxide, iodine, and sulfuric acid (Dawson et al., 1986).

Microalgal culture

To isolate the active compound and measure cell growth, the axenic prasinophyte flagellate *Tetraselmis suecica* (CCAP-66; P-4) was cultured in f/2 medium (Guillard and Ryther, 1962) with an initial cell density of 1.0×10^5 cells/mL. Purified compound was added to the medium and cultured under 70 μmol m^{-2} s^{-1} light intensity at 18°C for 5 days. Cells were counted under a microscope using a hemocytometer.

Statistics

Each independent assay was repeated at least three times. Treatment means were compared to controls using Student *t*-tests.

Results and Discussion

Monostroma nitidum powder (100 g) was extracted with DW after removal of the methanol-soluble fraction. The aqueous extract (800 mg; twofold growth activity at 2 mg/mL) was fractionated using Amicon diafilters (MW≥10,000) and DEAE-cellulose column chromatography (9.9 mg). The active growth enhancer was then separated using FPLC with a Superose-12 column (Fig. 1). The active growth enhancer peak was eluted at 14.5 elution volume (mL), and appeared as a powdery compound (8.3 mg; 1.6-fold growth activity at 2 mg/mL). The yield of the active compound from the seaweed powder was 8.3×10^{-3}%. The compound was identified using several biochemical detection reagents (Table 1). It reacted with both silver nitrate-sodium hydroxide and with iodine; both detect carbohydrates. However, it did not react with bromocresol green, ninhydrin, or sulphuric acid, which detect acidic compounds, amino acids, and lipids, respectively. Thus, the algal growth enhancer is a polysaccharide. By comparison with known proteins using FPLC with a Superose-12 column, the molecular weight of the compound is indirectly inferred to be approximately 24,000 (data not shown).

The purified polysaccharide compound was tested for microalgal growth-enhancement activity at various

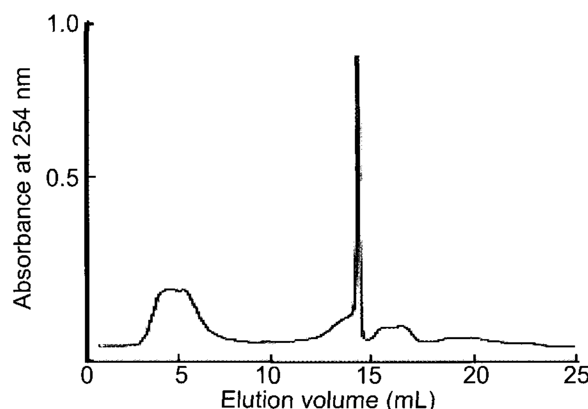

Fig. 1. Chromatogram from fast protein liquid chromatography using a Superose-12 gel filtration column Each sample (3 mg/100 μL) was eluted with distilled water for 25 min at a flow rate of 0.5 mL/min. UV absorbance was monitored at 254 nm.

Table 1. Identification of the purified compound using biochemical detection reagents. The "+" indicates a positive reaction; "–" indicates a negative reaction.

Biochemical detection reagent	Reaction
Bromocresol green	–
Ninhydrin	–
Silver nitrate–sodium hydroxide	+
Iodine	+
Sulfuric acid	–

concentrations in f/2 medium using *T. suecica*. *T. suecica* attained the stationnary growth phase within 2 mg/mL in f/2 medium under 70 μmol m^{-2} s^{-1} light intensity at 18°C. Cell numbers were counted after 5 days to determine the growth enhancement (Fig. 2). At 2 mg/mL concentration, the polysaccharide demonstrated strong growth enhancement of up to approximately 160%, relative to the reference culture without the extract. Nevertheless, the growth enhancement was lower than that obtained using the aqueous crude extract. This may be because another growth-enhancing compound of low molecular weight, present in the aqueous extract, is lost (Luyen et al., 2006). No changes in cell size (approximately 20 μm in diameter) occurred when the polysaccharide was added. In a previous study (Cho et al., 1999), we found that the gross biochemical composition (i.e., carbohydrate, chlorophyll *a*, lipid, and protein) and digestion efficiency of *T. suecica* (i.e., by the Pacific oyster, blue mussel, and Japanese littleneck clam) were similar between cultures grown with and without *M. nitidum* crude extract. The crude extract enhanced the cell growth of all feed microalgae that were tested, especially *Dunaliella salina* and *Isochrysis galbana*, with similar increases in cell density (Cho et al., 1999). Enhanced development

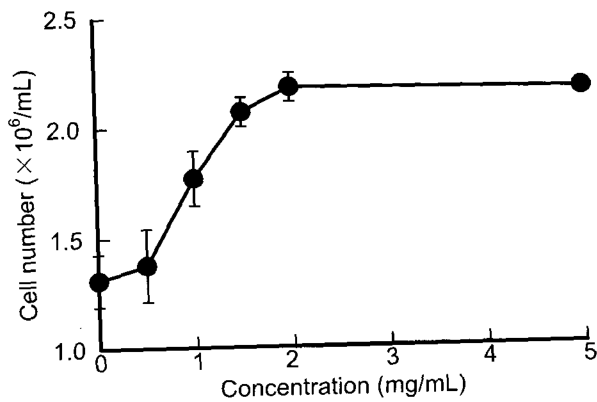

Fig. 2. Effect of various concentrations of the purified polysaccharide on cell growth (mean number of cells ± SD; n ≥ 3) of the microalga *Tetraselmis suecica*. The polysaccharide was added to f/2 medium inoculated with 1.0×10^5 cells/mL, and cultured at 18°C for 5 days.

and differentiation of macroalgae were observed on a *Pterocladia* agar, which may have toxic but growth-stimulating factors (Polne-Fuller et al., 1993). Polysaccharides, therefore, do not seem to be physiologically inert, and participate in stimulation or signal transduction in cell growth. The development of such a growth enhancer to increase the productivity of live-feed microalgae will be a useful additive in microalgal culture media.

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