PB - 12

Isolation of Growth Enhancer for Marine Microalga *Tetraselmis*suecica from the Seaweed Monostroma nitidum

Luyen Quoc Hai*, Ji Young Cho** and Yong Ki Hong*

- * Department of biotechnology, Pukyong National University, Busan 608-737
- ** Department of marine biotechnology, Soonchunhyang University, Asan 336-745

Introduction

The mass culture of microalgae is an important component of the mariculture industry. *T. suecica* possesses well-known nutritional qualities and is in great demand owing to its composition of protein, vitamins, pigments, and high quantities of EPA (Montaini et al., 1995 Robert et al., 2001). It is also a very mobile microalga and possesses four fragile flagella. Growth enhancer for *T. suecica* was isolated from water-soluble extracts from green alga *Monostroma nitidium*.

Materials and Methods

Algal material

M. nitidium thalli were collected in May 2002 and May 2004 in the intertidal zone from the open coast near Wando, Jeonnam, Korea. Seaweed tissue was dried completely for a week at room temperature and then ground to powder for 5 min using a grinder.

Isolation of compounds

M. nitidium powder (100 g) was extracted with 10 L of methanol three times and extracted with water. The crude extract of water was evaporated under vacuum to give a brown residue (800 mg). The extract was fractionated on a DEAE-cellulose (Pharmacia) column and reversed-phase HPLC ODS-5 column. The analysis was performed on a Waters 600 gradient liquid chromatograph monitored at 220 nm.

Analytical methods

The purified compound (5mg) was analyzed on a Jeol JNM-ECP 400 NMR spectrometer, operating at 400 and 100MHz for 1H and 13C, respectively, using methanol-d (CD3OD). IR spectra were measured on a Perkin-Elmer GX spectrophotometer by the KBr pellet method. Structures of the purified compounds were identified and confirmed to be identical to the spectral data from the Spectral Database for Organic Compounds.

Microalgal culture

The axenic Prasinophyte flagellate *Tetraselmis suecica* (CCAP-66; P-4) was cultured in f/2 medium with an initial cell density of 1.2x10⁵ cells/mL. Levoglucosan was added to the medium and cultured under 70 mmol/m²/s light intensity at 18°C for 8d. Cells were counted under a microscope with a hemocytometer.

Results

The purified active compound was analyzed on GC-MS, 1H and 13C NMR, and IR spectrometers. From these spectral data, the compound was identified as levoglucosan. When 10mM of levoglucosan was added to microalgae culture medium, it showed growth activations of 1.8-fold and 1.5-fold, *Tertaselmis suecica* and *Isochrysis glabana* respectively.

References

Cho, J.Y., H.J. Jin, H.J. Lim, J.N.C. Whyte and Y.K. Hong. 1999. Growth activation of the microalga *Isochrysis galbana* by the aqueous extract of the seaweed *Monostroma nitidum*. J. Appl. Phycol., 10, 561-567.

Montaini, E., G. Chini-Zittelli, M.R. Tredici, E. Molina-Grima, J.M. Fernandez-Sevilla and S. Sanchez-Perez. 1995. Long-term preservation of *Tetraselmis suecica*: influence of storage on viability and fatty acid profile. Aquaculture, 134, 81-90.

Robert, R., G. Parisi, L. Rodolfi, B.M. Poli and M.R. Tredici. 2001. Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostrea gigas* larvae. Aquaculture, 192, 333-346