PC-1

Axenic isolation procedure of the neutral spore and conchocelis from the seaweed *Porphyra yezoensis*

Jae-Suk Choi · Yong-Ki Hong
Department of biotechnology, Pukyong National University

Introduction

During in door or outdoor mass culture, *Porphyra* have been easily contaminated with bacteria, protozoa and microalgal species. Several axenic treatments for *Porphyra* thalli have been published (Polne-Fuller and Gibo 1984; Chen and McCracken 1993), but axenic techniques for neutral spores and conchocelis are not developed. In this work we describe the procedure for axenic isolation of neutral spores and conchocelis of *Porphyra yezoensis*

Materials and methods

① Epiphyte removement : *Porphyra yezoensis* fronds is sonicated in seawa to get rid of epiphytes for 60s at 2 times and then treated by dipping in a 1% betadine solution with 2% Triton X-100 in sterile seawater for 60s. Th chopped conchocelis was treated with a 5% Triton X-100 and with shor pulses in an ultrasonic water bath. After ultrasonication treatment for 60 seconds then conchocelis were washed by centrifugation with a 100uM mesh at 1500×g for 5 min. The algal materials were maintained in PES medium (Provasoli, 1968) under 70umol $m^{-2}s^{-1}$ light intensity (10L:14D) a 20℃.

② Antibiotic treatment : In the culture medium of neutral spores, we have added different concentrations of each antibiotics : amphotericin B, nystatin, ampicillin, gentamycin, kanamycin, neomycin, streptomycin, carbenicillin, chloramphenicol and tetracycline. Neutral spores were counted with a haemocytometer at 7 days after inoculation and dose-response curve was determined the MNLC, LC_{50}, and MLC.

Determination of the cell survival of conchocelis against several algicidal agent and antibiotics was produced same method as neutral spore experiment, except for using different algicidal agent and antibiotic : chitosan (Vanson Co), chitosan type L-40, chitosan type S, GeO_2, ampicillin, gentamycin, kanamycin, neomycin, streptomycin, carbenicillin, chloramphenicol and tetracycline.

③ Bacterial test : To count the bacterial numbers, a 500uL aliquot wa spread across the surface of Bacto marine agar (Difco Laboratories) plate Each test plate was allowed to incubate at 20℃ for 2wk to ensure slow-growing organisms also.

Results

1. Neutral spores : The MNLC of the neutral spore against amphotericin B, nystatin, ampicillin, carbenicillin, gentamycin, kanamycin, neomycin, and streptomycin were 0.12ug/mL, 7.0ug/mL, 4.0mg/mL, 2.75ug/mL, 0.04mg/mL, 0.8mg/mL, 0.1mg/mL, and 1.6mg/mL respectively. The half concentrations of MNLC for each of the 8 antibiotics had no detrimental effect on the cell growth and at the same concentration, numbers of contaminated bacteria had diminished to zero after 5d.

2. Conchochelis : To remove diatom it was the most effective to filtrate a 100uM mesh after ultrasonication treatment. The MNLC of chonchochelis against chitosan Vanson Co, Chitosan type L-40, Chitosan type S, GeO_2 ampicillin, carbenicillin, gentamycin, kanamycin, neomycin, and streptomycin were 0.015%, 0.25mg/mL, 0.1mg/mL, 0.1mg/mL, 0.01mg/mL, 0.5ug/mL, 0.01mg/mL, 0.04mg/mL, 0.005mg/mL, and 0.2 mg/mL respectively. The half concentrations of MNLC for each of the 10 antibiotics had no detrimental effect on the cell growth and at the same concentration, numbers of contaminated bacteria had diminished to zero after 6d.

References

Chen, L.C.M. and I. McCracken. 1993. An Antibiotic protocol for preparing axenic cell cultures of *Porphyra linearis*. *Bot. Mar.* 36:29-33.

Polne-Fuller, M. and A. Gibor. 1984. Developmental studies in *Porphyra* I .Blade differentiation in *Porphyra perforata* as expressed by morpholog enzymatic digestion and protoplast regeneration. *J. Phycol.* 20:609-616.

Provasoli, L. 1968. Media and prospects for cultivation of marine algae. *In* Watanabe, A. & Hattori, A. (eds) Cultures and Collections of Algae. Japanese Society of Plant Physiologists, pp. 63-75.