Primary Culture of Hemocytes From Japanese Black Abalone Nordotis discus discus

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In recent years viral diseases have caused serious problems in marine hatcheries1). Some causative viruses like yellowtail ascites virus (YAV)2) have been isolated from larval and juvenile marine fishes by established fish cell lines, however, the causative viruses of shellfishes could not be isolated due to the lack of cell lines of molluscs and crustaceans. A devastating disease named amyotrophia3) has been prevailing in juvenile abalones at several hatcheries in Japan. The etiology of the disease has not been settled, but a success in transmission experiment with a filtrate (0.22 μm membrane filter) prepared from homogenate of affected abalones indicates that a filterable agent is associated with the disease4). Isolation trials of the filterable agent were unsuccessful using established fish cell lines, RTG-2, CHSE-214 or EPC4). The purpose of this study was to prepare a primary culture of abalone cells for isolation of the filterable agent of amyotrophia. Following a past study on Bonamia ostrea in oyster5), hemocyte was aimed to primary cultures by using a modified Leiboviz’s L-15 medium6).

Materials and Methods

Apparently healthy wild Japanese black abalones Nordotis discus discus, 120 mm in average shell length, taken from western Japan Sea were used to collect hemocytes from hemolymph. The culture medium (modified L-15 medium) was prepared from a commercial L-15 medium (Sanko Junyaku) supplemented with 2.5% (w/v, final concentration) NaCl, 10% (v/v) fetal bovine serum, 1% (v/v) yeastolate solution (Gibco), 1% (v/v) lipid concentrate (Gibco), 100 μg/ml penicillin (Pentcillin, Toyama Chem., Tokyo) and 50 μg/ml kanamycin sulfate. The shell muscle of abalone was disinfected with 70% ethanol-cotton and blood vessel was cut across the pedal nerve by a sterile scalpel, and bled hemolymph was immediately suspended to the modified L-15 medium aseptically. The ratio in volume of hemolymph to the medium was approximately 1:10. The number of hemocytes were adjusted to 10^5 cells/ml using a hemocytometer and transferred to 250 ml culture flasks.

Some incubation conditions for primary culture of abalone hemocytes were investigated. The optimum concentration of NaCl was determined by culturing hemocytes in modified L-15 medium supplemented with 0.85%, 1.85%, 2.85% or 3.85% NaCl. The optimum pH of the medium was determined by comparing the cell growth at pHs 7.25, 6.65, 6.01, 5.50 and 4.80. The effect of temperature was also examined by culturing hemocytes at 15°C, 20°C and 25°C.

Results and Discussion

Approximately 10 ml of hemolymph was drawn from each healthy abalone 120 mm in average shell length and inoculated to about 20 flasks for primary culture. The hemocytes adhered to the bottom of tissue culture flasks after 3 h of incubation. Cell masses formed at first and fully spreading monolayer was observed after a few days (Fig. 1). Cultured hemocytes appeared to be fibroblastic and cell dimensions were approximately 7–10 μm x 100 μm. Cell proliferations were not observed in the hemocyte culture. Hemocytes obtained from apparently healthy abalones were usually maintained for about one month at 15°C or 20°C without renewal of the culture medium, although hemocytes from some abalones were maintained only for one week or less. It is not clear what factors were attributable to this difference in fate of the culture. It might be possible that the used abalones had already been infected with an unknown pathogen in spite of their healthy appearance, but further investigations on this problem have not been made. Both hyalinocytes and granulocytes were identified by May-Grünwald-Giemsa stain in the primary culture maintained (Fig. 2), but granulocytes were less frequently observed than hyalinocytes. It was not confirmed that

Fig. 1. Photomicrograph of a primary culture (24 h at 20°C) of Japanese black abalone hemocytes. Bar is 300 μm.
Hyalinocytes were selectively attacked by the virus or not. Investigations revealed that the optimum NaCl concentration and pH for the primary culture were 1.85–2.85% and 5.50–6.65, respectively. Among the temperature range tested (15, 20, 25°C), the culture was maintained for the longer period at the lower temperatures.

A virus was isolated from filtered homogenate samples prepared from affected abalone with amyotrophia by using this primary culture of hemocytes. The details of the virus isolation will be given elsewhere.

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References