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DEVELOPMENT OF A SUSPENSION CULTURE OF CHINOOK SALMON (*Oncorhynchus tshawytscha*) EMBRYO (CHSE-214) CELLS IN A SPINNER FLASK

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Cultivation of fish cells is generally carried out under static conditions using a flask or a dish providing the surface for cell adhesion. However, a notable report by Lidgerding (1981) described the development of suspension culture of fish cells such as chinook salmon (*Oncorhynchus tshawytscha*) embryo (CHSE-214) cells and fathead minnow (*Pimeohales promelas*) (FHM) cells, in a viscid culture medium using 0.15% carboxy methylcellulose (CMC). However, the potentiality of such cultures to support the replication of some viruses was poor.

In the present study we conducted the suspension culture of CHSE-214 cells in a spinner flask with normal culture medium in order to retain the susceptibility to viruses.

CHSE-214 cells (ATCC CRL 1681) (Nims *et al*, 1970), which had been continuously cultured with Eagle's minimum essential medium (MEM) supplemented with 5-10% fetal bovine serum (FBS) and 10 μg/ml kanamycin, and passaged over 400 times in our laboratory, were adapted to grow in Waymouth's MB 752/1 medium (Waymouth, 1959) containing 5% FBS, 0.05% $NaHCO_3$ and 10 μg/ml kanamycin adjusted to pH 7.4 by 14 mM Hepes-NaOH buffer system (Waymouth's static medium). After the adaptation to the new medium with several passages in static culture vessels for two months at 20°C, monolayers of CHSE-214 cells were dispersed by trypsin treatment and resuspended in Waymouth's MB 752/1 medium containing the same supplements described above except for $CaCl_2$ which was omitted (Waymouth's suspension medium). 200 ml of the cell suspension was placed, at 1 x 10^5 cells/ml, into a siliconized spinner flask with a rotating paddle. The suspension cultures were stirred at 60-70 rpm at 20°C. The majority of dispersed cells aggregated to form many tiny clumps. Viable cells declined gradually over three weeks. After the spinner culture for 15 days, a part of the cell suspension was transferred into a culture vessel for static incubation and propagated until the monolayer became confluent in Waymouth's static medium. The CHSE-214 cells were again suspended and cultured in spinner flask for 15 days by the same method described above. This process was repeated 5 times for the selection of cells which were adapted to suspension culture. Although this suspension culture consisted of tiny clumps and many single cells, they were able to adhere on static culture vessels and propagated as cell monolayers even in Waymouth's suspension medium. Probably the agitation by the rotating paddle releases single cells from cell clumps, because the gentle pipetting of the cell suspension in a spinner flask increases the proportion of single cells without any damage. The adapted cells in Waymouth's suspension medium in a spinner culture named CHSE-sp cells to distinguish them from the original CHSE-214 cell line.

The profiles of cell culture were examined after 6 months from the start of continuous suspension culture. When the cell suspension was seeded into static culture vessels, CHSE-sp cells adhered within a few hours and spread on the surface similar to the original CHSE-214 cells (Fig. 1-A). The cell population increased linearly up to day 30 and reached a density of approximately 1.4 x 10^6 cells/ml on day 38-40 without renewal of the culture medium (Fig. 1-B).

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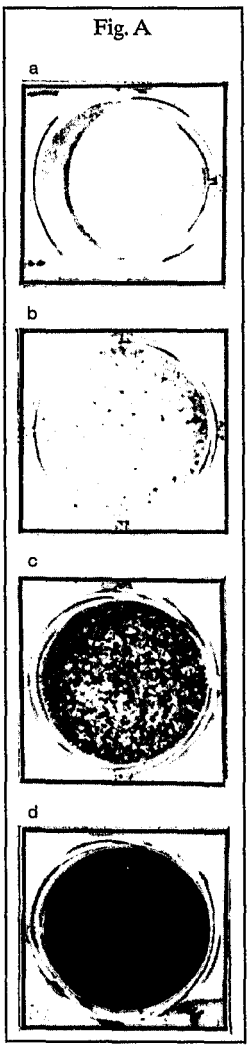
After the preparation of static cultures from suspension cells, the viral susceptibilities of CHSE-sp cells were tested with salmonid rhabdovirus and herpesvirus (IHNV and NeVTA; Sano 1976, Hasobe and Sano 1989).
Both produced cytopathic effects in the CHSE-sp cells similar to the original CHSE-214 cells.



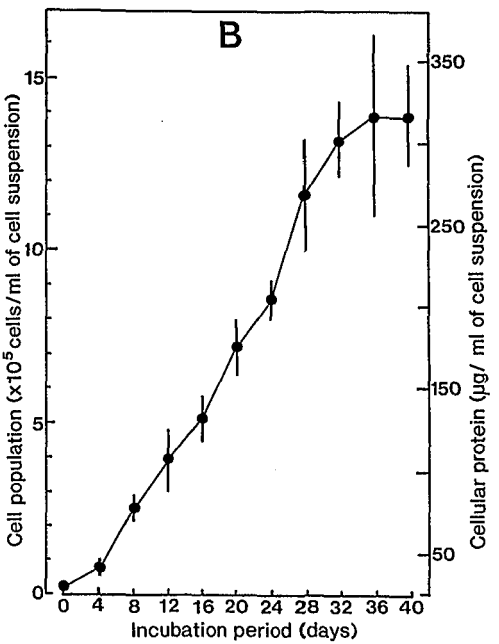


Fig. 1. 250 ml of dispersed CHSE-sp cells, approximately 1 x 10^4 cells/ml, were placed in two spinner flasks and the culture was stirred at 70 rpm in new Waymouth's suspension medium at 20°C.
After cultivation for 14 days, cell suspensions in two flasks were mixed and continuously cultured in one flask. At the indicated periods, two sets of static cultures were prepared by inoculating 0.5 ml of the cell suspension in a 1.5 cm dish and 2.0 ml in a 3.5 cm dish and then incubating for 6 hours at 20°C.
The cultures in these dishes were rinsed with phosphate buffer saline and stained with 0.1% crystal violet for small dishes or harvested for the determination of cell numbers with 500 µl of 0.1 N NaOH containing 0.2% Na_2CO_3 for large dishes. A: The cell monolayers (small dishes) from suspended CHSE-sp cells, incubated in the spinner flask for 0 day (a), 4 days (b), 10 days (c) and 20 days (d). B: Growth curve of CHSE-sp cells in the spinner flask. Cell populations per ml were evaluated from a linear correlation between cell numbers and cellular protein measured by the Bradford's method (1976). Bars represent the maximum and minimum values of cell numbers or cellular protein, and figures are averages of 4 dishes before day 14 and 2 dishes after day 14.

Summary

The cultivation of CHSE-sp in suspension culture was developed by a three-step procedure 1) the adaptation to Waymouth's MB 752/1 medium; 2) the selection of adapted cells by repeated suspension-static cultures; and 3) cell proliferation in the suspended condition. Culture has been continuously performed for 8 months in a spinner flask. The routine maintenance of this culture of CHSE-sp cells simply required medium renewal, the dilution of cell density and pipetting the cell suspension at adequate periods in order to disperse the cell clumps. Further study of the cell characteristics and possible applications of CHSE-sp cells are now in progress.

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