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Development of primary culture from heart tissue of *Chitala chitala* (Hamilton-Buchanan)

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ABSTRACT

A method for developing cell cultures from *Chitala chitala* heart tissue is described. The tissues were minced and seeded in culture flasks and grown in Leibovitz L-15 medium supplemented with 20% foetal bovine serum. The radiation started after 4th day in heart. The medium was changed after every five days. The radiations were able to withstand a wide range of temperatures from 25 °C to 28°C with an optimum temperature of 28°C. A partial monolayer was observed after 12 days in heart tissue. A primary culture was successfully obtained from heart tissue of *C. chitala* for the first time in India.

Key words: Chital, Heart, Primary culture

Ever since, the first cell line was developed from rainbow trout over 42 years ago (Wolf and Quimby 1969, Wolf and Ahne 1982), several short-term and continuous cell cultures from a variety of fish species have been developed (Nicholson 1988) and applied extensively in virological, toxicological and cytogenetic studies (Chen *et al.* 1986). It also offers the best potential source of chromosome preparation for the application of banding techniques (Blaxhall 1983).

Most of the established cell lines have been derived from cold water fish of European origin (Lakra and Bhonde 1996). Successful primary cultures from a variety of tissues from Indian major carp (Sathe *et al.* 1995, Lakra and Bhonde 1996, Sathe *et al.* 1997 Rathore *et al.* 2001, 2007), catfish (*Heteropneustes fossilis*; Singh *et al.* 1995) and mahseer (*Tor putitora*) were developed (Lakra *et al.* 2005). Cell lines from *Lates calcarifer* (Lakra *et al.* 2006, Hameed *et al.* 2006, Parameswaran *et al.* 2006) and characterization of 2 new cell lines from milkfish (*Chanos chanos*) and grouper (*Epinephelus coioides*) (Parameswaran *et al.* 2007) and a new cell line from heart muscle of *Catla catla* (Ishaq Ahmad *et al.* 2009) were reported.

The endangered featherback *Chitala chitala*, a game and food fish of commercial importance, is widely distributed in freshwaters of Ganges, Brahmaputra and Mahanadi river basins in India (Day 1878, Jayaram 1981, Froese and Pauly 2003). The species is fast depleting in natural waters. Recent success in captive breeding (Sarkar *et al.* 2006) has shown a great potential for its aquaculture in India. The lack of cell lines from *Chitala chitala* restricts the *in vitro* investigation on genetics and virology. Its cell line development will open new vistas of *in vitro* research in genetics and conservation biology of this prized fish which has also been adopted as the state fish of Uttar Pradesh. The present investigation deals with successful development of primary culture from heart tissues of *Chitala chitala* for the first time in India.

MATERIALS AND METHODS

To develop primary culture, the live fishes (fry and fingerlings) were collected from the Fish farm at Malda (West Bengal) and maintained at the National Bureau of Fish Genetic Resources (NBFGR), Lucknow. Fishes of around 10–15 g were transported live to laboratory and maintained in sterile, aerated water containing 1000IU/ml penicillin and 1000µg/ml streptomycin for 24 h at room temperature (25–28°C). Before experimentation, the fish were anaesthetized in ice-cold water, dipped in 5% cholerx for 5 min and wiped with 70% alcohol. The heart tissues were taken aseptically and washed 3 times in phosphate buffered saline (PBS) containing 500 IU/ml penicillin and 500µg/ml streptomycin and 2.5 mg/ml fungi zone. The tissues were minced into small pieces and seeded into 25 cm^2 cell culture flask with 50µl

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fetal bovine serum (FBS) and allowed to attach to the surface of the flask in an incubator at 28°C. Care was taken to avoid over drying of the tissue. After ascertaining tissue adherence, the explants were fed with L-15 medium culture media supplemented with 20% fetal bovine serum (FBS). The explants were then allowed to grow at 25–28°C in the incubator.

RESULTS AND DISCUSSION

Attempts to produce cultures using the explant method had limited success. There was attachment or outgrowth from chital heart which was a greater success but none of these outgrowths ever produced a confluent culture and eventually cell death occurred. Microscopic observations were carried out everyday under an inverted microscope. In all instances within in 24 h the explants got attached in the culture flasks. The radiation started after fourth day in heart (Fig. 1a). The medium was changed after every 5 days. Large number of cells emerged around the periphery of the explants.

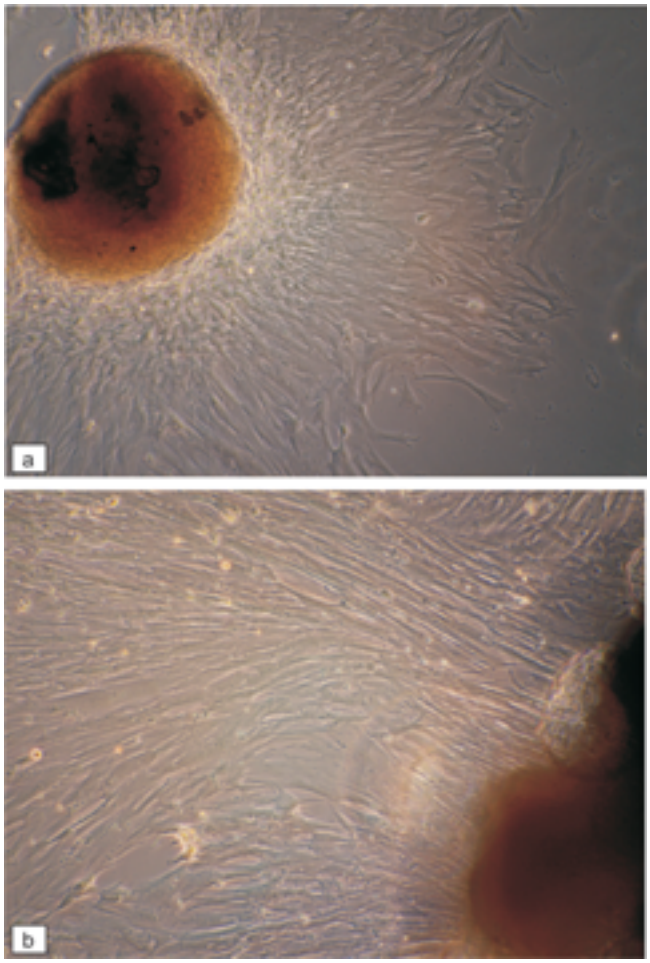

Fig. 1 a–b **1a.** Phase-contrast photomicrograph of radiated cells of heart explants of *Chitala chitala* (200X). **1b**. Phase-contrast photomicrograph of confluent cells of heart explants of *Chitala chitala* (a, b) (200X).

Microscopically, the cells were predominantly fibroblastic with a few epithelial like cells in heart. The widespread cell growth continued till it extended to the periphery of the neighbouring explants. A confluent monolayer around the explants was observed after 12 days in heart (Fig. 1. b). These cells multiplied and a good monolayer, comprising mostly of fibroblast like cells were obtained. A confluent monolayer comprising mainly of fibroblast like cells with a sharp and clear outline developed in a 12 day- period. After obtaining the monolayer, cells were passaged to new flasks by trypsinization. In the second phase of the experiment, cells were passaged to new flasks by trypsinization. The old medium was removed and replaced with 1 ml of 0.025% trypsin EDTA in PBS (pH 7.2) twice to detach the cells from the surface and washed with PBS (pH 7.2). Pellets were re-suspended in L-15 media into 25 cm^2 cell culture flask. The trypsinized cells attached to flasks in clumps and individually, retaining the original fibroblast like shape. Cells were in good condition for about a week, after which they died. The best growth was observed in the L-15 medium with 20% FBS. Fernandez *et al.* (1993) documented suitability of L-15 in supporting fish cell lines compared to that of other media.

Most of the previous workers preferred L-15 medium to develop cell cultures from fish and the results obtained using Leibovitz's L-15 for developing cell culture from chitala were in conformity with these workers (Lai *et al.* 2000, Kumar *et al.* 2001, Lai *et al.* 2003, Lakra *et al.* 2006, Hameed *et al.* 2006, Ye *et al.* 2006, Qin *et al.* 2006). The flasks were incubated at 25, 28 and 30°C to determine optimal growth temperature. The optimum growth temperature was 28°C. This corroborates the observations of Alvarez *et al.* (1991) who also reported the optimum *in vitro* growth temperature a few degrees above the preferred environmental level for live fish. Wolf and Ahne (1982) suggested that the difference between *in-vivo* and *in-vitro* temperature can be due to selection or adaptation of cells to growth of higher than normal temperature. In general, growth and development of cell monolayer from heart tissue explants were good and easy to maintain. Development of a cell line from *Chitala chitala* will open new vistas for research in conservation, genetics and management of fishery of this important species.

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