



Development of primary culture from gills of *Tor tor* (Hamilton-buchanan)

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ABSTRACT

Primary culture of gill cells was initiated from gill filament explants of game fish, *Tor tor*. The explants were cultured in Leibovitz L-15 medium with 5, 10 and 20% foetal bovine serum (FBS). The attachment efficiency of cells was serum-dependent, while increased FBS concentration did not stimulate further proliferation of cells. First radiation of typical gill epithelial cells was observed after 38 h, of attachment of explants. There was high proliferation for the first 7 days then a stable plateau till 10 days, followed by a decline phase from 14th day. Following removal of cells, the explants produced further cell outgrowth which was especially active at the proliferation phase (10 days). Removal of these cells caused the explants to produce a further proliferation of cells reaching confluency in 4–10 days. The use of gill explants to establish cell culture system of fish has advantages which include longevity of the culture and successive proliferations from explants, which could provide a useful tool for the investigation of toxicological and diagnostic studies.

Key words: Gill, Primary culture, *Tor tor*

The availability of fish cell lines, since the 1960s, has begun to make impacts in scientific research, but at a much slower rate than with mammalian cell lines. Earlier, work with fish cell lines was initiated with RTG-2, a gonadal cell line derived from rainbow trout (Wolf and Quimby 1962), mainly for virological studies. Some of the areas in which fish cell lines have made significant contributions are fish immunology (Clem *et al.* 1996, Bols *et al.* 2001), toxicology (Babich and Borenfreund 1991, Segner 1998), ecotoxicology (Fent 2001, Castano *et al.* 2003, Schirmer 2006), endocrinology (Bols and Lee 1991), virology (Wolf 1988), biomedical research (Hightower and Renfrow 1988), disease control (Villena 2003), biotechnology and aquaculture (Bols 1991), and radiation biology (Ryan *et al.* 2008).

The gills of aquatic organisms are involved in gas exchange, osmoregulation and other critical functions (Evans 2005) essential for survival of piscine and invertebrate species. Thus, damage to gills may reflect in impaired

functioning of the organism and eventual death. Studies of gill function have traditionally been performed *in vivo* in experimental animals. However, recent advancements in gill cell culture provide an alternate model system to study the gill (Fernandes *et al.* 1995, Wood and Part 1997, Sandbacka *et al.* 1999, Fletcher *et al.* 2000, Wood *et al.* 2002, Leguen *et al.* 2007). Till date, only 12 gill cell lines are reported from different fish species. All these cell lines have been initiated from normal gill tissue except FG-9307 which underwent *in-vitro* spontaneous neoplastic transformation (Guo *et al.* 2003).

In this paper, we describe a method of developing primary culture of epithelial cells from *T. tor* gills. Development of a cell culture system from *T. tor* will open new vistas of *in vitro* research in fish genetics and conservation.

MATERIALS AND METHODS

To develop primary culture, live *T. tor* fish (fry and fingerlings) were collected from the Narmada river, Hoshangabad (Madhya Pradesh) and were maintained at NBFGR, Lucknow. Live fingerlings (15–20g) were maintained in sterile, aerated water containing 1000IU/ml penicillin and 1000µg/ml streptomycin for 24 h at room temperature. Before experimentation, the fish were anaesthetized in ice-cold water, dipped in 5% chloerx for 5 min and wiped with 70% alcohol. The gill was taken out

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aseptically and washed with 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 cm² cell culture flasks with 50µl fetal bovine serum (FBS) and allowed to attach to the surface of the flask. The flasks were incubated at 28 °C after 24 h. L-15 medium supplemented with different concentration of serum (10, 15, 20%) was added and the medium was changed after every 5 days.

RESULTS AND DISCUSSION

Gills are unique organ involved in respiration and osmoregulation in piscinids as well as in many aquatic invertebrates. The availability of the trout-derived gill cell line, RTgill-W1, is the beginning to make impacts in fish health and toxicology (Bol *et al.* 1994). Primary gill cultures have been used for the evaluation of aquatic toxicants (Lilius *et al.* 1995, Part 1995, Sandbacka *et al.* 1999; Wood *et al.* 2002). In the present study, primary cell culture system was developed from gill tissue of *T. tor* for the first time.

The flasks were observed daily for attachment of explants, proliferation and spreading of cells and other morphological details using an inverted microscope (Olympus). All the explants were found to be attached properly after 18–24 h of explant preparation. The cells started showing radiation on fifth day of attachment whereas Lakra *et al.* 2006 (a) reported that caudal fin explant of *Lates calcarifer* showed cell outgrowth on 10th day. However the cell proliferation was higher during the first 7 days, the cells showed a stable plateau till 10 days followed by a steep declination phase from 14 days onwards. The medium was changed after an interval of 5 days. Large number of cells emerged around the periphery of the explants. The majority of cells were fairly heterogeneous in nature (Fig.A).

Heterogeneous nature of cells is reported during initial cell cultures (Lakra *et al.* 2010, Parameswaran *et al.* 2006). Following the change of medium, scattered colonies of cells as well as single cells remained attached to the dishes. 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 of explant preparation. Parameswaran *et al.* (2006) reported formation of monolayer around explant after 15 days in sea bass (*Lates calcarifer*). Upon reaching confluency, the cells lost their original shape and become flat with an irregular polygonal shape (Fig. B). During subculture, the trypsinized cells attached to flasks in clumps and individually, retaining the original epithelial like shape. Cells were in good condition for about a week, after which they could not survive. The best growth was observed in the L-15 medium with 20% FBS, which showed conformity with Lakra *et al.* (2006b). Suitability of L-15 in supporting growth of fish cell lines compared to that of other media has been reported by many researchers (Fernandez *et al.* 1993, Lai *et al.* 2000, Kumar *et*

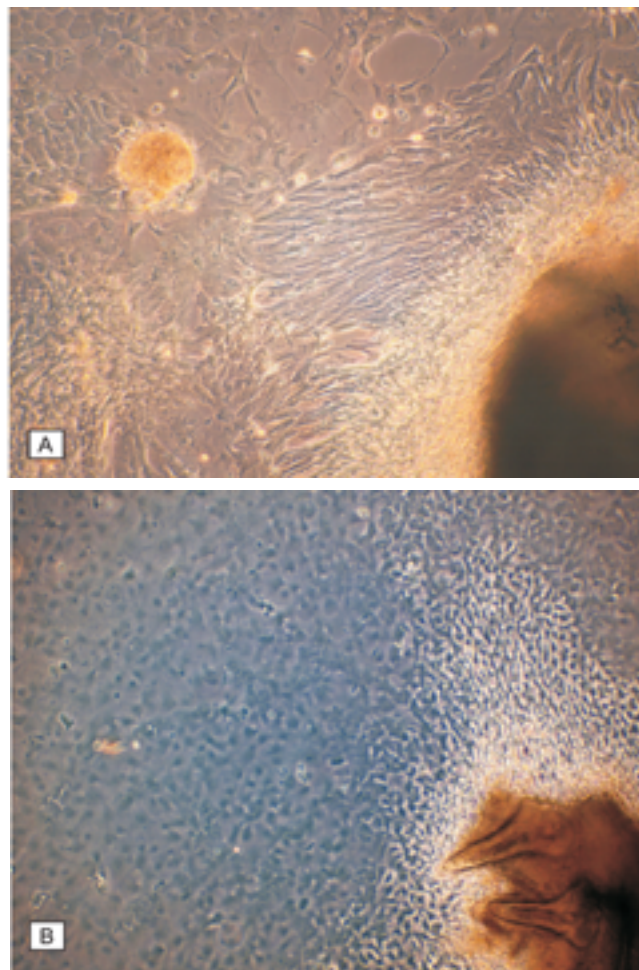


Fig. A–B. **A.** Cell radiation surrounding explant of *T. tor* (200×). **B.** Confluency of cells surrounding explant of *Tor tor* (200 ×).

al. 2001, Lai *et al.* 2003, Lakra *et al.* 2006b, Hameed *et al.* 2006, Ye *et al.* 2006, Qin *et al.* 2006). Flasks were incubated at 25, 28 and 30°C to determine optimal temperature for growth. The optimum growth temperature was 28°C, which was in conformity with other fish cell lines reported earlier (Tong *et al.* 1997, Lakra *et al.* 2006b).

In general, growth and development of cell culture system from gill tissue were successfully reported but the difficulty of their isolation, maintenance and reproducibility makes them cumbersome to use. The development of gill cell line from *T. tor* has not yet been reported so far. Thereafter development of a cell line from *T. tor* will open new vistas of *in vitro* research in fish genetics and conservation. Attempt to established cell line from gill primary culture of *T. tor* is under progress.

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