Development of a cell culture system from gill explants of the grouper *Epinephelus malabaricus* (Bloch and Shneider)

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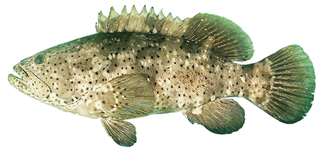
Background

In vitro cell culture systems are necessary for the isolation and characterization of viruses, development of diagnostic reagents, testing of therapeutics and for the production of material for immunological and vaccination studies. Fish cell lines have also found widespread application in cytogenetics; as *in vitro* models for studying cellular and physiological processes and also to evaluate the toxicity of pollutants.

With the development of mariculture activities in different parts of the world, diseases due to viral etiology, such as the viral nerval necrosis virus and iridoviruses have been reported. It is essential to develop suitable cell lines for the isolation and study of viral diseases affecting marine fish species for developing proper diagnostics and vaccines. In India successful marine fish cell lines/cell culture systems have been developed only from the sea bass, *Lates calcarifer* (Sahul Hameed *et al.*, 2006; Lakra *et al.*, 2006; Parameswaran *et al.*, 2006).

The present study describes the development of a successful cell culture system from the gill explants of *Epinephelus malabaricus.*



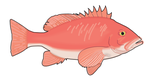
The Malabar grouper, ***Epinephelus malabaricus***

Methods

Preparation of fish and tissue removal

Healthy juveniles of *E. malabaricus* (average weight 62 ± 5 g), collected from the coastal waters of Cochin were used for developing primary cell culture. Fishes were acclimatized for a period of two weeks on a diet of marine shrimp/fish meat, in circular fibre glass tanks having *in situ* biological filtration system and holding 300 l of well aerated and dechlorinated sea water of 30 - 32 ‰ salinity. The fishes were subsequently transferred to rectangular perspex tanks holding 50 l of well aerated and dechlorinated sea water (30 ‰).

Before dissecting out the tissues for primary culture, the fishes were starved for two days and maintained overnight in sterile, aerated seawater containing 1000 IU ml^{-1} penicillin and 1000 µg ml^{-1} streptomycin. Prior to sacrifice, the fishes were tranquilized by plunging in iced water, then disinfected in sodium hypochlorite (500 ppm available chlorine) for 5 min, washed in sterile sea water and swabbed with 70 % ethyl alcohol. The gill tissue was aseptically excised and collected in sterile petridishes holding Leibovitz' L-15 medium (serum free) containing 500 IU ml^{-1} penicillin and 500 µg ml^{-1} streptomycin. The tissues were then washed three times in serum free medium containing 500 IU ml^{-1} penicillin and 500 µg ml^{-1} streptomycin. Tissue pieces were minced in to small fragments of approximately 1 mm^3 size using a sterile surgical scalpel and again washed thrice in serum free medium containing 500 IU ml^{-1} penicillin, 500 µg ml^{-1} streptomycin and 1.25 µg ml^{-1} amphotericin B.



Explantation

The tissue pieces were resuspended in about 2 ml of growth medium containing 20% FBS and 200 IU ml^{-1} penicillin, 200 µg ml^{-1} streptomycin and 0.25 µg ml^{-1} amphotericin B and subsequently transferred to 25 cm^2 tissue culture flasks and distributed uniformly. Excess medium was pipetted out and the flasks were incubated in semi-dried condition for 4 – 5 h at 28 ± 2 °C. L-15 medium (pH 7.2 ± 0.2) supplemented with 20% FBS, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 0.25 µg ml^{-1} amphotericin B was added to the flasks and incubated at 28 ± 2 °C. After 24 h the medium was replaced with fresh L-15 containing 20% FBS and antibiotic-antimycotic solution and incubated at 28 ± 2 °C. The tissue explants were observed for growth and formation of monolayer of cells using an inverted microscope .

Subculture and maintenance

When confluent monolayers were formed in primary culture, cells were dislodged from the flask surface by treatment with of 0.25% trypsin (0.25% trypsin and 0.2% EDTA in PBS). Two milliliter of fresh growth medium was then added to neutralize the action of trypsin. The cells were then split in to two portions, transferred to new culture flasks and incubated at 28 ± 2 °C.

Results

Explants of gill tissue readily got attached to the culture flask on incubation. Primary cultures initiated from gill explants showed promising results. Emergence of different types of cells from the attached gill explants was observed with in a day (Fig. 1). Cells were observed to spread and attach to the culture flask from the 2nd day onwards (Fig. 2).

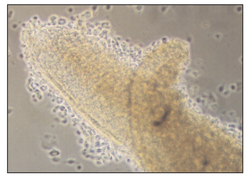


Fig. 1. Cells emerging from the gill explant of *E. malabaricus* (X100)

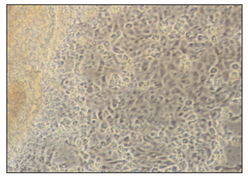


Fig. 2. Spreading and attaching cells from the gill explant of *E. malabaricus* (X100)

Growth of the cells was very fast and the cells formed a confluent monolayer within one week (Figs. 3 & 4).

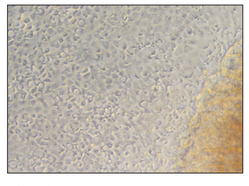


Fig. 3. Confluent monolayer formed along with the explant (X100)

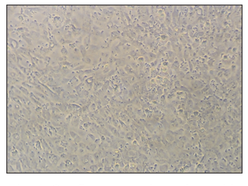


Fig. 4. Confluent monolayer formed in the primary culture (X100)

Trypsinisation to detach the monolayer yielded individual cells along with cell clumps. The subcultured cells attached well to the flask (Fig. 5) and formed monolayer consisting of epithelioid and fibroblast-like cells (Figs. 6 & 7).

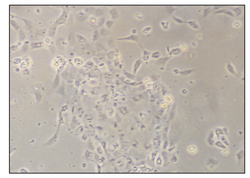


Fig. 5. Attachment and formation of monolayer by the subcultured gill cells in the 1st passage (X100)

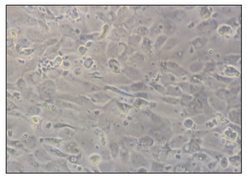


Fig. 6. Complete monolayer formed by the subcultured gill cells in the 1st passage (X200)

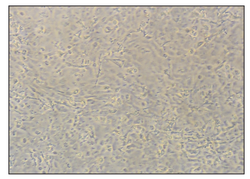


Fig. 7. Complete monolayer formed by the subcultured gill cells in the 1st passage (X200)

The cell monolayers could be successfully harvested for passage by trypsinization and produced confluent monolayers comprising predominantly epithelioid like cells in subsequent subcultures (Figs. 8, 9, 10 & 11).

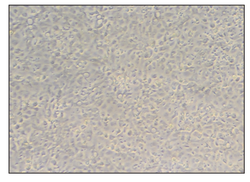


Fig. 8. Confluent monolayer comprising neatly packed, uniform epithelioid like cells in the 2nd passage (X100)

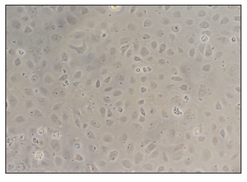


Fig. 9. Confluent monolayer comprising neatly packed, uniform epithelioid like cells in the 2nd passage (X200)

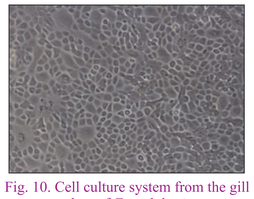


Fig. 10. Cell culture system from the gill explant of *E. malabaricus* at 4th passage (X200)

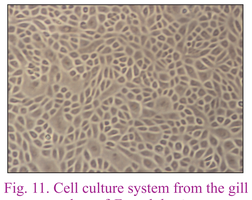


Fig. 11. Cell culture system from the gill explant of *E. malabaricus* at 6th passage (X200)

Conclusion

The results of the present study have clearly demonstrated good growth and formation of confluent monolayer of cells from gill tissue explants of *E. malabaricus,* which has been successfully subcultured. Gill tissue appears to be ideal for cell culture, as it is easy to collect aseptically. Hence there is scope and prospect for development of cell line from gill tissue of *E. malabaricus*.

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