DEVELOPMENT OF CELL CULTURE FROM CAUDAL FIN AND HEART OF TOR TOR (HAMILTON-BUCHANAN)

Kamalendra[1], W. S. Lakra[2], J. Sharma[3], M. Goswami[1] and B. S. Sharma[1]

[1] *National Bureau of Fish Genetic Resources (NBFGR), Lucknow (UP) 226002*

[2]*Central Institue of Fisheries Education,Versova, Andheri (W),Mumbai 400061*

e-mail: wslakra@gmail.com

[3]*Dept. of Biotechnology, Kurukshetra University, Kurukshetra (Haryana)*

ABSTRACT

Tor mahseer *(Tor tor),* possess high commercial and recreational value as they are potential game as well as food fish of India. Two cell culture systems were developed from fin and heart of *T. tor* (Hamilton-Buchanan). The explants excised aseptically from fingerling of *T. tor* were cultured in Leibovitz-15 (L-15) medium with 20% fetal bovine serum (FBS). Radiation of cells started after 72 hours and 48 hours of explant attachment from caudal fin and heart respectively. Confluent monolayer of cells with heterogeneous morphology around fin explants was observed after 7-10 days, where as a homogenous confluent layer of fibroblastic cells from heart explant was observed after 12-13 days. The establishment of cell culture systems from different organs and tissues of commercial important species would facilitates *in vitro* research.

Key words: Cell culture, Caudal fin, Heart, *Tor tor.*

INTRODUCTION

In-vitro cell culture system has been an invaluable tool in many areas of biological research. These experimental systems allow direct access and evaluation of specific functions with higher control of the conditions of assays, reducing variability of responses due to unavoidable stress responses. Since the first report of a fish cell line (Wolf and Quimby, 1962), many short-term and continuous cell cultures from a variety of fish species have been developed. Among the cell lines listed, more than 60% were established from Asian species, which contribute more than 80% of total fish production. This includes 59 cell lines from 19 freshwater, 54 from 22 marine and 11 from 3 brackish water fishes. Presently, about 283 cell lines have been established from finfish around the world (Lakra *et al.,* 2010b). Some of the areas in which fish cell lines have made significant contributions are fish immunology (Clem *et al.,* 1996; Bols *et al.,* 2001), 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).

Most of the cell lines have been developed from temperate fishes in the past (Fryer and Lannon, 1994) except some recent reports on *Tor putitora* (Lakra *et al.,* 2006a), *Etroplus suratensis* (Swaminathan *et al.,* 2010), *Epinephelus coioides* and *Chanos chanos* (Parameswaran *et al.,* 2007), *Lates calcarifer* (Lakra *et al.,* 2006b; Parameswaran *et al.,* 2006), *Labeo rohita* (Lakra *et al.,* 2010a), *Puntius densonii* (Lakra *et al.,* 2010b), Puntius *sophore* (Lakra and Goswami, 2011).

Tor tor commonly called as Tor mahseer in India, belonging to the Family Cyprinidae are described as the 'King of Indian freshwater systems'. Mahseer possess high commercial and recreational value as they are potential game as well as food fish. About 20 species are currently recognized within the genus, occurring throughout Asia from the trans-Himalayan Region to the Mekong River Basin to Malaysia, Pakistan, Bangladesh and Indonesia. Over-exploitation of the natural stocks and the deterioration of environmental conditions have resulted in significant decline of mahseers in the wild (Ogale, 2002). The present study reports the development of cell culture systems from fin and heart of *T. tor.*

MATERIALS AND METHODS

Experimental fish

Fingerlings of *T. tor* were collected from the Narmada River, Hoshangabad (M.P.) and were maintained at the National Bureau of Fish Genetic Resources (NBFGR), Lucknow. Live fingerlings (15-20g) were maintained in sterile, aerated water containing 1000IU/ml penicillin and 1000µg/ml streptomycin for 24 hours at room temperature before explant preparation.

Establishment of primary culture

The fish were anaesthetized in ice-cold water, dipped in 5% cholerx for 5 minutes and wiped with 70% alcohol before explant preparation. The fin and heart were taken out aseptically and washed with PBS containing 500IU/ml penicillin and 500µg/ml streptomycin and 2.5mg/ml fungi zone. The tissues were minced into small pieces and seeded into 25cm^2 cell culture flasks with 50µL fetal bovine serum (FBS) and allowed to get attached to the surface of the flask. The flasks were incubated at 28°C for 24 hrs in L-15 medium supplemented with 20% of serum .The medium was changed after an interval of 5 days.

Subculture and maintenance

The flasks were observed daily for attachment of explants, spreading and proliferation of cells, morphological details using an inverted microscope (Olympus Optical Co., Ltd., Tokyo, Japan). Upon reaching 90%-95% confluency, the cells were trypsinized using TPVG solution (0.1%trypsin, 0.2% ethylenediaminetetraacetic acid, EDTA, and 2% glucose in 1× PBS). The subcultured cells were grown in fresh L-15 with 20% FBS. In the initial subcultures, 50% of the culture medium was replaced with the fresh medium.

Growth studies

To check the optimum condition for cell growth, cells were grown at different temperatures and FBS concentrations in L-15 media. To determine the optimum temperature the flasks were incubated at 24, 28, and 32°C over 7 days at seeding concentration of 1x10^5 cells in 25 cm^2 tissue culture flasks. On alternate days, two flasks from different temperatures at which they were incubated, were trypsinized and cell counting was performed using a haemocytometer. Analogous procedures were performed for the effects of various concentrations of FBS (10, 15, and 20%) on cell growth at 28°C over 7 days.

RESULTS AND DISCUSSION

Cell cultures were developed from the caudal fin and heart tissue of *T. tor* using explant technique (Fig 1A and 1B). All the explants prepared from fin and heart explants

were found to be attached properly after 18-24 hrs of explant preparation. The radiation of cells started after 72 hours of explant preparation from fin and subsequently after 7-10 days a confluent monolayer around the explants was observed. The majority of the cells proliferating from fin explant were fairly heterogeneous in nature, *i.e.,* consist of both epithelial and fibroblastic cells (Fig 1A). Heterogeneous nature of cells has been reported by many researchers during early cell cultures (Lakra et al., 2010a; Parameswaran *et al.,* 2006).

Cells of heart explants started showing radiation after 48 hours of attachment and a confluent monolayer comprising mainly of fibroblast-like cells with a sharp and clear outline from heart explant was observed within 12-13 days. In addition, prominent heart beat was observed in heart explants, though the frequency of beating declined gradually with the culture time and ultimately stopped after 35 days. The cells radiating from heart explants were polygonal and homogenous in nature with fibroblast-like morphology (Fig 2A). Similarly, primary culture developed from heart explants of Indian major carps comprised mainly of fibroblast-like cells (Rao *et al.*1997). Lai *et al.* (2000) and Wang *et al.* (2010) also reported fibroblast-like cells from heart tissue of *Epinephelus awoara* and *Cynoglossus semilaevis* respectively.

During initial subculture from fin explants, mixed population of both epithelial and fibroblastic cells were present (Fig 1B). Population of fibroblastic cells dominates over epithelial cells resulting in homogenous population of fibroblastic cells during subsequent passages (Fig 1C). Predominance of fibroblastic cells over epithelioid cells in cell cultures from fish has been reported by many researchers also (Bejar *et al.,* 1997; Lai *et al.* 2003, Lakra *et al.,* 2006a). Ye *et al.* (2006) developed a fibroblast like cell line (LJH-2) from Lateolabrax japonicus. The cell culture system developed from fin tissue has been successfully subcultured upto 15th passage.

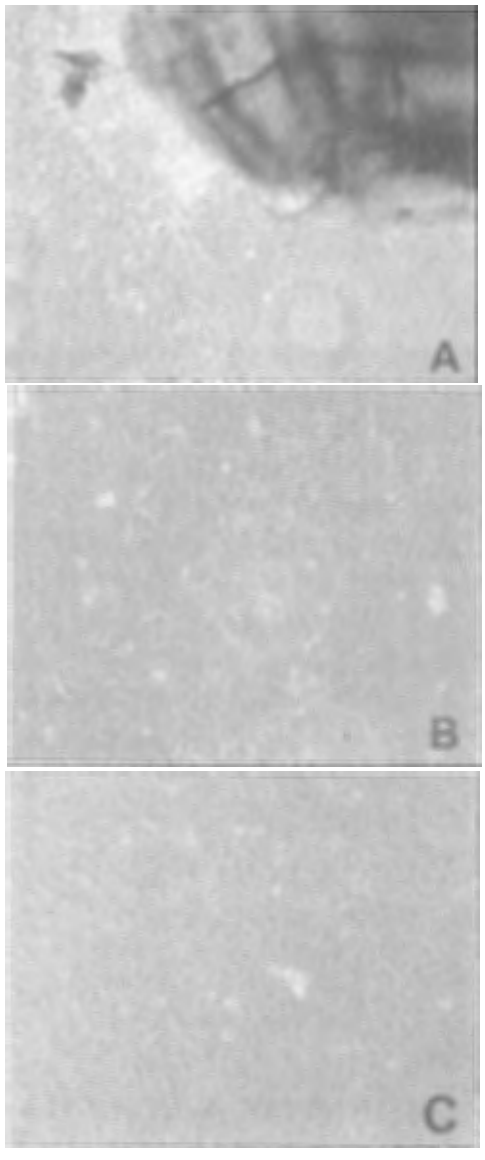

Fig 1: Phase-contrast photomicrographs of TTCF cells derived from the caudal fin of *T. tor (100x).*

(A) Confluent monolayer around the explants within 7-10 days.

(B) Heterogeneous nature of cells during initial subcultures and

(C) subcultured cells at passage 14.

The cells of heart explant retained their fibroblast-like morphology after subculturing. Similarly Lai *et al.,* (2003) and Wang *et al.,* (2010) reported fibroblast like cells from heart tissue of *Epinephelus awoara* and *Cynoglossus semilaevis* respectively. In contrast, SPH cells (Sea perch, heart) migrating from heart tissue has been reported to be epitheloid in morphology with no change during successive propagation (Tong *et al.,* 1998). The cell culture system developed from heart tissue has been successfully subcultured upto 13th passage.

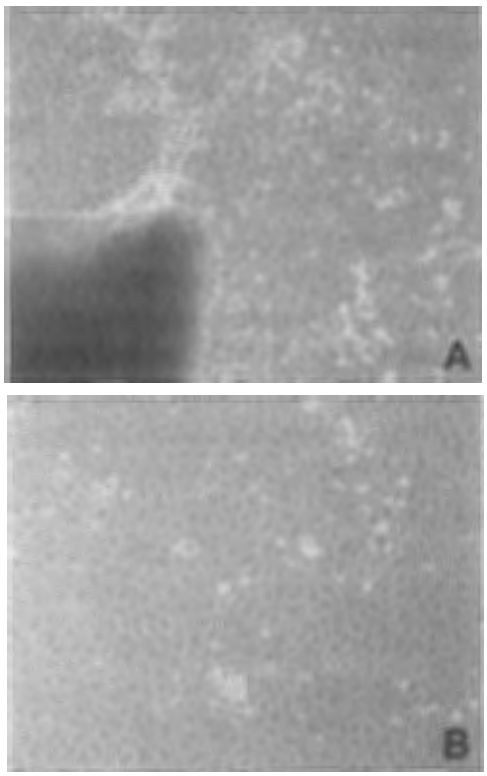

Fig 2: Phase-contrast photomicrographs of TTH cells derived from the heart tissue of *T. tor (100x).*

(A) Confluent monolayer around the explants within 7-10 days and

(B) Subcultured cells at passage 10.

The best growth was observed in the L-15 medium with 20% FBS which showed conformity with Lakra *et al.,* 2006b. Many researchers also reported about the suitability of L-15 for fish cell lines in comparison to that of other media (Fernandez *et al.*, 1993 Lai *et al.*, 2000; Kumar *et al.*, 2001; Lai *et al.*, 2003; Lakra *et al.*, 2006b; Hameed *et al.*, 2006; Ye *et al.*, 2006; Qin *et al.*, 2006), as L-15 is designed to maintain pH in the physiological range under normal atmospheric conditions without added CO_2. Faster growth and better proliferation was noticed in cell culture system with L-15 medium at pH 7.4. The optimum growth temperature was found to be 28^{0}C, which was in conformity with other fish cell lines reported earlier (Tong *et al.*, 1997; Lakra *et al.*, 2006b).

The results of the present study have clearly demonstrated the potential of the cell culture system in developing continuous cell line of *T. tor*. The development of cell line from fin and heart of *T. tor* has not yet been reported so far. Hence, the development of a cell line from *T. tor* will open new vistas of *in vitro* research in fish biotechnology and conservation.

ACKNOWLEDGMENTS

The authors are grateful to Dr. J. K. Jena, Director, National Bureau of Fish Genetic Resources, Lucknow for his support and encouragement. The Department of Biotechnology, Government of India is thankfully acknowledged for financial support.

REFERENCES

Bejar, J., Borrego, J. J. and Alvarez, M. C., 1997. A continuous cell line from the cultured marine fish gilt-head sea bream (*Sparus aurata*). *Aquaculture* 150:143153.

Bols, N. C., 1991. Biotechnology and aquaculture: the role of cell cultures. *Biotechnol. Adv.* 9: 3149.

Bols, N. C., Brubacher, J. L., Ganassin, R.C. and Lee, L. E., 2001. J. Ecotoxicology and innate immunity in fish. *Dev. Comp. Immunol.* 25: 853873.

Bols, N. C. and Lee, L. E. J., 1991. Technology and uses of cell cultures from the tissues and organs of bony fish. *Cytotechnol.* 6: 163187.

Castano, A., Bols, N. C., Braunbeck, T., Dierickx, P., Halder, M., Isomaa, B., Kawahara, K., Lee, L. E. J., Mothersill, C., Part, P., Repetto, G., Riego Sintes, J., Rufli, H., Smith, R., Wood, C. and Segner, H., 2003. The use of fish cells in ecotoxicology. *ATLA.* 31:317351.

Clem, L. W., Bly, J. E., Wilson, M., Chinchar, V. G., Stuge, T., Barker, K., Luft, C., Rycyzyn, M., Hogan, R. J., Van Lopik, T. and Miller, N. W., 1996. Fish immunology: the utility of immortalized lymphoid cellsa mini review. *Vet. Immunol. Immunopathol.* 54: 137144.

Fent, K., 2001. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicol. In Vitro.* 15: 477488.

Fernandez, R. D., Yoshimizu, M., Ezura, Y., and Kimura, T., 1993. Comparative growth response of fish cell lines in different media, temperatures and sodium chloride concentrations. *Gyobyo Kenkyu* 28(1): 27-34.

Fryer, J. L. and Lannan, C. N., 1994 Three decades of fish cell culture: a current listing of cell lines derived from fish. *J Tissue Culture Methods* 16:8794.

Hameed, S. S., Parameswaran, A. S., Shukla, V., Bright Singh, I. S., Thirunavukkarasu, A. R. and Bhonde, R. R., 2006. Establishment and characterization of India's first marine fish cell line (SISK) from the kidney of sea bass (*Lates calcarifer*). *Aquaculture* 257: 92103.

Hightower, L. E. and Renfrow, J. L., 1988. Recent applications of fish cell culture to biomedical research. *J. Exp. Zool.* 248(3): 290302.

Kumar, G. S., Bright Singh, I. S. and Philip, R., 2001. Development of a cell culture system from the ovarian tissue of African catfish (*Clarias gariepinus*). *Aquaculture* 194:51-32.

Lai, Y. S., John, J. A. C., Lin, C. H., Guo, I. C., Chen, S. C., Fang, K., Lin, C. H. and Chang, C. Y., 2003. Establishment of cell lines from a tropical grouper, *Epinephelus awoara* (Temminck and Schlegel), and their susceptibility to grouper irido and nodaviruses. *Journal of Fish Diseases* 26: 3142.

Lai, Y. S., Murali, S., Ju, H. Y., Wu, M. F., Guo, .I C., Chen, S. C., Fang, K. and Chang, C. Y., 2000. Two iridovirus-susceptible cell lines established from kidney and liver of grouper, *Epinephelus awoara* (Temminck & Schlegel), and partial characterization of grouper iridovirus. *Journal of Fish Diseases* 23: 379388.

Lakra, W. S., Bhonde, R. R., Sivakumar, N. and Ayyappan, S., 2006b. A new fibroblast like cell line from the fry of golden mahseer *Tor putitora* (Hamilton). *Aquaculture* 253: 238-243.

Lakra, W. S., Sivakumar, N., Goswami, M. and Bhonde, R. R., 2006a .Development of two cell culture systems from Asian seabass *Lates calcarifer* (Bloch). *Aquaculture Research* 37, 18-24.

Lakra, W. S., Swaminathan, T. R., Rathore, G., Goswami, M., Yadav, K. and Kapoor, S., 2010a. Development and characterization of thee new diploid cell lines from *Labeo rohita* (Ham.). *Biotechnology Progress* 26, 10081013.

Lakra, W. S., and Goswami, M., 2011. Development and characterization of a continuous cell line PSCF from *Puntius sophore. J Fish Biol.* 78:987-1001.

Lakra, W. S., Goswami, M., Yadav, K., Gopalakrishnan, A., Patiyal, R. S., and Singh, M., 2010b. Development and characterization of two cell lines PDF and PDH from *Puntius denisonii* (Day 1865) *In Vitro Cell.Dev.Biol.Animal* DOI 10.1007/s11626-010-9374-3.

Lakra, W. S., Swaminathan, T. R. and Joy, K. P., 2010b. Development, characterization, conservation and storage of fish cell lines: a review. *Fish Physiol Biochem.* 37:1-20.

Ogale, S. N., 2002. Mahseer ranching. In: *Riverine and Reservoir Fisheries of India* (Boopendranath M R, Meenakumari B, Joseph J, Sankar T V, Pravin P and Edwin L Eds.) 458 p.

Parameswaran, V., Ahmed, V. P. I., Shukla, R., Bhonde, R. R. and Hameed, A. S. S., 2007. Development and characterization of two new cell lines from milkfish (*Chanos chanos*) and grouper (*Epinephelus coioides*) for virus isolation. *Mar Biotechnol* 9:281291.

Parameswaran, V., Shukla, R., Bhonde, R. and Hameed, A. S., 2006. Establishment of embryonic cell line from sea bass (*Lates calcarifer*) for virus isolation. *Journal of Virology Methods* 137: 309-316.

Qin, Q. W., Wu, T. H., Jia, T. L., Hegde, A. and Zhang, R. Q., 2006. Development and characterization of a new tropical marine fish cell line from grouper, *Epinephelus coioides* susceptible to iridovirus and nodavirus. *J Virol Methods.* 131(1): 58-64.

Rao, K. S., Joseph, M. A., Shanker, K. M. and Mohan, C. V., 1997. Primary cell culture from explants of heart tissue of Indian major carps. *Current Science* 73, 374375.

Schirmer, K., 2006. Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish. *Toxicology* 224: 163183.

Swaminathan, T. R., Lakra, W. S., Gopalakrishnan, A., Basheer, V.S., Khushwaha, B. and Sajeela, K. A., 2010. Development and characterization of a new epithelial cell line PSF from caudal fin of Green chromide, *Etroplus suratensis* (Bloch, 1790). *In Vitro Cell Dev Biol Anim* doi:10.1007/s11626-010-9326-y.

Tong, S. L., Lee, H. and Miao, H. Z., 1997. The establishment and partial characterization of a continuous fish cell line FG-9307 from the gill of flounder *Paralichthys olivaceus*.*Aquaculture* 156, 327333.

Tong, S. L., Miao, H. Z. and Li, H., 1998. Three new continuous fish cell lines of SPH, SPS and RSBF derived from sea perch (*Lateolabrax japaonicus*) and red sea bream (*Pagrosomus major*). *Aquaculture* 169:143151.

Villena, A. J., 2003. Applications and needs of fish and shellfish cell culture for disease control in aquaculture. *Rev. Fish Biol. Fish*. 13: 111140.

Wang, X. L., Wang, N., Sha, Z. X. and Chen, S. L., 2010. Establishment, characterization of a new cell line from heart of half smooth tongue sole (*Cynoglossus semilaevis*). *Fish Physiol Biochem* doi:10.1007/s10695-010-9396-5.

Wolf, K., 1988. Fish viruses and fish viral diseases. Cornell University Press,NewYork.

Wolf, K. and Quimby, M. C., 1962. Established eurythermic line of fish cells *in vitro*. *Science* 135: 10651066.

Ye, H. Q., Chen, S. L., Sha, Z. X. and Xu, M. Y., 2006. Development and characterizationof cell lines from heart, liver, spleen and head kidney of sea perch, *Lateolabrax japonicus*. *Journal of Fish Biology* 69 (Supplement A): 115126.