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Coldwater fish cell line: Development, characterization and applications

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ABSTRACT

Fish cell lines have been used as important *in vitro* tools for carrying out research in different disciplines including toxicology, pathology, biotechnology, developmental biology and biomedical sciences. In recent years, the numbers of fish cell lines have been increasing tremendously covering a wide variety of species and tissues. Fish cell lines developed in the country have been maintained and cryopreserved in National Repositories with the financial assistance from the Department of Biotechnology, Govt. of India, New Delhi. Different projects supported by Department of Biotechnology, Govt. of India have been instrumental in boosting *in vitro* research in India using fish cell lines. Very few cell lines have been developed and characterized from cold water fish species and hence emphasis should be given to develop more cell lines from cold water fish to facilitate *in vitro* research in the area. The development, characterization and application of fish cell lines with special reference to cold water fish are reviewed in this paper.

Keywords: Coldwater fish, Cell line, Applications

Introduction

Cell line has been used as an important *in vitro* tool for carrying out various investigations in physiology, virology, toxicology and biotechnology. The adoption of intensive farming practices, unregulated use of inputs and inbreeding in hatcheries has led to increased disease incidence. There have been several incidences of mass mortality of carps in culture systems suspected to be caused by bacterial and viral diseases. Cell lines from fishes are particularly useful in detecting viruses and studying the molecular and cellular basis of physiological processes and toxicological mechanisms (Fryer & Lannan, 1994; Bols *et al*., 2005). In recent years, cell lines from aquatic animals have attracted considerable attention as a means of expediting disease diagnosis. Several viral diseases in cold water fish species have been reported (Dannevig *et al*., 1995, Crane and Hyatt, 2011). Hence, development of cell line from fish species is indispensable for virus isolation studies and understanding viral pathogenesis. The fish cell lines have been proven to be a valuable, rapid and cost-effective tool in the ecotoxicological assessment of chemicals and environmental samples**.**

The physiology and blood plasma constituents of teleosts are similar with those of terrestrial vertebrates; therefore, the methodology for culture of cells is also similar. Fish cell lines are more advantageous over mammalian cell lines in terms of its maintenance and versatile applications. Because of lower metabolic rates than eurythermic cells, fish cells can be maintained with little care for long periods of time. Thus, permanent fish cell lines, in contrast to the mammalian cells, are easier to maintain and manipulate, and unlike primary cultures, produce highly reproducible results (Wolf & Quimby, 1962). Embryonic and larval cells are the most easy to cultivate being mitotically activated.

The first fish cell line RTG-2 was developed in 1962 using the ovary of a coldwater fish, rainbow trout (Wolf and Quimby, 1962). Since then, an increasing trend in fish cell line development has been observed from a wide variety of tissues representing fish species from both tropical and temperate waters. A comprehensive review by Lakra *et al*. (2011) has reported 283 fish cell lines globally. The latest information enlisting 517 fish cell lines in Cellulosaurus; a knowledge resource on cell lines has been reported by Bairoch (2017). The Department of Biotechnology has been playing a pivotal role in promoting the fish culture related research in India by providing necessary grants to develop and maintain fish cell lines in the country. The first preliminary efforts to develop fish cell culture were made at Central Institute of Freshwater Aquaculture, Bhubaneswar in late eighties of the last century (Kumar, 1987) followed by successful primary cultures from gill tissue of mrigal, *Cirrhinus mrigala* (Sathe *et al*., 1995), kidney of the stinging catfish, *Heteropneustes fossilis* (Singh *et al*., 1995), and caudal fin of *Labeo rohita* (Lakra and Bhonde, 1996) at various laboratories providing a momentum to fish cell and tissue culture research in the country. In India, the research on the development and characterization of fish cell lines is mainly being conducted at several institutions like National Bureau of Fish Genetic Resources (NBFGR), Lucknow; Central Institute of Fisheries Education (CIFE), Mumbai; Central Institute of Freshwater Aquaculture, Bhubaneswar; Central Marine Fisheries Research Institute, Kochi; C. Abdul Hakeem College, Vellore; National Centre

for Cell Science, Pune; Fisheries College and Research Institute (FCRI), Tamilnadu Veterinary & Animal Sciences University, Tuticorin; Cochin University of Science and Technology (CUST). Approximately, 50 fish cell lines have been developed by different research groups in the country (Goswami *et al*., 2015). A National Repository of Fish Cell Lines (NRFC) was established during 2010 at ICAR-NBFGR, Lucknow with the financial assistance from DBT, New Delhi for a project National Repository for Conservation and Characterization of Fish Cell Lines at NBFGR, Lucknow (Principal Investigator: Dr. M Goswami). About 50 fish cell lines have been maintained and cryopreserved in the NRFC.

Development and characterization of coldwater fish cell lines in India

Teleost cell lines have been developed from a broad range of tissues such as ovary, fin, swimbladder heart, spleen, liver, eye muscle, vertebrae, brain and skin. A simple and reproducible short-term fish cell culture technique was described for *Tor putitora* by Prassana *et al*., 2000. A TP-1 cell line from cold water fish golden mahseer *Tor putitora* was developed for the first time in India by Lakra et al. 2006. Since then, consistent efforts have been made to develop cell line from cold water fish. The cell line exhibited best growth in the L-15 medium with 20% FBS at 28 ^{0}C.

Three cell culture systems were developed from caudal fin, heart and gill of Tor tor (Kamalendra *et al*., 2010, Kamalendra *et al*., 2011). Subsequently, a permanent cell line TTCF was developed from caudal fin of *Tor tor* by Yadav *et al*., 2012. TTCF cell line was cryopreserved and presently maintained at NRFC with an accession number NRFC003.

The cell culture system from eye, heart, fin and swim bladder of *Puntius (Tor) chelynoides* was developed from eye of *Puntius (Tor) chelynoides* by Goswami *et al*., 2012. The monolayer formed from heart explants exhibited rhythmic heartbeat. The cells were grown in Leibovitz' L-15 media supplemented with 20 % fetal bovine serum (FBS) at 24 °C. The PCE cell line from eye was characterized by DNA barcoding based on amplification of mitochondrial cytochrome oxidase subunit I (COI) & 16S rRNA genes and cytogenetic analysis. The cell line demonstrated expression for GFP reporter gene suggesting that this cell line can be used for transgenic and genetic manipulation studies. Further, genotoxicity assessment of PCE cells illustrated the utility of this cell line as an in vitro model for aquatic toxicological studies. The PCE cell line was successfully cryopreserved and revived at different passage levels. Another PCF cell line was developed from the caudal fin of *Puntius (Tor) chelynoides* by Goswami *et al*., 2014. Immunocytochemistry of PCF cells confirmed its fibroblastic morphological nature. Upon exposure of PCF cells to bacterial extracellular products, significant cytopathic effects were observed which validated the usefulness of the PCF cell line for toxicity assessment as *in vitro* model. The cell line has been maintained in NRFC with an accession number NRFC001.

A SRCF cell line from caudal fin of snowtrout, *Schizothorax richardsonii was developed and characterized* by Goswami *et al*., 2013. The cell line has been maintained in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum (FBS) at 24 ◦C. Transfection of SRCF cells with pEGFP-C1 plasmid showed bright fluorescent signals, suggesting the application of cell line in transgenic and gene expression studies. The cell line has been maiantained in NRFC with an accession number NRFC002.

Applications of fish cell lines

Early work with fish cell lines was initiated with RTG-2, a gonadal cell line derived from rainbow trout (Wolf and Quimby, 1962). Fish cell lines have enormous applications in biomedical research, toxicology, gene regulation, gene expressions and gene transfer (Hightower and Renfro, 1988, Babich *et al*., 1986, Driever *et al*., 1993). The main advantage of cell culture is that cell lines allow higher control of conditions of experiments and at the same time reduces the variability of the in vivo responses that arise due to the responses of fish to stress and environmental influences and to disparate genetic background of farmed fish and shellfish species. Fish cell cultures have been increasingly used in toxicology research for evaluating effects of various chemicals, pesticides and industrial wastes. The effects of different inorganic and organic pollutants on the metabolism of aquatic biological systems have been studied using cell cultures and hence fish cell lines can be used bio-indicators in environmental monitoring. Due to the good correlation found between the *in vitro* data and the *in vivo* fish data, the use of established fish cell lines can represent an alternative tool to acute fish bioassay for toxicity screening of chemicals. RTgill-W1 is an epithelial cell line derived from the gill explants of normal adult rainbow trout (*Oncorhynchus mykiss*) (Bols *et al*., 1994). RTgill-W1 has been used in toxicity testing of industrial effluents (Dayeh *et al*., 2002), including petroleum refinery effluents (Schirmer et al. 2001), polycyclic aromatic hydro-carbons (Schirmer et al.1998) and metals (Dayeh et al.2005) including Cu, Cd, Zn, Fe, and Ni.

In aquatic toxicology, *in vitro* investigations with both freshly isolated cells and permanent cell lines have been used for screening of chemicals or environmental samples (Gagne *et al*., 1996; Mori and Wakabayashi, 2000; Davoren *et al*., 2005; Tan *et al*., 2008). Genotoxicity and cytotoxicity studies have been carried out with many primary fish cells

and with different permanent fish cell lines (Brunbeck and Neumuller, 1996; Kamman *et al*., 2000; Abdul *et al*., 2013, Goswami *et al*., 2014; Taju *et al*., 2014; Dubey *et al*., 2015). Proteomic techniques, in particular, offer great potential for insight into chemical modes of toxic action and are useful tools in biomarker discovery (Wetmore and Merrick, 2004; Benninghoff, 2007). Protein expression signatures (PES) of fish cell lines have been developed using 2-DE and image analysis (Wagg and Lee, 2005; Goswami *et al*., 2016).

The utilization of fish cell lines in fish health management focuses on prevention and control of infectious diseases. Cell lines help to understand the pathogenesis of virus, and other intra cellular pathogens like Rickettsia sp. etc. Moreover, cell culture methodologies are useful in diagnosis of viral pathogen by production of cytopathic and syncytial effects and aid in isolation and characterization of virus. The most widely employed application of fish cell lines is the isolation and characterization of viruses. Most commonly used fish cell lines for diagnosis and characterization of intracellular fish pathogens are RTG-2, EPC, FHM, CHSE-214, CCO and BF-2. RTgill-W1 cell line demonstrated its ability to support the growth of a novel paramyxovirus isolated from the gills of disease seawater-reared Atlantic salmon (Kvellestad *et al*., 2003). The complete genome sequence of the virus, dubbed Atlantic salmon paramyxovirus or ASPV, was made possible due to the growth support of RTgill-W1 (Nylund *et al*., 2008). RTgill-W1 cell line could be useful for studies of gill infecting microsporidia such as Loma salmonae (Kent and Speare, 2005), the causative agent for microsporidial gill disease of salmonids affecting aquaculture-raised chinook salmon in Canada (Speare *et al*., 2007). Fish leukocyte cell lines with cells of specific lineage and function like T-cells, B-cells and macrophages developed from catfish, *Ictalurus puntatus*, *Onchorhynchus mykiss* etc. have been employed to generate immunological information useful for disease prevention in farmed fish. Cell lines of gut, skin and gill origin have been used to study the local defense responses. The immuno-response potential of DNA vaccines, recombinant protein and synthetic peptide vaccines and immunostimulants can be studied with the aid of cell lines.

Fish cell lines have useful applications as *in vitro* models for studying the replication and genetics of the viruses, the establishment and maintenance of virus carrier states, effects of antiviral drugs and production of experimental vaccines. *In vitro* fish RBC cultures have been used in investigations of the replication of the viruses and as *in vitro* models of viral induced anemia (Reno and Nicholson, 1980). Fish cell lines have been developed as *in vitro* models for studying various biological processes. The fish cell lines have been used for determining karyotypes and other aspects of cytogenetics including chromosomal polymorphism and speciation, chromosome abnormalities and evolution (Roberts, 1970). Organ cultures from tilapia, eel and trout pituitary glands have been used for studying the production of growth hormone prolactins (Baker and Ingleton, 1975). Tolerance to cold water is important trait which has tremendous economic interest in aquaculture because the success of aquaculture is in many areas restricted by cold winter temperatures (Hinder, 2010). Cell line would be an ideal *in vitro* for facilitating transgenic studies using cold tolerant gene.

Conclusion

The number of fish cell lines has been increased tremendously during the last decade covering wide ranges of tissues in India. Cold water fish cell lines are available in very limited numbers and more cell lines including stem cell should be developed from prioritized cold water fish species to facilitate in vitro genetic and biotechnological research in cold water fisheries and aquaculture. Species specific and tissue specific cell lines are need of the hour for virological and toxicological studies. The fish cell line repositories would be very useful in maintaining the cell lines for in vitro research in the country. This would be the right step for conservation of germplasm and other genetic material of cold water fish species. The cell lines would be valuable complement to whole animal studies for various investigations and thus will resolve many ethical issues associated with biological studies.

References

Abdul, M. S., Nambi, K. S., Taju, G., SundarRaj, N., Madan, N. and Hameed, A. S. S. 2013a. Establishment and characterization of permanent cell line from gill tissue of *Labeo rohita* (Hamilton) and its application in gene expression and toxicology. *Cell Biology and Toxicology* 29(1):59-73.

Babich, H., Puerner, J. R. and Borenfreund, E. 1986. *In vitro* cytotoxicity of metals to blue gill (BF-2) cells. *Arch. Environ. Contam. Toxicol* 15:31-37.

Bairoch, A. 2017. The Cellosaurus: a cell line knowledge resource. http://web.expasy.org/cellosaurus/

Baker, B. I. and Ingleton, P. M. 1975. Secretion of prolactin and growth hormone by teleost pituitaries *in vitro*. *Journal of Comparative Physiology* 100:269-282.

Benninghoff D.A. 2007. Toxicoproteomics—The Next Step in the Evolution of Environmental Biomarkers? *Toxicological Sciences*. 95 (1): 1–4

Bols, N. C. and Lee, L. E. J. 1994. Cell lines: availability, propagation and isolation. In: Hochachka, P. W.; Mommsen, T. P. (eds) Biochem. Molec. Biol. Fishes. Vol. 3: Analytical Techniques. Amsterdam. Elsevier Science. 145–159.

Brunbeck, T. and Neumuller, D. 1996. The comet assay in permanent and primary fish cell cultures – a novel system

to detect genotoxicity. In Vitro Cellular Developmental Biology 32, 61A.

Dannevig B.H., Falk K. and Namork, E. 1995. Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *J Gen. Virol.* 76:1353–1359.

Davoren, M., Ni, S. S., Hartl, M. G. J., Sheenan, D., O'Brien, N. M., O'Haloran, J., VanPelt, F. N. and Mothursill, C. 2005. Assessing the potential of fish cell lines as tools for the cytotoxicity testing of estuarine sediment aqueous elutriates. *Toxicology In Vitro.* 19(3): 421–431.

Dayeh, V. R.; Schirmer, K. and Bols, N. C. 2002. Applying whole-water samples directly to fish cell cultures in order to evaluate the toxicity of industrial effluents. *Water Res.*36: 3727–3738.doi:10.1016/S0043-1354(02)00078-7.

Dayeh, V. R., Schirmer, K., Lee, L. E. J. and Bols, N. C. 2005. Evaluating the Toxicity of Water Samples with the Rainbow Trout Gill Cell Line Microplate Cytotoxicity Test, In : Small-Scale Freshwater Toxic-ity Investigations. Vol I. Ed. C. Blaise and JF Ferard. Springer.473–504.

Driever, W. and Rangini, Z. 1993. Characterization of a cell line derived from zebrafish *Brachydanio rerio* embryos. *In Vitro Cellular and Developmental Biology* 29A:749-754.

Dubey, A., Goswami, M., Yadav, K. and Chaudhary, D. 2015a. Oxidative stress and nano-toxicity induced by TiO_2 and ZnO on WAG cell line. *PLoS ONE* 10(5): e0127493. doi:10.1371/journal.pone.0127493.

Fryer JL and Lannan, C.N. 1994. Three decades of fish cell culture: A current listing of cell lines derived from fishes. *Methods in Cell Science*. 16(2): 87-94.

Gagne, F., Blaise, C. and Bermigham, N. 1996. Lethal and sublethal effects of marine sediment extracts on rainbow trout hepatocytes. *Toxicology Letter* 87(2): 85–92.

Goswami, M., Dubey, A., Yadav, K., Sharma, B. S. and Lakra, W. S. 2016a. Identification of Fish Cell Lines Using 2-D Electrophoresis Based Protein Expression Signatures. *Current Proteomics* 13(4): 245 – 252.

Goswami, M., Nagpure, N. S. and Jena, J. K. 2015. Fish Cell Line Repository: an enduring effort for conservation. *Current Science India* 107 (50): 738-739.

Goswami, M., Sharma, B. S., Bahuguna, S. N. and Lakra, W. S. 2013. A SRCF cell line from snowtrout, *Schizothorax richardsonii*: Development and characterization. *Tissue & Cell* 45(3):219-26.

Goswami, M., Sharma, B. S., Tripathi, A. K., Yadav, K., Bahuguna, S. N., Nagpure, N. S., Lakra, W. S. and Jena, J. K. 2012a. Development and characterization of cell culture systems from *Puntius (Tor) chelynoides* (McClelland). *Gene* 500(1):140-147.

Goswami, M., Sharma, B. S., Yadav, K., Bahuguna, S. N. and Lakra, W. S. 2014a. Establishment and characterization of a piscean fibroblastic cell line from *Puntius (Tor) chelynoides* suitable for toxicity and gene expression studies as in vitro model. *Tissue and Cell* 46: 206–212.

Hightower, L. E. and Renfro, J. L. 1988. Recent applications of fish cell culture to biomedical research. *Journal of Experimental Zoology* 248 (3)**:** 290–302.

Kamlendra, J, Sharma J, Goswami M and Sharma B. S. 2010. Development of cell culture culture from caudal fin and heart of Tor *tor. Indian Journal of Animal Sciences* 37(12):37-43.

Kamlendra, Kapoor S, Sharma J., Goswami, M. and Rathore, G. 2011. Development of primary culture from gills of Tor tor. *Indian Journal of Animal Sciences* 81(12):75-78.

Kamman, U., Chaparro Riggers, J.F., Theobald, N., Steinhart, H., 2000. Genotoxicity potential of marine sediments from the North Sea. *Mutation Research* 467, 161–168.

Kent, M. L. and Speare, D. J. 2005. Review of the sequential development of Loma salmonae (Microsporidia) based on experimental infections of rainbow trout (Oncorhynchus mykiss) and Chinook salmon (O.tshawytscha). *Folia Parasitol*. (Praha).52(1–2): 63–68..

Kumar, D. 1987. Fish Cell Culture. Annual Report, Central Institute of Freshawter Aquaculture,

Lakra, W. S. and Bhonde, R. R. 1996. Development of primary cell culture from the caudal fin of an Indian major carp, *Labeo rohita* (Ham). *Asian Fisheries Science* 9:149-152.

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

Lakra, W. S., Swaminathan, T. R. and Joy K. P. 2011. Development, characterization, conservation and storage of fish cell lines: a review. *Fish Physiology and Biochemistry* 37(1):1-20.

Mori, M. and Wakabayashi, M. 2000. Cytotoxicity evaluation of synthesized chemicals using suspension-cultured fish cells. *Fisheries Science* 66 (5) 871–875.

Nylund, S., Karlsen, M. and Nylund, A. 2008. The complete genome sequence of the Atlantic salmon paramyxovirus (ASPV). *Virology* 373: 137–148. doi:10.1016/j.virol.2007.11.017.

Prasanna, I., Lakra, W.S., Ogale, S.N. and Bhonde, R.R. 2000. Cell culture from fin explant of endangered Mahseer *Tor putitora* (Hamilton). *Current Sci.* 79 (1):93-95.

Reno, P. and Nicholson, B.L. 1980. Viral erythrocytic necrosis (VEN) in Atlantic cod *(Gadus morhua):* In vitro studies. *Can J Fish Aquat Sci*; 37:2276–2281.

Roberts, F. L. 1970. Atlantic salmon (*Salmo salar*) chromosomes and speciation. *Transaction of the American Fisheries Society* 99:105-111.

Sathe, P. S., Maurya, D. T., Basu, A., Gogate, S. S. and Banerjee, K. 1995. Establishment and characterization of a new fish cell line, MG-3, from the gills of mrigal *Cirrhinus mrigala*. *Ind J Exp Biol* 33:589–594.

Schirmer, K., Dixon, D. G., Greenberg, B. M. and Bols, N. C. 1998. Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. *Toxicology* 127: 129–141; doi:10.1016/S0300-483X(98)00030-4..

Schirmer, K., Tom, D. J., Bols, N. C. and Sherry, J. P. 2001. Ability of fractionated petroleum refinery effluent to elicit cyto- and photocytotoxic responses and to induce 7-ethoxyresorufin-O-deethylase activity in fish cell lines. *Sci. Total Environ*. 271(1–3):61–78. doi:10.1016/S0048-9697(00)00831-7.

Singh, I.S.B., Rosamma, P., Raveendranath, M. and Shanmugam, J. 1995. Development of primary cell cultures from kidney of freshwater fish *Heteropneustes fossilis*. *Indian Journal of Experimental Biology* 33:595-599.

Speare, D. J., Markham, R. J. and Guselle, N. J. 2007. Development of an effective whole-spore vaccine to protect against microsporidial gill disease in rainbow trout (Oncorhynchus mykiss) by using a low-virulence strain of Loma salmonae. *Clin. Vaccine Immunol.* 14(12): 1652 –1654. doi:10.1128/CVI.00365-07.

Tan, F., Wang, M., Wang, W. and Lu, Y. 2008. Comparative evaluation of the cytotoxicity sensitivity of six fish cell lines for four heavy metals in vitro. *Toxicology In Vitro* 22 (1):164–170.

Wagg, S. K. and Lee, L. E. J. 2005. A proteomics approach to identifying fish cell lines. *Proteomics* 5: 4236–4244.

Wetmore, B.A. and Merrick, B.A. 2004. Toxicoproteomics: Proteomics Applied to Toxicology and Pathology. *Toxicologic Pathology* 32:619–642.

Wolf, K. and Quimby, M.C. 1962. Established Eurythermic Line of Fish Cells *in vitro. Science* 135(3508):1065 – 1066.

Yadav, K., Lakra, W. S., Sharma, J., Goswami, M. and Singh, A. 2012. Development and characterization of a cell line TTCF from endangered mahseer *Tor tor* (Ham.). *Fish Physiology and Biochemistry* 38(4):1035-45.