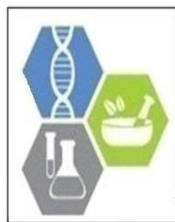

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Current scenario and advances in fish cell lines development and characterization in India

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Abstract
Cell lines are a valuable biological tool for carrying out researches in physiology, virology, pharmacology, toxicology, cancer/carcinogenesis and transgenic fields. Several "fish cell lines" have been developed from a broad range of tissues by explant techniques, e.g., ovary, fin, swim bladder, heart, spleen, liver, eye muscle, vertebrae, brain, skin. At present, 50 fish cell lines from 24 different fish species are maintained and cryopreserved in the National Repository of fish cell line (NRFC), NBFGR. In the current review, the authors have discussed the national status and advances in the development cell lines from different fish species. Besides these, the importance of cell line characterization, applications and cross-contamination have also been discussed briefly in this review. The author feels that this review article will undoubtedly serve an updated database for the scientists/researchers in the field of Pathology, Toxicology, Fishery Science, and other Life Science disciplines wherever needed for research.

Keywords: Fish cell line, immunohistochemistry (IHC), protein expression signatures (PES), karyotype, barcode of life database (BOLD)

1. Introduction
Animal cell culture as an essential branch of life science has been dealing with removal of cells, tissues or organs from animals and they are *et al* propagation in an artificial environment. Cell culture systems are biological entities with specific physiological needs, much like any other laboratory animals. Over the last decade, considerable advancements have occurred in the large-scale cultivation of animal cells and their storage in cell line repositories. Many fish cell lines have been established from fish tissues for detection and isolation of fish viruses. The cell lines from different tissues of different species are valuable for studying species-specific responses to viral infection at the cellular level. Fish cell lines serve as essential *et al* tools for pathological, toxicological and immunological studies. Fish cell lines have enormous applications in biomedical research, toxicology, gene regulation, gene expressions and gene transfer. 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. Fish cell lines are more advantageous over mammalian cell line in many aspects, and hence fish cell line has been intensively used in *et al* research. In recent years, the number of fish cell lines has increased greatly and currently represents a wide variety of species and tissue types. Proper characterization and identification of the cell lines are critical for scientific usage. Characterization of a cell line is necessary to authenticate the origin of the cell line, to detect the absence or presence of cross-contamination and to establish its applicability for various research works.

2. National Status
The development of fish cell lines has got momentum from previous efforts at Central Institute of Freshwater Aquaculture, CIFA, Bhubaneswar in the early eighties and others attempt to develop cell line from *Cirrhinus mrigala* (Sathet *et al.,* 1997) [1], *Heteropneustes fossilis* (Singh *et al.,* 1995) [2] and *Labeo rohita* (Lakra and Bhonde, 1996) [3] in early nineties. Department of Biotechnology, Govt. of India has played a pivotal role in expanding fish cell culture research in India. As a result, more than 50 fish cell line has been developed by different research groups in the country. Fish cell lines have been developed from many tissues such as ovary, fin, spleen, swim bladder, heart, liver, eye muscle, brain, and skin (Table 1). Development of cell lines have been reported from important fish species like *Etroplus suratensis*, *Clarias batrachus* (Babu *et al.,* 2011), *Schizothorax richardsoni* (Goswami *et al.,*

2013) [5], *Catla catla* (Chaudhuri *et al*. 2014) [6], *Channa striatus, Puntius chelynoides* (Goswami *et al.,* 2014) [7] and *Wallago attu* (Dubey *et al.,* 2014) [8].

Table 1: Details of Indian cell lines from different tissues of fish species

Designed cell lines	Developed from tissues/Organs	Species	Developed By
SISK	Kidney	*Lates calcarifer*	Hameed *et al*. (2006) [9]
TP-1	Fry	*Tor putitora*	Lakra *et al.,* (2006), [10]
SISE, and SBES	blastula	*Lates calcarifer*	Parameswaran *et al.,* (2006) [11]
SIGE	Eye	*Epinephelus coincides*	Parameswaran *et al.,* (2007) [12]
SIMH	heart	*Chanos chanos*	Parameswaran *et al.,* (2007) [12]
SICE	Eye	*Catla catla*	Ahmed *et al.,* (2008) [13]
RH, RF,and RSB	heart, fin, and bladder	*Labeo rohita*	Lakra *et al.,* (2010) [14]
PDF and PDH	caudal fin and heart	*P. denisonii*	Lakra *et al.,* (2010) [15]
CCF and CCH	fin and heart	*Cyprinus carpio*	Lakra *et al.,* (2010) [15]
IEE,IEG) and IEK	eye, gill, and kidney,	*Etroplus suratensis*	Sarth *et al.,* (2012) [16]
PCE	eye	*Puntius chelynoides*	Goswami *et al.,* 2012 [17]
RESC	blastula	*Labeo rohita*	Goswami *et al.,* 2012 [17]
SRCF	Caudal fin	*Schizothorax richardsonii*	Goswami *et al.,* 2013 [18]
WAF	Caudal fin	*Wallago attu*	Dubey *et al.,* (2014) [19]
PCF	Caudal fin	*puntius chelynoides*	Goswami *et al.,* 2014) [20]
TTCF	Fin	*Tor tor*	Yadav *et al*., (2012). [21]
RTF	Fin	*Puntius denisoni*	Swaminathan, *et al*. (2012) [22]
FSP	spleen	*Paralichthys olivaceus*	Kang, M. S., *et al*. (2003) [23]
CF	Caudal fin	*Chitala chitala*	Kapoor *et al.,* 2013 [24]
DRM	Muscles	*Danio rerio*	Kumar *et al*., (2016). [25]
WAM	Muscle	*Wallago attu*	Dubey *et al*., (2015). [26]
CSG	Gill	*Channa striatus*	Majeed, *et al*., (2014) [27]
CSK	Kidney	*Channa striatus*	Majeed, *et al*., (2013) [28]
RE / CB	Eye and Brain	*rohita, Catla*	Ishaq Ahmed *et al*., (2009). [29]
CTE	Thymus	*Catla catla*	Chaudhary *et al*., (2013) [30]
CTM	Thymus	*Catla catla*	Chaudhary *et al*., (2014) [6]
LRG	Gill	*Labeo rohita*	Majeed, S. *et al*., (2013) [31]
ICG	Gill	*Catla catla*	Taju, G. *et al.* (2013) [32]
ICF	Fin	*Clarius batrachus*	Babu *et al*., (2011) [4]
SICH	Heart muscle	*Catla catla*	Ahmed, VP. *et al*. (2009) [13]
SISS	Spleen	*Lates calcarifer*	Parameswaran, V., *et al*., (2006) [11]
HBF	Caudal fin	*Horabragus brachysoma*	Swaminathan, T. *et al*., (2016) [33]
CCKF	Fin	*Cyprinus carpio koi*	Swaminathan, T., *et al*., (2015) [34]
ASBB	Brain	*Lates calcarifer*	Hasoon, Mauida F., *et al*., (2011) [35]
SRF	Fin	*Schizothorax richardsonii*	Goswami *et al*. (2013) [18]
CFFN2	Dorsal fin	*Amphiprion sebae*	George *et al*. (2016) [36]
CFBR	Brain	*Amphiprion sebae*	George *et al*. (2016) [36]
CFSP	Spleen	*Amphiprion sebae*	George *et al*. (2016) [36]
CFCP1	Caudal Peduncle	*Amphiprion sebae*	George *et al*. (2016) [36]
IGK	Kidney	*Epinephelus coioides*	Parmeshwaran *et al*. (2007) [12]
AFF	Caudal fin	*Pterophyllum scalare*	Swaminathan T. R. *et al*., (2016) [33]

3. Various parameters using for Characterization of Cell Lines

3.1 Test for Detection of Contamination

Elimination of contamination from a cell line is time-consuming and laborious, and after all, it doesn't work always. Discarding the contaminated flask, taking care to prevent contamination and starting over is preferred. Most commonly occurred contamination are bacterial, fungal, mycoplasma and cross contamination. Bacterial and fungal contaminations are usually checked regularly by microscopic examination as well as using standard kits Gopalkrishna *et al*., (2007) [37] and mycoplasma detection was done by PCR-RFLP method.

3.2 Morphological Confirmation

Epithelioid or fibroblastic morphology of cells can be confirmed by immunohistochemistry (IHC) using a cell-specific marker. Basically, monoclonal antibodies directed against Vimentin and Cytokeratin are used for conforming fibroblastic and epithelioid morphology respectively. (Lakra and Goswami 2010) [15].

3.3 Cell Counting and Measurement of Cell Doubling Time

Cell counting is usually done by using hemocytometer. Cell doubling time is an important factor to understand the features of a cell line.

3.4 Molecular Characterization

Various markers are used for authentication of fish cell lines include mitochondrial 16S rRNA and COI gene (Lakra and Goswami 2011) [38], and microsatellite DNA profiling (Lakra and Goswami 2011) [39]. Molecular characterization methods, *e.g.* amplification of 16S, 18S ribosomal RNA and cytochrome oxidase subunit I (COI) region of mitochondrial gene followed by sequence analysis are generally used for authenticating the origin of a fish cell line (Lakra *et al.,* 2010) [15]. These molecular methods are routinely used for

authenticating established fish cell lines. DNA barcoding through the Barcode of Life Database (BOLD), with universal primers for mitochondrial COI gene, used for fish species identification as reported by caudal fin (PDF) and heart (PDH) cell lines from *Puntius denisonii* caudal fin (PSCF) cell line from *Puntius sophore* Fin (RTF) cell line from *Puntius denisonii* (Swaminathan *et al.,* 2015) [35].

3.5 Cytogenetic Markers

Cytogenetic analysis is used to establish the common chromosome complement or karyotype of a species or cell lines. Cell culture offers several advantages for chromosome studies such as a large number of metaphases can be obtained, chromosome morphology is generally better than obtained from direct tissue preparation. These advantages will make cell culture a useful tool for improving the current status of fish chromosome research. The study of the chromosomal morphology of related species may permit detection of changes, which modified an ancestral karyotype as it evolved into new lines. Lakra and Goswami, 2011 demonstrated distribution of diploid chromosomes with a modal peak at 52 chromosomes in PSCF cell developed from *Puntius sophore*. Hameed *et al*. (2006) [9] reported that the continuous cell line (SISK) developed from *L. calcarifer* showed a modal value at diploid chromosome number 48 and 46 at passage 37 and 61, respectively.

3.6 Plating Efficiency

Other methodologies were used to characterize various cell lines include cell plating efficiency which is a measure of the number of colonies originating from the single cells. The number of colonies was counted (x) under the microscope, and plating efficiency (y) was calculated using the formula: $Y=100X/Z$ where the Z^{-1}= number of cells seeded (Freshney, 1994).

3.7 Proteomic Analysis

The proteomic approach is used to identify the fish cell lines derived from tissues of the same or different species. Protein expression signatures (PES) of the evaluated fish cell lines were developed by using 2-DE and image analysis (Goswami *et al.,* 2015).

3.8 Growth Studies

Growth studies are another important characterization parameter which describes the optimum condition required for growth of cell line i.e. temperature, FBS concentration, type of media to be used and requirement of other growth factors. Basically, the temperature ranged from 24 to 32 °C supported the growth of cell lines with an optimum temperature of 28 °C has been described by many researchers (Lakra *et al.,* 2006). Fetal bovine serum (FBS) is importantly supplemented with the tissue culture media as serum contains various known and unknown growth factors that stimulate both attachment and growth of cells. The fish serum can also be used in combination with FBS in developing fish cell lines (Lakra *et al.,* 2006) [10]. Serum concentration can also affect primary cultures and growth of cell lines. Concentration varies from 5% to as high as 20%. Serum concentration may inhibit cell growth. Kumar *et al.,* (2001) [25] used a large list of additives, for example, fish muscle extract, sucrose prawn shell extract, which were explored during developing a primary culture from ovary tissue of African catfish.

3.9 Transfection Studies

Transfection efficiency of a cell line is another important parameter that must be determined as exogenous DNA delivery to the cultured cell is very useful for both basic research and applied research related to gene expression studies. When the cell lines were transfected with pEGFP vector DNA, significant fluorescent signals were observed by many researchers indicating their potential utility for transgenic and genetic manipulation studies (Lakra and Goswami 2010) [15].

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