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Research Article

From *In Vivo* to *In Vitro*: An Intriguing Journey of Fish Cell lines

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Abstract

Cell culture techniques have largely replaced the traditional protocols for efficient and precise research. In fisheries sciences, research based on the development and application of fish cell lines has become popular over a period of two decades. Fish cell lines find wide application in the development of vaccines, toxicological studies, drug development, and biotechnological manipulations that have set new landmark. The use of fish cell lines is thought to be one of the best alternatives for the studies carried on fishes, because the cell lines have the host's cellular genetic homogeneity and have low variability. The available articles for finfish cell lines were used to examine worldwide distribution. The cell lines have been observed an emerging research topic to have an important role in identification and prevention of viral and bacterial diseases, evaluation of treatment options and proactive management strategies that are critical for optimal fish health management.

This review briefs the trend of development and potential use of fish cell lines in advanced research in the fisheries sector. Genomic methods implied for early disease diagnosis, isolation of new pathogens, identification of toxicity mechanism, early attainment of growth and, maturity by fish in culture systems and conservation of threatened/endangered fish species is the propensity of cell line development.

Keywords: Cell Culture; Virus Isolation; Toxicological Studies; In-Vitro Studies; Germplasm Conservation

Introduction

In-vitro raising of living cells (template cells taken from an organism) in a favourable artificial culture medium is known as cell culture [1]. Cells are dislodged from the prototype organism either mechanically or by enzymatic disaggregation and seeded in a cell culture medium that contains all nutrients required for their survival. When the cells reach the stage of confluence, they start proliferating and gradually occupy the available substrate. This stage is called primary culture; the cells are then sub-cultured (shifted to fresh medium) for continued growth [2]. After the successful completion of first sub-culture, the primary cell culture is called a cell line, which is finite but has a limited life span. Generally, cells with highest growth predominate, and this results in genotypic and phenotypic uniformity in the population. If a sub-population of a cell line is positively selected from the culture by cloning or another method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line [3].

With some ethical constraints, cell culture technique has widespread application in life sciences, especially cytogenetics, physiology, the developing assays for disease diagnosis, and the development of medications and vaccines to combat fish disease, toxicology and host-pathogen interactions [4-6]. Main types of cells cultured in cell lines include primary cells, transforming cells and self- renewing cells. Primary cells undergo senescence, a genetically determined stage after which, their ability to proliferate ceases and the cell lines are called finite cell lines [7]. However, the chemically or virally transformed cells become immortal and achieve the capability to divide indefinitely, and give rise to a continuous cell line [8]. Impulsive as well as genomic manipulation may also result in chromosomal aberrations and non-functional phenotypes. The immortalized cell lines are easy to maintain and multiply. The embryonic, induced pluripotent and intestinal stem cells have self-renewing property, and differentiate into many other cell types countenancing their permanent *in-vitro* conservation and make stem cell lines

[9]. These cell lines have also been found physiologically relevant for modelling of *in-vivo* biochemical processes [10,11]. Cell lines have proven effective in identifying genotoxic effects, and are currently being used to study toxic mechanisms and biomarkers like cytochrome P4501A. This review will cover the importance of fish cell lines, the characterization process, and issues related to cell line contamination. Furthermore, a thorough exploration of the various applications of fish cell lines will be discussed.

Importance of cell lines in fisheries sciences

Researchers are developing a strong interest in piscine primary cell cultures because of the wider incubation temperature range, easy handling and sustenance of cells for longer periods in contrast to poikilothermic fish [12]. Approximately 20,000 species of bony fish have been used for developing primary cell cultures or cell lines which have been maintained in mammalian sera for various uses. Research done in finfish cell culture throughout the world in the last decade has resulted in the establishment of 280 new fish cell lines drawn from 20 distinct tissue types of 44 fish families, bringing the total number of finfish cell lines to 783 [13]. Cyprinids are most frequently grown in Asian nations and around 60% of the fish cell lines have been developed in Asia, accounting for more than 80% of the global output. In 2011, Lakra., *et al*. [12] reported 59 cell lines from 19 freshwater fishes, 53 cell lines from 22 marine fishes, and 11 cell lines from three brackish water species of Asia.

Development of piscine cell lines started with the development of RTG-2 cell lines from the gonad of *Salmo gairdneri* [14]. The cells were sub-cultured during the second and the third week after establishing monolayers of mixed epithelial and fibroblast-like cells. The RTG-2 cells exhibit a variety of morphologies depending on the medium and location in the cell sheet, but exhibit a fibroblastic appearance under most circumstances. Till 2010, only 283 cell lines were developed from the finfishes around the world. In addition to the previously existing cell lines, 25 new cell lines including five from pituitary gland [15-18], spleen, eight from ovary, heart, three from milt, liver [19], two from intestine, one each from head kidney, ovarian fluid, fin [20,21] and gill of rainbow trout were developed. Globally up to 2022, 280 new cell lines originating from various finfish tissues have been added, bringing the total number of cell lines to 783 [11]. These cell lines derived from various tissues of diverse fish species hold significant importance in comprehending how different species react differently at the cellular level to viral infections.

In recent years, though intensive efforts were made in some parts of the world to develop fish cell lines from various fish species but a limited number of fish cell lines were developed in India [22,40]. Indian researchers have developed primary cell cultures rather than the continuous cell lines**.** Cell cultures from the heart of Indian Major Carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) and Chirruh (*Schizothorax esocinus*) have been successively established [23] (Figure 1). A fin explant from a mahseer (*Tor putitora,* an endangered fish) was developed without sacrificing the fish by using L-15 medium supplemented with 20% foetal calf serum (FCS) and 10% of fish muscle extract (FME), at 28ºC. Culture was also developed from the ovarian tissue of African catfish (*Clarias gariepinus*) but it perished after 15 rounds of the primary cell culture system. Then a new cell culture system was developed using *Lates calcarifer* fry, an economically important brackish water fish and *Tor putitora* fry, which is a sport fish, where-in confluency was observed in different serums at different concentrations.

First successful initiative was made to establish and characterize continuous cell line (SISK) from kidney of sea bass (*Lates calcarifer*), which is India's 1st marine fish cell line [8]. These cells showed strong positive reactivity for epithelial markers such as cytokeratin 19 and pancytokeratin, thus, confirming epithelial characteristics of cells. Majority of these cell lines have been submitted to the National Centre for Cell Sciences in Pune, a DBT-funded national repository that facilitates animal cell lines and tissue cultures for *in-vitro* research and development, or to the Institute of Microbial Technology in Chandigarh's Microbial Type Culture Collection and Gene Bank Facility. The main aim of the repository is to receive, identify, preserve, develop, and supply cell lines to researchers for R&D purposes. Researchers at C. Abdul Hakeem College of Tamil Nadu, are now a days working hard to develop and maintain fish cell lines, the unit has more than 40 cryopreserved fish cell lines [13].

Development of Primary Cell Culture from Schizothorax esocinus for germplasm conservation - A Case Study: [23]

Schizothorax esocinus is an indigenous and preferred fish of Kashmir valley. Till now, no cell line has been established from *S. esocinus* in India. To preserve the germplasm of this valuable fish species and for the isolation and identification of viral pathogens, an attempt was made at SKUAST-K to establish a primary cell culture from the tissues of *S. esocinus*.

A total of 40 live fish were used for the study. Heart tissue showed a prominent visible growth with the formation of monolayer of fibroblast like cells. The monolayer was then sub-cultured up to 6 passages (Figure 3). Best growth of the heart cell cultures was observed in L-15 media supplemented with the increment of amino acids and vitamins. Two important parameters viz., FBS (Foetal Bovine Serum) and temperature were optimized for the development of a primary cell culture. FBS is an important component of cell culture medium. It is known to contain various growth factors which aid in survival, maintenance and proliferation of cells. Temperature on the other hand, is an important physical factor that sustains growth of cells in a culture medium.

At 15 %, FBS and 24ºC, highest increment was observed, in cellular growth (Figure 4 and 5). It was also observed that the explants method of cell culture resulted in optimum growth and pro-

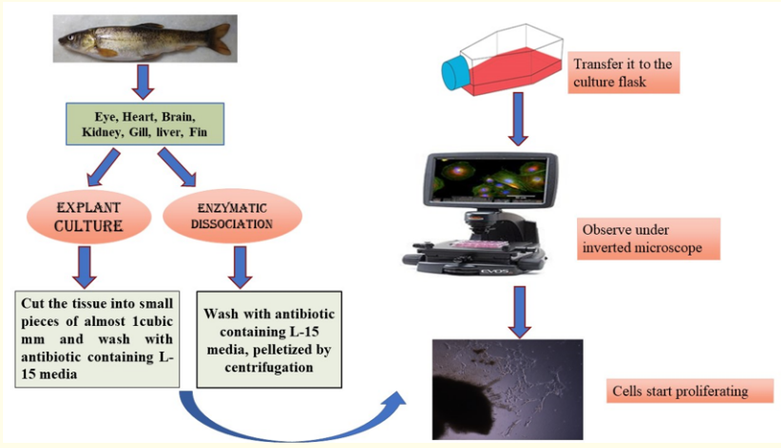

Figure 1: Basic steps process involved in the development of piscine primary cell culture.

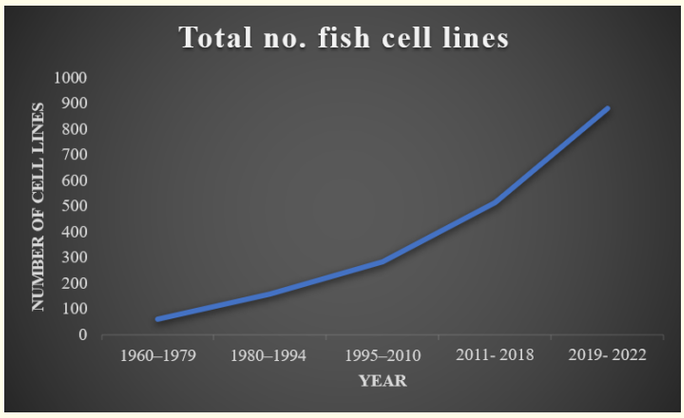

Figure 2: Trend of the number of fish cell lines in the world.

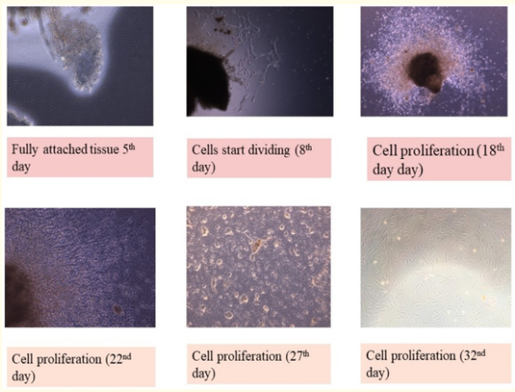

Figure 3: Development of monolayer in primary cell culture of *S. esocinus*.

liferation of cells. The best method of sub culturing was found to be trypsinization at 0.25% trypsin-EDTA solution which resulted in efficient dislodgement and then attachment of cells to the culture flasks. Karyotyping revealed chromosome number of 98, confirming the species identity of the developed primary cell culture from *S. esocinus* (Figure 6). This study offers a great scope for further refinement of methodology for the development of cell lines from cold water fishes.

Characterization of cell lines

Microsatellite DNA profiling, sequencing of mitochondrial 16S and 18S rRNA, random amplified polymorphic DNA methods (RAPD), and other techniques, like molecular characterization by DNA barcoding, immuno-cytochemistry, cell plating etc, have been used to characterize fish cell lines. The identification of several fish

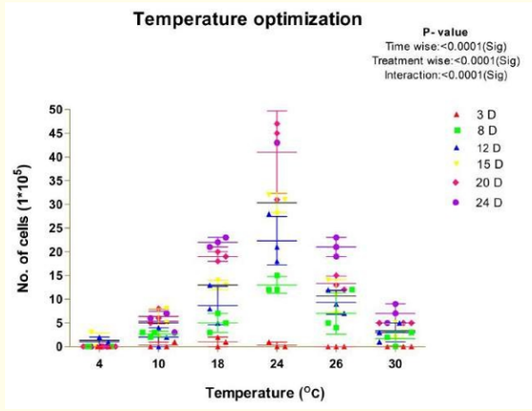

Figure 4: A Scatter-plot showing the effect of varying temperature on the growth of cells.

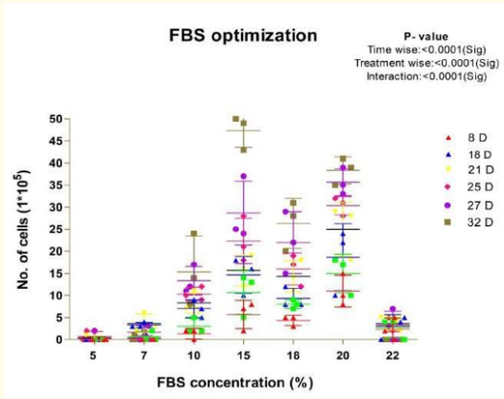

Figure 5: A Scatter-plot depicting the effect of varying FBS concentration on the growth of cells.

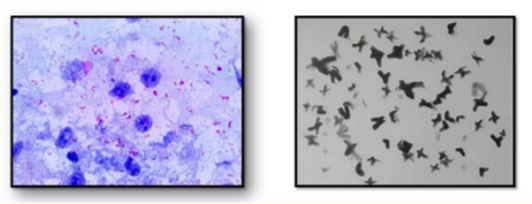

Figure 6: Karyotype of *S. esocinus* (at 100X).

cell lines generated from tissues of one species or from different species has been done using 2-DE (2-dimensional gel electrophoresis) and image analysis [24,25]. Protein expression signatures (PES) of the tested fish cell lines have been established, and can be used as a reliable method for identifying fish cell lines. Karyotyping chromosomal abnormalities, polymorphism and species identification can be done with the help of these established fish cell cultures. For characterization of cell lines, it is important to check for contamination, identify the species of origin and to ascertain whether or not the cell lines are transformed [26-29].

Contamination in cell line culture

The most frequent contaminants of cell lines are bacteria, fungi, viruses, and parasites. It is critical to check biological contamination since microbes have an ability to change the phenotype and genotype of a cell line through nutritional competition, production of toxic by-products, or interference with the cell's genome. Other contaminants include chemical impurities (such as plasticizers in cell culture containers) or different cell types cocultured in the lab.

Mycoplasma or moulds are the members of Kingdom fungi that are commonly found in cell cultures and produce multicellular, highly connected, thin filaments (hyphae). Cell culture supernatants contaminated with yeasts or moulds appear turbid and although the pH remains stable during the initial stages of infection, it increases as contamination increases (Figure 7A&B). Yeast contaminations may also be accompanied by a distinct smell [27]. Mycoplasma is less noticeable than bacteria since they are tiny, stationary, and difficult to detect. Usually, quality of the cultivated cells tends to decline over time, but mycoplasma infections can sometimes remain unnoticed for a longer period of time before becoming evident. For monitoring mycoplasma infections in cell cultures, it is recommended to routinely perform PCR, Enzyme-Linked Immunosorbent Assays (ELISA), or Immuno-staining of cell cultures. The cell cultures that have been contaminated with bacteria are also turbid, have altered pH and can be detected by the change of the colour of phenol red to yellow. Bacteria are generally visible in the cultures at higher magnification (Figure 7C&D).

Intracellular viruses may induce cytopathic effects in the cultured cells, while others may integrate into the cellular genome and alter the phenotype of the cell line [30]. Viruses can enter cell cultures, with animal-derived cell culture medium components such as trypsin or foetal bovine serum and are a serious health concern for laboratory workers. Confirmation of viral contaminants in challenging but generally relies on PCR, ELISA, Immunocytochemistry, or electron microscopy.

Regardless of the type of contamination, affected cell cultures should be removed from the cell culture room and discarded to prevent the spread of infection to other cultures. Furthermore, it is important to identify the source of contamination and rectify it. It is advisable to dispose-off the culture media and other cell culture components that may have come in contact with the contaminated cells and to clean the surfaces that may have been used for the contaminated culture containing vessel (e.g., incubator, biosafety cabinet, microscope, and aspirator). It is not recommended to treat the infected cells, since any handling of contaminated cultures will increase the potential spread of contaminants, especially airborne fungal spores. Use of antifungal compounds is not recommended as these can interfere with the metabolism of cultured cells.

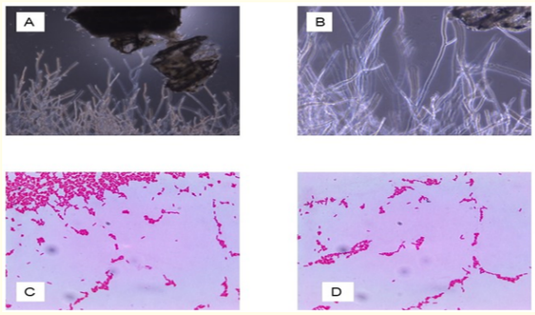

Figure 7: Fungal (A, B); and Bacterial (C,D) Contamination of cell cultures.

Advantages of cell lines

Cell culture offers a wide range of options that are difficult to obtain in the *in-vivo* experiments as different methods and experimental conditions are required for various organisms. The most important ones being that individual cells of a complex tissue can be used to study the pharmacodynamics and pharmacokinetics of various drugs and toxins under different conditions and variations in the culture conditions, population density, composition of culture medium and growth rates [31]. In addition to this, transfection can also be used to understand the role of specific vital genes in cell physiology or cell transformation or onset of malignancy. The degree of toxicity, carcinogenicity and interactive effects of various drugs, viruses, and physical or chemical carcinogens, can also be determined easily and efficiently [32]. Clones can be created from a mixed cell population (most native cell lines are known to contain a heterogeneous cell population) and the patterns of individual clones be analysed effectively [24]. Specific proteins or peptides generated or released by different cells can be assessed qualitatively and quantitatively in, a conditioned medium under varied growth conditions. Immunohistochemistry, molecular biology, and immune-electron microscopy are other techniques that can be used to understand the mechanism of cell dynamics easily and efficiently [27].

Applications of cell lines

Cell lines have a wide application in biomedical research, basic research, gene regulation, gene expression and gene transfer studies, pathological studies, radiation biology and germplasm conservation studies [32-34]. Their significant importance and most extensive use are observed in isolation of fish viruses that are agents of epizootic diseases in economically important fish species and for studying host-pathogen interactions [27]. Fish cell lines can also be used for Genomic engineering, Genomic research, immunological investigations, toxicity, and vaccine development [22].

Fish cell lines and virus isolation

Several viruses such as catfish herpesvirus, tilapia lake virus, largemouth bass virus, Koi herpesvirus and gold shiner virus severely impact aquaculture. Fish cell lines are very useful alternative to animals in the field of virology and are known as gold standard for viral disease diagnosis [33]. Diagnosis and confirmation of viral diseases, mode of propagation, isolation, verification, and characterization of viruses can be done easily when cell lines are used [52]. For viral disease diagnosis and confirmation of viral nucleic acids in cells, cell cultures are used for molecular identification assays by the OIE (*Office International des Epizootics*) [33-35]. Fish cell lines have helped to understand in detail the aetiology and pathogenesis of Infectious Pancreatic Necrosis (IPN) and Infectious Hematopoietic Necrosis (IHN), Viral Nervous Necrosis (VNN) causing fish pandemics, and thus helped in improving health of aquatic animals and production efficiency of aquacultural systems [36,38,39,41,48]. To control emerging viruses, the primary step is to understand their pathophysiology, the infectious cycle, method of infection, pathogenicity, probable host range, and measures for inhibition of viral replication, these steps are essential for establishing a complete plan, which involves development of vaccines, antiviral drugs and discovery of efficient management techniques [30,40]. Viral-host cell interactions and virus localization investigations are also important for better understanding of the pathophysiology of viruses. Various cell lines used for isolation of viral pathogenic strains that have been used till date are presented in table 1.

S. No	Cell line designation	Species	Virus identified
1.	CHSE-214	Chinook salmon embryo	IPNV, Salmonid alphavirus and Salmon pancreas disease virus
2.	SAF-1	Gill-head Seabream	LDV IPNV IHNV VHSV
3.	GH GE GSB GL	Tropical Grouper, *Epinephelus awoara*	Susceptible to GIV
4.	FEC	Japanese flounder, *Paralichthys olivaceus*	LCV TRBV
5.	MFF-1	Mandarin fish fry, *SynchiropusSplendidus*	Susceptible to ISKNV
6.	BM BSB	Barramundi, *Lates calcifer*	Susceptible to GIV
7.	GBC 1 GBC 4 GS GS-1	Orange-spotted grouper, *Epinephelus coioides*	Susceptible to GNNV, GSIV-R, ISKNV and GIM
8.	BF-2	*Bluegill Fry*	Lymphocystis virus VHSV EHNV
9.	RTG-2	*Rainbow trout*	VHSV EHNV
10.	FHM	Fathead minnow	VHSV EHNV
11.	EPC	Epithelioma papillosum cyprini	VHSV EHNV

12.	OLCAB-e21	Medaka, *Oryzias latipes*	Resistant to VNNV
13.	OLKaga-e1	Medaka, *Oryzias latipes*	Resistant to VNN
14.	CB CF	Cobia, *Rachycentron canadum*	Susceptible to GIV
15.	Cod ESC	Atlantic cod, *Gadus morhua*	NM
16.	CRF-1 RSBF-2	Red seab bream, *Pagrus major*	Susceptible to RSIV
17.	EAGL	Red spotted grouper, *Epinephelus akaara*	Susceptible to SGIV
18.	BEF-1	North American burbot, *Lota lotamaculosa*	Susceptible to North American genotypes of IHNV, IVa, and IVb of VHSV
19.	CSEC CSH	Half smooth tongue sole, *Cynoglossussemi-laevis*	Susceptible to LCDV
20.	TK TF	Turbot, *Scophthalmus maximus*	Susceptible to TRBIV and Noda-virus
21.	SHK	Spotted halibut, *ve-rasper variegates*	LCDV
22.	bmGH	Brown-marbled grouper, *Epinephelus fuscoguttatus*	Susceptible to TRBIV and LCDV
23.	SPB	Snubnose pompano, *Trachinotusblochii*	Susceptible to GSIV, RSIV, GNNV, CSV and HVA.
24.	SFK	Southern flounder, (*Paralichthys lethostig-ma*)	LCDV
25.	MEF	Mandarin fish	Megalocytiviruses, KHV, SVCV, Chum salmon reovirus and IPNV.
26.	SISK SISS	*Lates calcarifer*	Susceptible to No-davirus
27.	CSK	*Channa straitus*	Susceptible to ma-rine fish nodavirus
28.	SKF-9	Potted knifejaw, *oplegnathus punctatus*	Susceptible to RSIV
29.	CFF	*Pristolepis fasciatus*	Susceptible to TiLV
30.	OnIB OnIL	Nile Tilapia, *Oreo-chromis niloticus*	Susceptible to TiLV
31.	KOK	Common carp, *Cyprinus carpio*	Susceptible to KHV
32.	EMK EMB	Kelp grouper, *Epineph-elus moara*	Susceptible to SGIV and NNV
33.	GiCF	Gibel carp, *Carassius auratus*	Susceptible to CyHV-2
34.	PHF	Iridescent shark, *Pangasianodonhypoph-thalmus*	Cells were refrac-tory to TiLV virus
35.	Neural cells	Senegalese sole, *Solea senegalensis*	Susceptible to beta-nodavirus RGNNV/ SJNNV
36.	KB	Koi carp, *Cyprinus carpio L.*	Susceptible to KHV
37.	TiB	Nile Tilapia, *Oreochromis niloticus*	Susceptible to TiLV
38.	ARBB	Green terror, *Andino-acararivulatus*	Susceptible to CSV, GSIV, RSIV and MEIPNV
39.	OLM	Medaka, *Oryzias latipes*	Susceptible to SVCV, SGIV and GCRV.
40.	Fr994	Rainbow trout, *Oncorhyn-chus mykiss*	Susceptible to ISAV OR KHV
41.	BGA	Mosquito fish, *Gambusia affinis*	Susceptible to RGNNV and SJNNV
42.	AOF	Oscar, *Astronotus ocel-latus*	Not susceptible to CyHV-2
43.	GrE	Chinese rare minnow, *Gobiocyprisrarus*	Susceptible to CRV
44.	TG TH TE	Mozambique tilapia, *O. mossambicus*	Susceptible to TiLV
45.	DMEPF-5	Giant mottled eel, *An-guilla marmorata*	Susceptible to MEAdoV and MERV
46.	CrCB	Silver crucian carp *Caras-sius auratus*	Susceptible to CyHV-2
47.	FtGF	Goldfish, *Carassius auratus*	Susceptible to CyHV-2. High viral titre of 107.8 ±0.26 TCID50/ mL
48.	SGA	Mosquito fish, *Gambusia affinis*	Susceptible to RGNNV and SJNNV
49.	CSK	Tongue sole, *Cynoglossussemilaevis*	Susceptible to GNNV
50.	FuB-1	Killifish, *Fundulusheteroclitus*	Susceptible to SVCV and IPNV
51.	SaB-1	Gilthead seabream, *Sparus aurata*	Susceptible to NNV, IPNV, VHSV and SVCV
52.	CAMB	Hybrid snakehead (*Channa argus* (♂) x *Channa maculata*(♀))	Susceptible to TiLV
53.	OCF	Ocellaris clownfish, *Amphiprion ocellari*	Susceptible to NNV
54.	EL	European eel, *Anguilla anguilla*	Susceptible to Rana grylio virus and the Herpesvirus anguillae

Table 1: Isolation of different virus species using fish cell lines [10,56].

Cell lines and gene expression

Now-a-days, genomic methods are increasingly being used in aquaculture research related to health, toxicity, and early development of fish. A precise amount of gene product is produced in a dynamic manner through a process known as gene expression [42]. The transcriptional regulation, splicing, end modification, export, and destruction are only a few of the stages that are completely regulated [37,42]. Selection arising from harvest procedures has been linked to genomic technologies correlating impacts on functional genes involved in growth, maturity, and life cycle development.

The introduction of new technology and advancement in understanding of fish genomes is building up a fresh viewpoint which is beneficial for conservation and management of wild fish [36]. Cell lines are also being used for studying viral gene expression. The expression of various viral genes using different primer sequences is represented in table 2.

S. No.	Name of the Pathogens	Target viral gene	Primer Sequence	Annealing temperature(°C)	Product size (bp)
1.	FNV	RNA2 Capsid	F-CGTGTCAGTCATGTGTCGCT R-CGAGTCAACACGGGTGAAGA	55	426
2.	TiLV	RNA segment	F-TTGCTCTGAGCAAGAGTACC R-TATGCAGTACTTTCCCTGCC	58	491
3.	CyHV-2	Capsid	F-GAATTCATGTCTAGTCAACAGTACA R-AAGCTTGTTGTAGATGACGCCAGA	55	751
4.	COI	Mitochondrial DNA	F-GTTGGAGGTCTGACTGGA R-GTGTAGGCGTCTGGGTAG	55	296

Table 2: Different cell lines for gene expression [53].

Cell lines in toxicological studies

Cell cultures, especially the cultures produced from liver and kidney are widely used to test the effects of novel medications and chemicals to identify the maximum allowable dosage, either alone or in combination with animal studies. Relative sensitivity of cell cultures from various fish species to medications or pollutants can be investigated easily [37,38]. Cell lines are particularly well suited for chemical fractionation studies due to the modest sample quantities required for cytotoxicity evaluation [49,50]. *In-vivo* investigations in fish are currently the mainstay of toxicological research including fundamental toxicological study, toxicity testing for regulatory purposes, and surveillance and monitoring of the environment [39-41,43-47]. Animal cell cultures may be utilised as a quick and affordable screening technique to assess the toxicity of a variety of different chemicals as well as environmental samples [51]. Automated and high-throughput technologies are used along with cellular test systems as the foundation in ecotoxicological studies [54]. For screening of contaminants and hazard assessment in aquatic toxicology, use of fish models has been demonstrated to be an efficient and sensitive approach. It holds good for the evaluation of genotoxicity of single compounds and complex chemical combinations. Fish cell lines, in particular, have been effectively used to identify genotoxic effects and potentially replace animal testing in early ecological/geno-toxicological research. For this purpose, comet test has been widely employed in fish cell lines [55]. Studies have shown that compared to mammalian cells, fish cells are more sensitive to the genotoxic effects of some environmental contaminants. Fish and mammalian cells differ in their potential for cell development and DNA repair mechanisms, which may be used to explain why fish cells are more sensitive to DNA damage and are more likely to induce it than mammalian cells [58].

Fish cell lines in vaccine development

In recent times, increase in antibiotic resistance and poor import quality has hampered the aquaculture production, which demand vaccine development to control fish infections. Several vaccines are being developed against fish diseases caused by bacteria and viruses. Currently, around 26 fish vaccines have been approved and made available for commercial use. The development of vaccines is based on a thorough understanding of diseases and the immune responses they elicit. Fish cell lines are essential for development of vaccines and optimizing two fundamental parameters that is adjuvant selection and delivery method. Additionally, it is easy to understand and examine the immune responses of cell lines to different vaccine components. Saint-Jean., *et al.* [59] reported SSP-9 cell line as an important candidate for vaccine development against infectious pancreatic necrosis virus (IPNV) and infectious hematopoietic necrosis virus (IHNV). A bivalent DNA vaccine, p Ch-IHN/IPN which provides immunity against IHNV and IPNV has been developed by utilizing EPC (*Epithelioma papulosum cyprini*) [60]. After identifying and characterizing new viral infections from epidemiology, pathology, aetiology and host immune responses, cell culture has been extensively used for vaccine development against the fish pathogens. Additionally, preparation techniques and immunisation strategies are cited in the creation of specialised vaccines in relation to these disorders. Transcriptomic and proteomic investigations of vaccines in cell lines have been largely utilized to identify differentially expressed genes and vaccine targets. Cell culture research revealed new immunogenicity pathways in teleost, such as the TANK binding kinase 1 (TBK1) during DNA vaccine induction. In addition to the fish vaccines, human and animal vaccines have also been developed by using fish cell lines. Zebrafish cell lines have recently been used to investigate the safety and efficiency of human vaccines because of their important genetic, anatomical, and physiological similarities with humans [61].

Cell cultures may be used to produce vaccines in large quantities for their commercial availability [57]. Koi fin cell culture from wild type koi has been used to develop koi herpesvirus vaccine. The use of fish cell cultures in the research and manufacturing of bacterial vaccines appears to be promising as well. A live-attenuated *Edwardsiella ictaluri* vaccine has proved to be effective when given orally to prevent Enteric septicaemia of catfish (ESC), that is caused by *E. ictaluri* [62-64]. Table 3 shows use of cell lines for developing various vaccines.

Disease	Pathogen	Major Fish Host	Vaccine Type	Antigen Target	Country/Region
Infectious hematopoietic necrosis	IHNV	Salmonids	DNA	G Glycoprotein	Canada
Infectious pancreatic necrosis	IPNV	Salmonids, Sea bass, Sea bream, Pacific cod, Turbot	Inactivated	Inactivated IPNV	Norway, Chile, UK
			Subunit	VP2 and VP3 capsid proteins	Canada, USA
			Subunit	VP2 proteins	Canada, Chile, Norway
Infectious salmon anemia	ISAV	Atlantic salmon	Inactivated	Inactivated ISAV	Chile, Norway, Ireland, Finland, Canada
Pancreatic disease virus	SAV	Salmonids	Inactivated	Inactivated SAV	Chile, Norway, UK
Spring viremia of carp virus	SVCV	Carps	Subunit	G Glycoprotein	Belgium
			Inactivated	Inactivated SVCV	Czech Republic
Koi herpesvirus disease	KHV	Carps	Attenuated	Attenuated KHV	Israel
Infectious spleen and kidney necrosis	ISKNV	Asian seabass, grouper, Japanese yellowtail	Inactivated	Inactivated ISKNV	Singapore

Table 3: Vaccines developed against different viral infections by using cell lines [11,57].

Conclusion

Cell line development is gaining speed from the past two decades in biological sciences in order to facilitate absolute subjects for research and development. Cell lines especially fish cell lines are becoming increasingly important for the studies related to conservation, toxicology and vaccine development. Therefore, these should be made available in international cell repositories, such as, ATCC, EACC or other appropriate institutions, so that a high-quality consistent source of cells is accessible to the researchers worldwide.

Currently, methods for developing fish cell lines for the production of recombinant therapeutic proteins are mostly empirical. With the passage of time and improved procedures, highly productive cell lines are expected. Grade products may also be obtained through genetic engineering of parent cell lines or by optimizing culture conditions. Since the researchers are now effortlessly developing primary cell cultures, and in combination with recent technologies like 3D cell culture it is expected to make a better platform for more precise results in future. Collection, maintenance, and distribution of biomaterial for conservation programmes are a considerable problem. Prioritizing species is necessary so that the selected endangered species may be safeguarded in the bio-bank. In order to successfully implement assisted breeding techniques like bio-bank gametes, the preplanning phase of the bio-bank should also entail the creation of a wealth of information on more fundamental integrative research on the species physiology and reproductive biology.

Cell features can alter and sometimes become very different from those present in the initial population over a period of continuous expansion. By altering the activity of their enzymes, cells may also adapt to various culture conditions (such as changing nutrients, temperatures, salt concentrations, etc.). Expertise is required to handle and check for cross contamination, microbiological contamination, and chemical contamination. There is a need to set up a controlled atmosphere at work so that biohazards may be incubated, contained, and disposed off. In addition, capital equipment, consumables, medium, serum, and plastics used for cell culture are ten times more expensive than employing animals directly. Variability across passages caused by heterogeneity in growth rate and capability for differentiation within the population is another major drawback.

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