

National Repository of Fish Cell Lines:

A potential fish cell line depository for tomorrow's cellular agriculture

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Cell lines developed from various fish organs are largely used as in vitro models in aquatic animal disease surveillance, pharmacology, toxicology and resolving metabolic abnormalities. Cellular agriculture is another promising sector to increase agrarian production through use of cell culture. Recently, in vitro model is also tested for cell-based meat production that may help in reducing the pressure on open water fishing and create a safe and sustainable food industry. Further, the augmented use and development of cell lines open the avenue of establishing cell line depositories, where cells could be deposited, stored/preserved and made available to researchers for further research and applications. The National Repository of Fish Cell Line (NRFC) at ICAR-National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, is a cell line depository that holds the world's largest collection of fish cell line accessions. This facility holds several fish muscle cell lines too that could be utilized for developing cell-based fish meat in labs. The depository provides easy access of fish cell lines to researchers on demand for various R&D applications, such as disease diagnosis, drug screening, cytotoxicity, cytology, gene expression studies, cell-based meat production etc. This article presents the status, and applications of fish cell lines and summarizes potential, issues and ways forward.

Keywords: Cell-based meat, Cell line, Disease, NRFC, Repository

THE availability of food to everyone in the desired quantity is always of great concern worldwide. Today, food and nutritional security are important challenges globally due to increasing population pressure, declining resources and a degrading environment. Among other food items, fish is considered as 'nature's superfood', being a rich source of proteins, healthy fats (such as omega-3 fatty acids), iodine, vitamin D, calcium etc. Per capita fish consumption in India was 5-6 kg in the year 2019-20 (<https://pib.gov.in/FactsheetDetails.aspx?Id=148557>) against the global consumption of 20.5 kg in 2018 (<https://www.fao.org/state-of-fisheries-aquaculture/2020/en/>). The World Health Organization (WHO) recommends the consumption of 12

kg of fish in a year, which India is expected to achieve by 2024-25. The rate of growth in fish production in India was over 10% from 2020-21 to 2021-22 with a 19.0 million tonnes fish market size in 2022, which is expected to reach 31.7 million tonnes by 2028 at 8.81% compound annual growth rate during 2023-2028. The fishery and aquaculture sectors have added over ₹ 1.5 trillion in gross value to agriculture across India in the year 2020. Per capita fish consumption is continuously increasing over the past few decades and is expected to increase too in coming years. FAO opined the world's appetite for fish and fish products shows no sign of slowing. To meet the growing demand for the blue food, we need to utilize the diversity of blue foods and stand-

up with the new innovations using cutting-edge technologies, such as cellular agriculture.

Cell line applications

As limiting factors, the pathogens (mainly bacteria, fungi and viruses) present in aquatic surroundings cause diseases and hamper fish production from freshwater, brackish water or marine ecosystems. There has been amplified incidences of fish diseases, especially viral infections, with the increase in intensive aquaculture systems and transboundary movement of high-value ornamental fishes. Accurate identification and isolation of these pathogens and testing of suitable chemicals/ drugs for their extermination is possible with the help of fish cell lines. These cell lines

are important potential biological resource with several applications in areas, like production of cell-based meat, disease diagnosis and virus isolation, toxicology and gene expression studies, cytogenetic and genotoxicity screening studies, biobanking and cryopreservation for conservation efforts etc. They are the ethical alternative to whole fish specimen for biological research.

Disease monitoring: Many fish cell lines have been tested for their susceptibility to fish viruses, such as nodavirus (NV), infectious pancreatic necrosis (IPN) virus, nervous necrosis virus (NNV), cyprinid herpes viral hematopoietic necrosis virus (HVHN), tilapia lake virus (TiLV), red seabream iridovirus (RSIV), white spot syndrome virus (WSSV) etc. The identification and isolation of viruses using fish cell lines have helped to screen infected individuals and chalk out strategies to reduce infection and boost aquaculture production. As per FAO, the TiLV, reported in Israel, Columbia, Ecuador, Egypt and Thailand, is associated with significant mortalities in farmed tilapia, valued at over US\$ 9.8 billion. Researchers were able to alert the Indian aquaculture industry by isolating and identifying this virus using fish cell lines. Another example is the isolation and first report of RSIV disease from India, which affects marine species, mainly red sea bream. With global production exceeding 50,000 tonnes of this species and the availability of its cell lines, it is expected that a better understanding of the pathogen and its mechanism of pathogenicity will be attained to manage the disease caused by the virus. Identification and isolation of fish nodavirus, responsible for severe mortality in sea bass hatchery, for the first time in India is another example. Other than

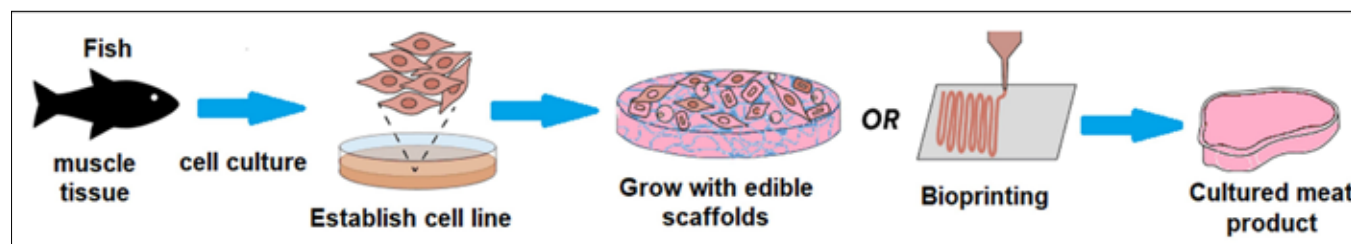
viruses, fish cell culture has also been used in the detection of bacterial pathogens, like *Estrella lausannensis*, *Parachlamydia acanthamoebae*, *Photobacterium damsela* subsp. *damsela*, *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *Rickettsia* spp., *Vibrio anguillarum*, *Waddlia chondrophila*, *Yersinia ruckeri* etc., which are causing huge losses in fish production.

Environmental monitoring: There has been an increased usage of artificial inputs in the new aquaculture systems. Farmers facing fish health problems have resorted to manage their ponds involving the use of chemicals and therapeutics. Primary cell cultures and cell lines can be important tools in ecotoxicology studies, like the assessment of cytotoxicity and estrogenic activity of chemicals and environmental samples. One of the important advantages of using cell lines, as *in vitro* model, is that it enables control over both the macro and micro-environments, such as pH, osmolarity, temperature and nutrient components. Cell line produces a higher number of cells that can be replicated in low volumes and stored for a long time with good recovery. Studies on cells isolated from living organisms are routinely used to assess the safety and possible action mechanisms of drugs and other compounds. Researchers have applied cell culture methods to perceive the underlying mechanisms of the immunomodulation effects of heavy metals, pesticides, fungal toxins etc. and their relationships with the fish health. Fish cell lines also widely allow comparing different species for their relative sensitivity at the cellular level to various environmental contaminants, like heavy metals, pesticides and other aquatic pollutants coming

from agricultural runoffs. Such information will be helpful in ranking pollutants on the basis of their cytotoxic effects and help in monitoring aquatic environments. The development of recombinant fish cell lines may further support their role as a bioanalytical tool for environmental assessment.

Cellular agriculture: Cellular agriculture is one of the promising and potential technologies to increase the production of some agricultural commodities. It basically applies the cell culture tools along with a combination of molecular and synthetic biology approaches to produce specific agricultural foodstuffs *in vitro*, which are otherwise harvested from natural means. The cultured meat sector has driven a long way since 2013, when the first cultured meat prototype was demonstrated by Dr Mark Post of Maastricht University in the Netherlands. Recent reports indicate an increasing trend of companies dealing with cultured meat. There are around 99 companies reported worldwide till the start of the year 2023, in comparison to 4 in the year 2016, which are involved in different aspects of developing cultured meat. It is also predicted that about 60% of meat will come from culture-based meat industries by the year 2040 and will be sold worldwide by grocery stores, retail shops, restaurants etc.

This cellular technique, through a safe and sustainable food industry, would also help in reducing the fishing pressure on certain fish stocks. Technology potential is an exciting opportunity and emerging solution to support the increasing population and also to address animal welfare and sustainable environmental challenges associated with the intensive aquaculture systems. Cell-based fish meat techniques may also be employed



Schematic presentation of procedure of cultured/ lab meat production

for the species that have commercial demand, but are either expensive to harvest or are not bred in farms, like mahi-mahi, crustaceans etc. Researchers in the USA, Israel and the Netherlands have advanced the idea of producing lab-grown meat from cell culture rather than whole animals. As proof of concept, lab-grown chicken meat has been developed and is fast approaching commercial viability. It has been reported by many researchers that the physiological properties of fish cell lines, like tolerance to hypoxia, high buffering capacity and low-temperature growth conditions, are most suited to *in vitro* cultivation making fish cell culture an attractive opportunity for the production of cell-based fish meat. Certain legislative and regulatory framework for the production and distribution of cell-based fish meat are already underway in Singapore, Europe and America. Recently, Singapore approved cultured meat produced in bioreactors for sale. A few start-up companies are already working on developing cell lines from a few commercial species, like salmon, yellowtail, bluefin tuna and shrimp, and have conducted taste test of their products for consumer adaptation.

Cell line depositories

The increased use of cell lines in such potential areas is also reflected by the increased interest in developing fish cell lines, wherein 918 fish cell lines have been reported worldwide (<https://www.cellosaurus.org/> Release 47 of October 2023). In the past decade alone, 280 new fish cell lines were established from 44 fish families in 21 countries. Maintenance and long-term storage of cell lines are as important as developing these cell lines. Depositing a cell line in a depository not only provides recognition to the developer, but also enables other researchers to obtain and use them, preventing duplication of work. These depositories are important not only to store and preserve genetic material but can also be useful as a conservation tool in case of certain

exigencies, such as species loss due to natural calamities, over fishing etc. American Type Culture Collection (ATCC), European Collections of Cell Cultures (ECACC), German Collection of Microorganisms and Cell Cultures (DSMZ) etc. are some popular and trusted depositories of cell lines. It is observed that the majority of the fish cell lines in these depositories belong to temperate freshwater species, having commercial importance in Europe and America. With extensive research in aquaculture and fisheries in Asian countries, like China, India, Vietnam, Thailand etc., there has been an increase in the development of cell lines from the Asian continent. Thus, there emerging needs to cater the demand of the cell lines of Asian fish species, which led to the establishment of cell line depositories.

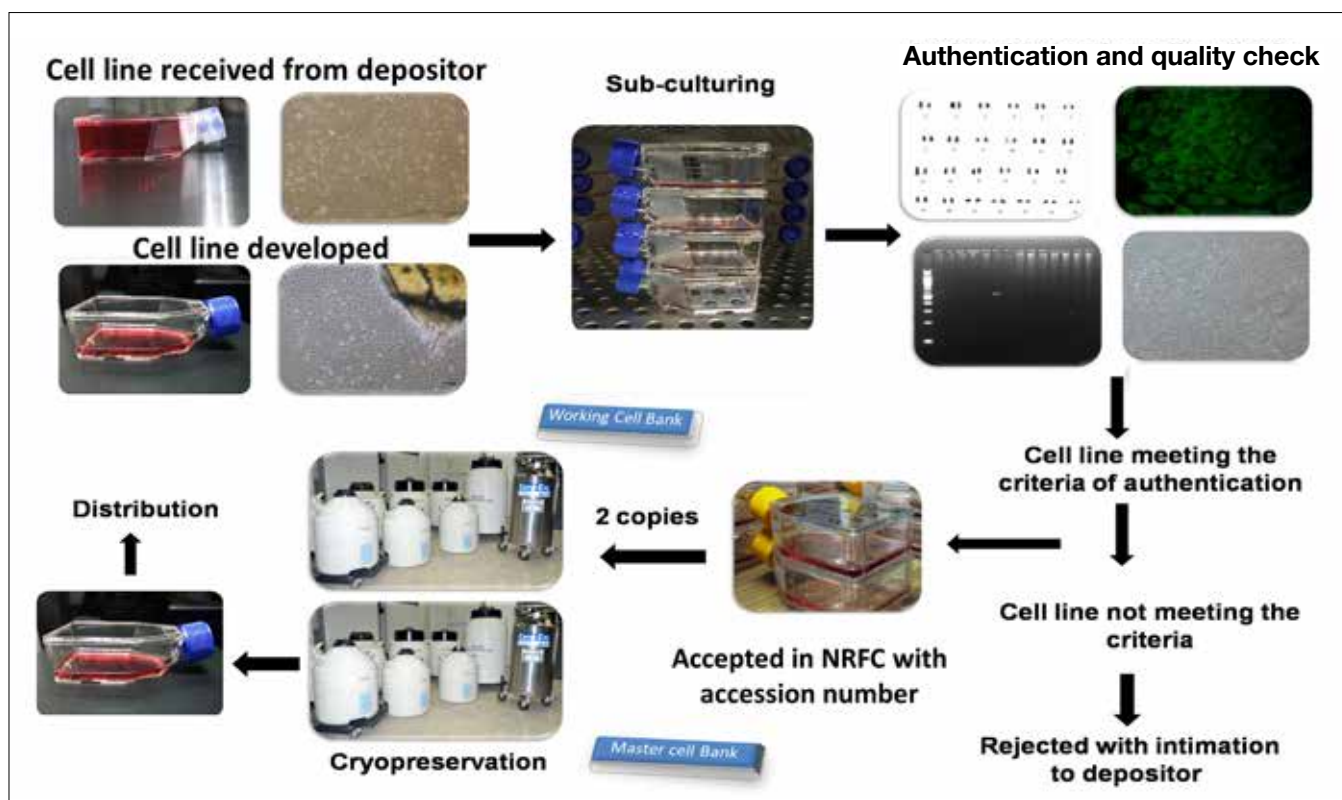
To provide a platform to deposit fish cell lines developed in India, a depository, named National Repository of Fish Cell Lines (NRFC), was established at ICAR-National Bureau of Fish Genetics Research (NBFGR), Lucknow, India, in 2010 with the financial support of Department of Biotechnology, Ministry of Science and Technology, Govt. of India, New Delhi. The aim of NRFC is to develop, deposit, maintain and distribute authenticated fish cell lines on request. It is a designated repository by the Ministry of Environment and Forests, Government of India, under sub-section (1) of Section 39 of the Biological Diversity Act, 2002 in the Fish Genetic Resources category. It has state-of-the-art facilities for cell-transfection, automated cell-counting, DNA sequencing capabilities etc. Today, NRFC has the world's largest collection of fish cell lines with 81 cell line accessions developed from 16 tissues of 39 fish species (29 freshwater, 3 brackish-water and 7 marine species) belonging to 32 genera, 16 families and 8 orders. The Bureau has deposited 44 of these cell lines, while 19 cell lines is being contributed by C. Abdul Hakeem College, Vellore, Tamil Nadu; 12 by ICAR-CMFRI, Kochi, Kerala; 4 by

FCRI, Tamil Nadu, and 2 by ICAR-DCFR, Bhimtal, Uttarakhand. Many of the cell lines are derived from the fin tissue (Table 1). The NRFC has a detailed strategy to develop, deposit, sub-culture, authenticate and distribute cell lines on demand. Researchers developing fish cell line may also submit their cell line(s) to NRFC for effective maintenance and distribution to other researchers/ academicians for research and development works.

Table 1. Details of cell lines originated from different fish tissues and maintained in NRFC

Tissue	Number
Fin	27
Gill	10
Eye	6
Heart	7
Spleen	5
Kidney	4
Caudal peduncle	4
Brain	4
Muscle	4
Thymus	4
Liver	2
Testes	1
Barbels	1
Peritoneal	1
Blood lymphocytes	1

These cell lines have extensively been used to study the susceptibility of cells to various viruses, such as *viral nervous necrosis virus* (VNVN), *cyprinid herpes viral haematopoietic necrosis virus* (CyHV2), *tilapia lake virus* (TiLV), *red sea bream iridovirus* etc. and also to assess cytotoxic effects of extracellular components from various bacteria and numerous heavy metals as well as pesticides. The repository is continuously supplying authenticated and contamination-free fish cell lines to researchers through a Material Transfer Agreement (MTA) within 15–20 days of request. The NRFC has provided critical inputs to India's National Surveillance Programme on Aquatic Animal Disease (NSPAAD) to tackle the threat of aquatic diseases. It enabled the researchers to study the first report of *Cyprinid herpes virus-2* in goldfish



Strategy followed at NRFC for collection and distribution of fish cell lines

in India. Since August, 2013, a total over 130 fish cell line requests have been processed and distributed to researchers all over India for R&D works. ICAR-NBFGR through NRFC has conducted several short-term hands-on training programmes to build human resource capacity and supported researchers and students working on cell lines.

Some of the issues like cross-contamination with other cell lines or with mycoplasma is a serious problem faced by researchers working on cell culture. It has been reported that almost 1/3rd to 1/5th of the cell lines have been misidentified. Hence, accessing authentic cell lines becomes important for the right interpretation of experimental results. NRFC ensures that the developed/ submitted cell lines are authenticated and fully characterized before assigning the accession number to them. Once a cell line is received from a depositor, it is subjected to various checks. A mycoplasma detection

test is performed to ensure that only mycoplasma-free cell lines are accepted. DNA sequencing of COI and 16S region is undertaken to authenticate the species of origin of the cell line. Chromosome preparation and immunostaining is performed to offer more information about the cell line. Only when the cell lines clear all the tests, an accession number is provided and the cell line accepted is for deposition in the depository. This ensures that researchers are able to work on authentic and contamination-free cell lines. For long-term storage, the cell lines are cryopreserved using liquid nitrogen. There is scope to further expand NRFC based on the intensity of application in future.

SUMMARY

New and innovative technologies are always needed to meet the food security and nutritional requirements of every individual in the growing global population. Cellular agriculture

is one such technology to increase the production of agricultural commodities. Fish cell lines are important biological resources with several applications in key areas, like disease diagnostics, toxicology studies, genetic material conservation and lab-grown meat, that can supplement food and nutritional security. It is expected that the use of fish cell lines will increase in such innovative areas in the coming years. Cell line depositories, like NRFC, would help in screening pathogens, especially those originating from the cross-boundary movement of fishes. The availability of many fish cell lines at NRFC also opens up avenues for Indian researchers to use it for various applications in genomics, cytology, toxicology and disease management and to explore cell-based meat development for increasing the food basket.

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