National repository of fish cell lines: accelerating fish cell line research in fisheries and aquaculture

Fish cell line is an important biological resource with potential applications in several fields of life sciences. After the development of the first fish cell line (i.e. rainbow trout, *Salmo gairdneri*, gonadal cell line, named as RTG-2) by Wolf and Quimby in 1962, several fish cell lines have been developed[1]. These cell lines became popular due to certain advantages over the traditional animal cell line models. Fish cell lines are being widely employed for numerous applications such as virus isolation, disease diagnosis, eco-toxicology, cytology, developmental biology and gene expression studies.

Fish cell lines, being of poikilothermic origin, grow well at room temperature without thermo-regulated incubators, unlike mammalian cell lines. Moreover, the Leibovitz-15 (L-15) medium, used in fish cell lines, does not require CO_2 buffering action, thus CO_2 incubators are not strictly necessary and the cells can grow in BOD incubators itself. In recent years, changing aquaculture practices has made fish cell culture an important tool for researchers. Lakra *et al.*[1] listed 283 fish cell lines developed across the world encompassing both marine and freshwater species. But maintenance and storage of cell lines are as important as developing the cell line. If proper care and checks are not maintained, it is possible that the cell lines may be lost due to natural senescence, contamination, breakage, electrical or equipment failure, etc. Possibility of loss of cell lines may also occur due to unforeseen natural calamities such as floods and earthquakes. Hence, it is imperative that these valuable cell lines need to be stored at multiple places, in addition to the developer's lab. This is where the concept of a repository becomes essential. Depositing a cell line in a repository not only provides recognition to the developer, but also enables other researchers to obtain and use the cell lines preventing duplication of work.

American Type Culture Collection (ATCC), European Collections of Cell Cultures (ECACC), German Collection of Microorganisms and Cell Cultures (DSMZ), RIKEN (Japan) and CellBank (Australia) are the major repositories in the world with large collections of human, animal, microbial, hybridomas, etc., but few fish cell lines are also being maintained by them. The National Centre for Cell Sciences (NCCS), Pune, is one of the most popular and trusted cell line repositories in India, but it is maintaining very few fish cell lines.

Since only a few fish cell lines were available with popular repositories, the National Repository of Fish Cell Lines (NRFC) was established in 2010 at ICAR-NBFGR, Lucknow, with financial support from DBT, to accelerate fish cell culture research. At present NRFC (http://mail.nbfgr.res.in/nrfc/) with 63 fish cell line accessions, is one of the largest collections of authenticated fish cell lines in the world (Figure 1). Of these, 28 cell lines were developed, characterized and deposited by ICAR-NBFGR and the rest were deposited by other researchers across India.

Fish cell lines can be used in a variety of applications. The most widely used applications are in virology, toxicology and cytological studies. The institute is regularly employing cell lines to determine inhibition constant (IC50) values of toxicants such as like heavy metals (mercury, cadmium, arsenic, chromium), pesticides (pethoxamid, tembotrione) and other chemicals/pollutants. The institute has worked extensively on studying susceptibility of the fish cell lines to the viruses such as viral nervous necrosis virus (VVNV), cyprinid herpesviral haematopoietic necrosis virus (CyHv-2), tilapia lake virus (TiLV), etc. and also on the cytotoxic effects of extracellular components from various bacteria using cell lines. Bureau is also actively working on developing new cell lines of fish species with potential aquaculture upscaling. The institute is planning to develop cell-specific lines, like hepatocytes, etc., in the future.

Germplasm conservation is a complex subject that requires a combination of scientific research, effective management strategies and coordination among various implanting agencies to achieve upright results. Cell lines act as a tool for *ex situ* germplasm conservation. Stem cell lines/induced pluripotent stem cells (iPSCs) can support conservation programmes. All the authenticated and available cell lines in the repository have been stored at ultra-low temperature of –196°C in LN2 for future use. NRFC has six cell line accessions of fishes that are vulnerable, endangered and near threatened according to IUCN Red List categories. It is expected that in the years to come, when the success rate of cloning improves, the availability of cell lines of endangered species can help in conservation strategy.

Figure 1. Screenshot of ICAR-NBFGR, NRFC web-page.

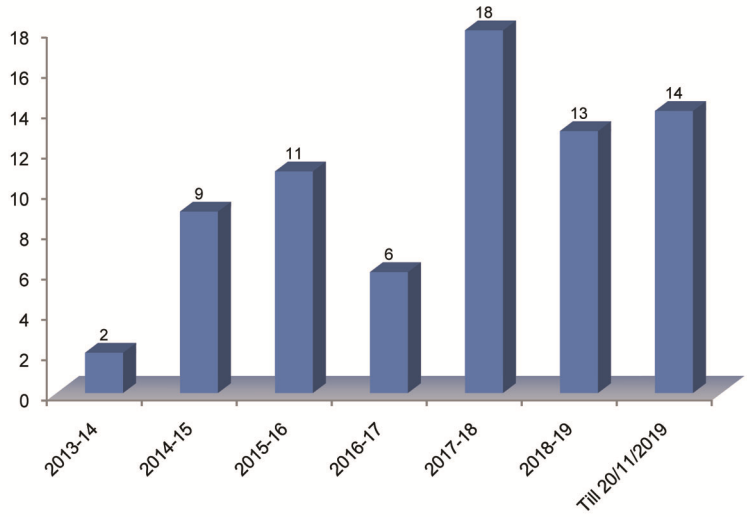

Figure 2. Year-wise total fish cell lines distributed.

The repository has served to provide continuous supply of authenticated and characterized fish cell line to researchers, in India with a transparent Material Transfer Agreement (MTA). Mycoplasma-free cell lines are supplied within 15–20 days of request. From August of 2013 till date, a total of 73 fish cell line requests have been processed and distributed to researchers all over India for R&D works (Figure 2). Currently, out of 63 cell lines belonging to 33 fish species, 24 species are freshwater, 7 marine and 2 brackish-water. In the last 7 years, majority of the cell line requests were mainly for freshwater and brackish water aquaculture species. Further, most requests were for conducting virological and toxicological research on these cell lines. This year, the most frequently requested was OnlL cell line to conduct research on Tilapia lake virus. This indicates that fish cell line research is shifting from basic to application-orientated work.

The development, characterization and maintenance of a cell line require specialized skill which comes with proper training and practical experience. ICAR-NBFGR realized that there is a paucity of skilled workforce in the field of fish cell culture in the country. The fruits of fish cell line repository can be reaped only when the cell culture techniques are transferred to researchers. Hence, the institute has conducted several short term training programmes to support researchers and students working on cell lines. About 68 researchers have successfully been trained in the last few years. It is hoped that more researchers would get training support in the years to come.

It is expected that the use of cell lines, especially fish, is bound to increase in the coming years. Intensification of aquaculture practices and increase in the global ornamental trade may bring in new challenges and novel pathogens which may need to be screened. Cell line repository would be helpful to screen the pathogens against many authenticated fish cell lines. Researchers would be able to easily access the relatively large collection of fish cell lines at NRFC and use it for various applications such as disease diagnosis, cytotoxicity, cytology and gene expression studies.

1. Lakra, W. S., Swaminathan, T. R. and Joy, K. P., *Fish Physiol. Biochem.*, 2011, **37**(1), 1–20.
2. Wolf, K. and Quimby, M. C., *Science*, 1962, **135**, 1065–1066.

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