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FISH CELL LINES AND ITS APPLICATIONS FOR DISEASE MANAGEMENT IN MARICULTURE

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Fish cell lines have an important role in the expanding aquaculture industry of the world. The main impetus for the development of many of the continuous fish cell lines was to provide the means for isolating and identifying viruses that are the causative agents of epizootics of commercially important species. Cell lines are indispensable for development of precise diagnostics and prophylactics of the viral pathogens. Besides diagnosis, cell lines are important in the much required national and international quarantine and certification programme for producing virus free fish stock. Fish cell lines have also found widespread application in cytogenetics, transgenics, toxicology, as *in vitro* models for studying cellular physiological processes and also in comparative immunology.

One of the major limiting factors in successful hatchery rearing, marine fish farming and aquariculture is the occurrence of diseases, specifically those caused by pathogenic viruses. Disease losses have traditionally been high in some groups of fish, particularly those that are emerging species of importance. Increasingly, new diseases of probable viral origin have been growing in groups of marine fish causing severe losses. In many parts of Asia with mariculture activities, diseases due to viral etiology, such as the viral nerval necrosis virus (VNN) and iridoviruses have been reported. Relatively little is known about the epidemiology of viral infections in tropical fish from our region. Many of these viruses may be capable of causing latent infections in wild and cultured fish with onset of disease, triggered only under poor husbandry and management conditions. The increase in trade and movement of fish amplifies the potential for spread of these viruses through latently infected fish.

Viruses are known to be important pathogens to many species of aquacultured fish and often cause mass mortality of affected species. Unlike other microorganisms, which can be readily grown in artificial nutrient medium, viruses are obligatory intracellular pathogens and their isolation and propagation are totally dependent on the availability of a live host, such as permissive cell cultures. In addition, most viruses are host-specific and tissue-specific, and they can only be isolated and propagated using cell lines established from tissues of the same/related host species.

An appropriate cell line is the most important laboratory tool to grow, isolate, characterize, and identify pathogenic fish viruses. However, cell lines currently do not exist for most of the farmed marine food fish and ornamental fish. The lack of appropriate cell culture systems hinders the development of preventive strategies for viral diseases and the inspection of batches of juvenile fish for health certification. Thus, appropriate cell lines necessary for the cultivation and isolation of pathogenic viruses from aquaculturally important fish species need to be developed. These cell cultures will become an essential tool for the detection and surveillance of pathogenic fish viruses and will be important to the fish health assurance programme of the aquaculture industry.

Most of the established fish cell lines were derived from cold water fish, such as salmonids, channel cat fish and common carp. However, many new continuous cell cultures are constantly being developed as a result of intensive efforts in several parts of the world, to provide cell cultures from local species utilized in aquaculture. Although many fish cell lines have been established for isolating and identifying fish viruses, relatively few marine fish cell lines are available. Since cell cultures derived from the same species or a species closely related to that in which the disease occurs would be the most sensitive for virus isolation, cell lines derived from local species should be given high priority. The host and tissue specificity of virus underlines the need for developing cell lines from different species in different regions.

The present knowledge on various aspects of fish viral diseases has come mainly from temperate countries from fishes like salmon, trout and channel cat fish. The rapid expansion of aquaculture and associated viral diseases in

North America, Europe and Japan led to the subsequent development of several fish cell lines for health management purposes. Most of the early cell lines were derived from cold water fish species.

RTG-2 cell line of rainbow trout *Salmo gairdneri* gonad origin initiated in 1960 was the first permanent fish cell line to be developed (Wolf and Quimby, 1962). Clem *et al.* (1961) initiated trypsinised blue-striped grunt, *Haemulon flavolineatum*, fin cultures which provided GF-1 cells, the first line of marine fish origin. Several cell cultures and cell lines from a variety of fishes have been developed since the first cell line from rainbow trout. Teleost cells are the 2nd most numerous among the animal cell lines which have been developed, mammalian cells being the most numerous. A comprehensive list of most fish cell lines developed before 1980 has been published (Wolf and Ahne, 1982). In addition, several comprehensive reviews of maintenance and application of cell cultures all from temperate fishes are available.

Although a large number of fish cell lines (~159) have been established for isolating and identifying fish viruses (Fryer and Lannan, 1994), relatively few marine fish cell lines (~34) are available (Chi *et al.*, 1999). Recent interest in marine fish cytogenetics, immunology and pathology, together with other *in vitro* applications, has given rise to the need for improved methods for the isolation, handling and culture of cells from marine fish. The limited numbers of reports on viruses from marine fish compared with those from freshwater fish are due to the shortage of fish cell lines derived from marine fish. The research on marine fish cell lines has progressed rapidly in recent years and several cell lines from tissues of commercially important marine fish have been described since 1980.

Three continuous cell lines have been established from gonads of Japanese striped knife jaw, *Oplegnathus fasciatus* (JSKG cell line), embryos of a hybrid of kelp (*Epinephelus moara*) and red spotted grouper *E. akaara* (KRE cell line) and skin of greater amberjack (also called purplish amberjack) *Seriola dumerili* (PAS cell line) (Fernandez *et al.,* 1993). A continuous cell line (SAF-1) was developed from fin tissues of an adult gilt head sea bream, *Sparus aurata* (Bejar *et al.*, 1997). The GF-1 cell line derived from the fin tissue of a grouper, *Epinephelus coioides* (Hamilton) (Chi *et al.,* 1999) can effectively proliferate fish nodavirus and is a promising tool for studying fish nodavirus. Two iridovirus-susceptible cell lines were established and characterised from kidney and liver tissues of the grouper, *Epinephelus awoara.* These cell lines have been designated GK and GL, respectively (Lai *et al.*, 2000). A tropical marine fish cell line (SF) was established from fry of Asian seabass, *Lates calcarifer* (Chang *et al.,* 2001).

Pluripotent embryonic stem cells (PES) provide an efficient approach for genome manipulation with many applications in marine biotechnology and developmental studies. Chen *et al.* (2003) developed a pluripotent cell line, LJES1 from blastula stage embryos of the sea perch, *Lateolabrax japonicus*.

Kang *et al.* (2003) established and characterized two cell lines, FFN cells from the fin tissue and FSP from the spleen tissue of the flounder, *Paralychthys olivaceus*. Both the cell lines were found susceptible to a wide range of fish viruses such as IPNV, marine birna virus, chum salmon virus, IHNV, SVCV and hirame rhabdovirus. Four tropical marine fish cell lines were established from the eye, fin, heart and swim bladder of the grouper *Epinephelus awoara* by Lai *et al.* (2003).

A continuous cell line TEC (turbot embryonic cell line) was established from embryos at the gastrula stage of a cultured marine fish, turbot (*Scophthalmus maximus*) (Chen *et al.*, 2005a). Qin *et al.* (2006) described the development and characterization of a tropical marine fish cell line (GS), derived from the spleen of orange spotted grouper, *Epinephelus coioides.* It is suggested that the GS cell line has good potential as a diagnostic tool for isolation and propagation of iridovirus and nodavirus. When the GS cells were transfected with pEGFP vector DNA, significant fluorescent signals were observed suggesting that the GS cell line can be used as a useful tool for transgenic and genetic manipulation studies.

Fish tissue culture work is relatively new in India. Sathe *et al.* (1995) established a cell line (MG-3) from gills of mrigal, *Cirrhinus mrigala* and characterized it with respect to isoenzyme pattern and chromosome number. Moreover, electron microscopic studies were also carried out which revealed the cellular structure and also secretory nature of the cultured cells. Sathe *et al.* (1997) have also developed a cell line from gill of rohu, *Labeo rohita.* Primary cultures

were developed from the kidney of freshwater fish, *Heteropneustes fossilis*, by employing certain modifications in conventional procedures and a number of clones of cells have been developed (Singh *et al.,* 1995). Lakra and Bhonde (1996) were successful in developing primary cultures from the caudal fin of rohu, *Labeo rohita.* Primary cultures from various tissues of Indian major carps (Rao *et al.,* 1997), caudal fin of *Tor putitora* (Prasanna *et al*., 2000), and ovary of *Clarias gariepinus* (Kumar *et al*., 2001) have also been reported. Unfortunately, none of these cell lines/ cell cultures were maintained for long-term applications (Kumar *et al*., 2001). Kumar *et al*. (2001) developed a cell culture system from the ovarian tissue of African catfish, *Clarias gariepinu*s. The cell culture could be passaged 15 times after which they ceased to multiply and consequently perished.

Lakra *et al*. (2005) assessed the potential of six tissues of *Labeo rohita viz.* kidney, liver, heart, gill, caudal fin and swim bladder for attachment, cell growth and proliferation by explant culture and trypsinisation methods. Lakra *et al.* (2006) reported the development of a diploid cell line (TP-1) for the first time from fry of golden mahseer, *Tor putitora*. Sahul Hameed *et al*. (2006) established and characterized India's first marine fish cell line (SISK) from kidney of sea bass, *Lates calcarifer.* The cell line was found to be susceptible to two marine fish viruses. Parameswaran *et al.* (2006), described the establishment of an embryonic cell line from Indian sea bass (SISE) derived from blastula-stage embryos of *L. calcarifer*. Parameswaran *et al.* (2006) also described the development and characterisation of a marine fish cell line (SISS), derived from the spleen of sea bass. Two cell culture systems namely epithelioid cells of *Lates* (LCE) and fibroblastic cells of *Lates* (LCF) have been developed from fry and fingerlings of *L. calcarifer* (Lakra *et al.,* 2006).

Cell culture systems are biological entities with specific physiological needs, much like any other laboratory animals. They require ongoing care, adequate nutrition, a proper environment and regular check ups. The fish health biologists must provide the cultures with an optimum environment for survival. If this environment is not provided, the cells can be unacceptable for viral testing. Every cell line cultured must also be backed up by cells in frozen storage. Therefore cryopreservation of fish tissue cell lines is very important. Freezing in liquid nitrogen or in ultra cold freezers is the method of choice for storage of fish cell lines. The medium used for freezing fish cells generally contains 10% or more serum and either of the two cryoprotectants – glycerol or dimethyl sulphoxide (DMSO) added to a final concentration of 5 – 10%.