

Development and application of fish cell lines: a review

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ABSTRACT

Cell culture has become one of the major tools used in the life sciences today. Tissue Culture is the general term for the removal of cells, tissues, or organs from an animal or plant and their subsequent placement into an artificial environment conducive to growth. This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semisolid medium that supplies the nutrients essential for survival and growth. The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called Organ Culture. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called Cell Culture. In this paper, development and application of fish cell lines are discussed.

Key words: Fish, Cell Lines, Stem Cells

Since 1965, some 157 fish cell lines have been established which represent 34 families of fishes (Fryer and Lannan, 1994). A comprehensive list of most fish cell lines developed before 1980 has been published (Wolf and Ahne, 1982) and several comprehensive reviews of maintenance procedures and applications of fish cell cultures are available (Sigel and Beasley, 1973; Wolf, 1973; Wolf and Ahne, 1982). Most of fish cell lines were derived from freshwater or anadromous fish species. The limited number of reports on the viruses from marine fish compared with those from freshwater fish is due to the shortage of fish cell lines derived from marine fish. The studies of marine fish cell lines have developed rapidly in recent years and at least 17 cell lines from tissues of commercially important marine fish have been reported since 1980 (Fernandez *et al.* 1993).

Studies on fish cell lines carried out abroad

Gravell and Malsberger (1965) developed first fish cell line from fathead minnow (*Pimephales promelas*). A monolayer culture of fibroblast-like cells was established from a marine fish (*Caranx mate*) and its susceptibility to poikilothermic viruses such as FV-3 and IPN viruses were studied (Lee and Loh, 1975). A cell line designated SP-1 was established from tissue of the silver perch, *Bairdiella chrysura* and this cell line supported the growth of lymphocystis virus from the silver perch (Wharton *et al.* 1977). Fibroblast-like cells from primary culture of the caudal fin of gold fish (*Carassius auratus*) was cultivated and characterized (Shima *et al.* 1980). Klaunig (1984) developed primary cell culture from rainbow trout (*Salmo gairdneri*)

and channel catfish (*Ictalurus punctatus*). Nine permanent cell lines was established from five species of salmonids native to America's Pacific Northwest and all these cell lines effectively supported the growth of one or more of the common salmonid viruses (Lannan *et al.* 1984).

Two fibroblast-like cell lines (OL-17 and OL-32) were established from fins of adults of the medaka, (*Oryzias latipes*) (Komura *et al.* 1988). Lu *et al.* (1990) developed three new cell lines from different tissues (fin, snout and swim bladder) of the grass carp, *Ctenopharyngodon idella* and these cell lines were found to be sensitive to different fish viruses such as (*Rhabdovirus carpio*) (RVC), infectious hemotopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus serotype VR299 (IPNV), chum salmon virus (CSV) and golden shiner virus (GSV) and these cell lines were refractory to channel catfish virus (CCV), channel catfish reovirus (CRV) and Chinook salmon paramyxovirus (CSP).

Vallejo *et al.* (1991) developed active monocyte-like cell lines from channel catfish and found that these cell lines morphologically resemble mammalian monocytes or macrophages. Collodi *et al.* (1992) developed methods for the culture of cells from blastula-stage diploid and haploid zebrafish embryos, as well as cells from the caudal and pelvic fin, gill, liver and viscera of adult fish. A fibroblast like cell line, ZF4, was developed from 1 day old zebrafish embryos and characterized, and this cell line was found to be a useful tool for the analysis of gene regulation in zebrafish (Driever *et al.* 1993). A cell line RTL-W1, was developed from the normal liver of an adult rainbow trout by proteolytic

dissociation of liver fragments and grown routinely in the basal medium, L-15, supplemented with 5% fetal bovine serum (FBS) (Lee *et al.* 1993). A long-term macrophage cell line from the goldfish was established and characterized, which was maintained in culture for more than 2 years (Wang *et al.* 1995). The macrophage cell line from the gold fish will be invaluable in studies of pathogen/macrophage interactions, the mechanisms of macrophage antimicrobial effector's functions and the contribution of macrophages to the specific immune responses of teleosts. Faisal *et al.* (1995) developed continuous cell cultures from marine teleost, *Leiostomus xanthurus* and identified the optimal conditions for spot liver cell survival and propagation *in vitro*.

Ostrander *et al.* (1995) developed long-term primary cultures of epithelial cells from rainbow trout (*Oncorhynchus mykiss*) liver and these cells showed three types of cell populations. A new stromal adherent cell line, called trout pronephric stroma (TPS) was initiated from a long-term pronephric culture of an adult rainbow trout, and sub-cultured 104 times over a period of 4 years (Diago *et al.* 1995). This study also described the culture conditions and characterization by enzyme-cytochemistry, electron microscopy, isoenzyme profile, cytogenetic techniques, and viral susceptibility. Ganassin and Bols (1996) developed long-term rainbow trout spleen cultures and found them to be useful for studying the regulation of haemopoiesis and the functions of specific immune cells in fish. Hong *et al.* (1996) established pluripotent embryonic stem cell line from the medakafish (*Oryzias latipes*) and these cells were able to form embryoid bodies under appropriate conditions and differentiate into a variety of cell types. A long-term cell line (SHK-1) was developed from the head kidney of Atlantic salmon (*Salmo salar* L.) and characterized with respect to phenotypic and functional properties (Dannevig *et al.* 1997). Bejar *et al.* (1997) developed a continuous cell line SAF-1 from the fin tissues of an adult gilt-head seabream (*Sparus aurata*) without immortalizing treatments. This cell line was found to be susceptible to Infectious Pancreatic Necrosis Virus (IPNV), Infectious Haematopoietic Necrosis Virus (IHNV) and Viral Haemorrhagic Septicaemia Virus (VHSV). A continuous cell line, designated FG-9307, was developed from the gill tissue of the flounder (*Paralichthys olivaceus*) and was propagated for 96 passages over 3 years (Tong *et al.* 1997). Bradford *et al.* (1997) established and characterized cell cultures from fry of *Fugu niphobles* and eye of *F. rubripes*. The continuous cell lines SPH and SPS were derived from the heart and spleen tissues of sea perch (*Lateolabrax japonicus*) and the cell line RSBF from fin tissue of red sea bream (*Pagrosomus major*) (Tong *et al.* 1998). These cell lines were found to be susceptible to fish birnavirus and IPNV. Ganassin and Bols (1998) developed a long-term haematopoietic culture from spleen and these cells appeared both to produce and respond to an autocrine growth

factor(s). Flano *et al.* (1998) described the culture conditions and the phenotypic feature of different types of splenic cultures established from splenic explants of rainbow trout (*Oncorhynchus mykiss*). A new continuous cell line (GF-1) derived from the fin tissue of a grouper (*Epinephelus coioides*) was established and this line was found to be susceptible to different strains of IPNV (AB, SP, VR299 and EVE), Hard Clam Reovirus (HCRV), Eel Herpes Virus Formosa (EHVF) and Grouper Nervous Necrosis Virus (GNNV) (Chi *et al.* 1999).

Bejar *et al.* (1999) developed a long-term embryonic cell culture, derived from the commercial fish (*Sparus aurata*) and these cells were *in vitro* characterized for totipotency and transfected with a GFP plasmid for expression studies. Two cell lines susceptible to iridovirus were developed from grouper (*Epinephelus awoara*) kidney and liver tissues and these cell lines were designated GK and GL for kidney and liver, respectively (Lai *et al.* 2000). Chang *et al.* (2001) developed a tropical marine fish cell line from Asian seabass (*Lates calcarifer*) fry and this cell line was maintained in Earle's minimum essential medium (EMEM). This cell line had been found to be susceptible to IPNV, lymphocystis virus (LDV), grouper iridovirus (GIV), grouper nodavirus (GNV), reovirus from guppy (GRV) and reovirus from threadfin (ThRV). Bejar *et al.* (2002) have developed a long-term stable cell line, derived from embryonic cells of a marine fish (*Sparus aurata*) which has been characterized for cell proliferation, chromosome complement, molecular markers and *in vitro* tests of pluripotency by alkaline phosphatase (AP) staining. Four tropical marine fish cell lines have been established from the eye, fin, heart and swim bladder of the grouper, *Epinephelus awoara*, and characterized (Lai *et al.* 2003). Kang *et al.* (2003) have established and characterized two new cell lines from the flounder, *Paralichthys olivaceus*, fin and spleen. These cells have multiplied well in EMEM, supplemented with 10% FBS and have been sub-cultured more than 100 times, becoming continuous cell lines. Two cell lines from liver and heart of juvenile pilchards (*Sardinops sagax neopilchardus*) have been established and the origin of the cell cultures has been confirmed by PCR analysis using primers designed to be specific for pilchard mitochondrial DNA (Williams *et al.* 2003). Shimizu *et al.* (2003) have reported a cell line (WBE) derived from white bass (*Morone chrysops*) embryos, that has been grown for more than 80 passages over 21 months in Dulbecco modified Eagle medium supplemented with fetal bovine serum. Holen and Hamre (2003) have developed a long-term embryonic stem cell like cultures from a marine flatfish, (*Scophthalmus maximus*) and has reported the expression of Oct-4 in embryonic cells by immunofluorescence staining. A pluripotent embryonic cell line has been developed from embryonic stem-like cells of red sea bream (*Chrysophrys major*) blastulae and named SBES1 (Chen *et al.* 2003b). These cells have shown positive result for alkaline

phosphatase activity and differentiated into a variety of cell types by adding all-*trans* retinoic acid. Chen *et al.* (2003a) have established pluripotent embryonic stem cells from blastula stage embryos of sea perch (*Lateolabrax japonicus*) and named LJES1. These cells successfully expressed GFP reporter gene.

A continuous embryonic cell line has been established from gastrula-stage embryos of the Japanese flounder (*Paralichthys olivaceus*) a marine cultured fish, cells were cultured for more than 200 days with 60 passages (Chen *et al.* 2004). Pombinho *et al.* (2004) have described the development and characterization of two new cell lines, designated Vsa 13 and Vsa 16, derived from the vertebra of the marine teleost (*Sparus aurata*). Chen *et al.* (2005) have developed and characterized a continuous embryonic cell line from turbot (*Scophthalmus maximus*) and named TEC. A new tropical marine fish cell line (GS), derived from the spleen of orange spotted grouper, *Epinephelus coioides* has been established and characterized (Qin *et al.* 2006). The expression of GFP in the cells suggests that the GS cell line can be used as a useful tool for transgenic and genetic manipulation. Chi *et al.* (2005) have developed a novel cell line (BB) from the brain tissue of a barramundi, (*Lates calcarifer*), which had survived viral nervous necrosis disease. This is the first cell line reported to be persistently infected with NNV, and would be a useful model for understanding the mechanisms of NNV-persistent infection *in vitro* and *in vivo*. Buonocore *et al.* (2006) have developed a continuous anchorage dependent adherent cell line, named DLEC, derived from early embryos of the European sea bass (*Dicentrarchus labrax* L.) and these cells are transfected using liposomes with a commercial plasmid vector containing a reporter gene. Zhao and Lu (2006) have established and characterized two new cell lines from body muscle and fins of bluefin trevally, *Caranx melampygus*, and these cell lines have been found to be susceptible to IPNV, IHNV, spring viremia of carp virus, viral hemorrhagic septicemia virus, snakehead rhabdovirus and channel catfish virus.

Fish stem cells

Embryonic stem (ES) cell lines are undifferentiated long-term cell cultures derived from inner cell mass of developing embryos (Evans and Kaufman, 1981; Martin, 1981). These cells retain stem cell characteristics and can be induced to differentiate into a variety of cell types. When introduced into host embryo, the ES cells can participate in normal development and contribute to several tissues of the host including cells of the germ line (Bradley *et al.* 1984). These characteristics make embryonic stem cell ideal experimental systems for *in vitro* studies of embryo cell development and differentiation and a vector for the efficient transfer of foreign DNA into the germ line of an organism (Goossler *et al.* 1986). In addition, ES cells provide an attractive strategy for the

preservation of biodiversity (Hong *et al.* 1996). ES cell lines have so far been limited to mice (Evans and Kaufman, 1981; Martin, 1981; Bradley *et al.* 1984) and rat (Iannaccone *et al.* 1994). Human embryonic stem cell lines were first isolated by Thompson *et al.* (1998). These cells have the potential to produce any type of cells of the body and can be propagated in unlimited quantity for clinical applications such as cell and gene therapy (Trounson, 2005).

Most attempts to culture ES cells have been based on the original methods established for mice (Evans and Kaufman, 1981; Martin, 1981). To prevent differentiation in culture, mouse ES cells have been derived and maintained on feeder layers (Robertson, 1987) in conditioned media (Martin, 1981; Handyside *et al.* 1989) or in a medium supplemented with leukemia inhibitory factor (LIF) (Pease *et al.* 1990; Hasty *et al.* 1991; Wurst and Joyner, 1993) or with LIF-related cytokines (Conover *et al.* 1993; Nichols *et al.* 1994; Piquet-Pellorce *et al.* 1994; Yoshida *et al.* 1994). In mammalian species other than mice and rat, however, ES cells could be cultured only for a limited period (Sims and First, 1993) or their pluripotency could only be partially maintained after long-term culture (Sukoyan *et al.* 1992; Campbell *et al.* 1996). It has been suggested that the differentiation-inhibiting conditions suited for mice do not adequately prevent differentiation of stem cells in species other than rodents (Anderson, 1992). To develop ES cell lines and gene targeting technique in fish, extensive studies have been done in small model fishes, such as zebra fish (*Danio rerio*) and medaka (*Oryzias latipes*), because they offer the possibility of combining embryological, genetic and molecular analysis of vertebrate development. ES-like cell lines have been established in medaka (Wakamatsu *et al.* 1994; Hong *et al.* 1996) and zebrafish (Collodi *et al.* 1992; Sun *et al.* 1995). One medaka ES-like cell line, MES1, was shown to retain a diploid karyotype and the ability to form viable chimeras (Hong *et al.* 1998b). Attempts towards the derivation of ES cell lines from the zebra fish (Collodi *et al.* 1992; Sun *et al.* 1995) and medakafish (Wakamatsu *et al.* 1994) have achieved only partial success by using feeder layer techniques. Bejar *et al.* (1999) have derived a long-term embryonic cell culture from *Sparus aurata* and these cells have been *in vitro* characterized for totipotency and transfected with a GFP plasmid. Chen *et al.* (2003a) have established a pluripotent cell line, LJES1, from blastula-stage embryos of *Lateolabrax japonicus* and these cells were found to be differentiated into different types of cells under the treatment of retinoic acid.

Another important aspect of piscine cells is their lowest position in the ladder of evolution, which makes them suitable for xenotransplantation in mammals (Wright and Yang, 1997; Laue *et al.* 2001; Wright and Pohajdak, 2001). The islet tissue called Brockmann bodies in certain teleost fish like Tilapia, has been shown to restore normoglycemia upon transplantation into diabetic nude mice (Wright and Pohajdak,

2001). Similarly reversal of streptozotocin-diabetes has been achieved after transplantation of piscine islets to nude mice (Laue *et al.* 2001). These reports indicate potential of piscine cells for clinical applications.

Applications of fish cell culture

Fish cell cultures have both fundamental and practical importance. A diversity of questions can be addressed with fish cell cultures. These include applications in biomedical research (Hightower and Renfro, 1988), toxicology (Babich *et al.* 1986) and basic fish research (Nicholson, 1989), viral isolation (Chi *et al.* 1999; Chang *et al.* 2001; Kang *et al.* 2003) and, gene regulation, expression and gene transfer (Driever *et al.* 1993). The most widely employed application of fish cell cultures and, until recently, the rationale for developing most such cultures, is the isolation of fish viruses that are agents of epizootics of commercially important aquaculture fish species. The first fish cell line was developed to facilitate the isolation of infectious pancreatic necrosis virus (IPNV) and other viruses of trout (Nicholson, 1989). Because of the importance of trout and other salmonid fish cultures in North America, Europe, and Japan, this has led to the development of other salmonid cell lines for similar purposes. New cell lines from a variety of fish species are being used for the isolation and characterization of previously unknown viruses.

In addition to serving solely as a means of virus isolation, fish cell cultures are very useful as *in vitro* models for studying the replication and genetics of these viruses, the establishment and maintenance of virus carrier states, the effects of antiviral drugs, and the production of experimental vaccines. Also, *in vitro* culture techniques have been used to investigate unique viruses that do not replicate in standard fish cell lines but require highly differentiated cells. One example is viral erythrocytic necrosis (VEN), a viral infection of the red blood cells of several species of marine and anadromous fish. Although fish red blood cells will not divide, viable *in vitro* red blood cell cultures have been maintained for several weeks and used in investigations of the replication of these viruses and as *in vitro* models of viral induced anemia (Reno and Nicholson, 1980).

Primary cell cultures form an increasingly important tool in understanding fundamental aspects of fish immunology (Faisal and Ahne, 1990). Primary cultures have been used for studies on the mitogenic response of fish lymphocytes (Faulman *et al.* 1983), the *in vitro* generation and detection of antibody-secreting cells (Miller and Clem, 1984; Kaattari *et al.* 1986). Macrophage cultures have been used to study phagocytosis (Sovenyi and Kusuda, 1987), the respiratory burst (Chung and Secombes, 1988), lipoxin synthesis (Pettitt *et al.* 1989), the formation of multinucleate giant cells (Secombes, 1985), bactericidal activity (Olivier *et al.* 1986), and association with fish pathogens (Trust *et al.* 1983). Nonspecific cytotoxic cells, which may be the fish equivalent

of mammalian NK cells, have been defined through cell cultures (Graves *et al.* 1985; Cleland and Sonstegard, 1987).

Cell cultures are being used to identify fish cytokines and antibacterials. Both classes of products are being used or being considered for use in mammalian medicine (Meager, 1990; Elsbach, 1990) but are poorly characterized in fish. An interleukin-1 that elicits a response from catfish peripheral blood lymphocytes is produced by a carp epidermal cell line (Sigel *et al.* 1986). An interferon gamma-like molecule has been detected in the medium of rainbow trout leucocytes stimulated by concanavalin A (Graham and Secombes, 1990).

Fish cell cultures are also being developed as *in vitro* models for studying various biological processes. They have been used for determining karyotypes and other aspects of cytogenetics including chromosomal polymorphism and speciation, chromosome abnormalities, and evolution (Roberts, 1970). They are also finding widespread use in studying cellular physiology. Examples include: organ cultures from tilapia, eel and trout pituitary glands for studying the production of the growth hormone prolactin (Baker and Ingleton, 1975; Benjamin and Baker 1976); carp pituitary organ cultures to determine the efficiency of hypothalamus extracts on the production of gonadotropin and a continuous carp pituitary cell line producing gonadotropin (Benjamin and Baker 1976).

Fish cell cultures are finding application in toxicology for evaluating effects of various chemicals, pesticides and industrial wastes and in the study of carcinogenesis as *in vitro* models for investigating cell transformation by fish viruses, chemical agents, and the interaction of viruses and chemical carcinogens. Also, fish cell cultures are now being used for studying the immune response in fish, both from the practical view of vaccine development and for the study of the comparative evolutionary aspects of the immune response. Examples include *in vitro* stimulation of antibody production for basic studies and testing the potency of vaccines, *in vitro* models of immunoglobulin synthesis, and the role of natural killer (NK) cell activities in disease resistance in fish (Kaattari and Irwin, 1985).

Cell cultures can be developed to complement recombinant DNA technologies for the transfer of genes to fish, such as those for growth hormone (GH). Transgenic fish have been created, but the technology for their creation could be improved (Chen and Powers, 1990; Cloud, 1990; Ozato *et al.* 1989). In mammals pluripotent embryonic stem (ES) cells can be placed into culture (Evans and Kaufman, 1981), where they can be genetically modified (Roberston *et al.* 1988). When injected into embryos, ES cells have been found capable of developing into a wide variety of somatic tissues and the germ line of the adults (Hooper *et al.* 1987).

Cell cultures are being used to understand the hormonal regulation of fish reproductive cycles. Both pituitary and gonadal cell cultures have been prepared. Primary pituitary cell cultures are being used to study the regulation of

gonadotropin hormone (GTH) secretion in response to gonadotropin releasing hormone (GnRH) (Fahraeus-van Ree *et al.* 1982; Weil *et al.* 1986). Also, a carp pituitary cell culture that releases GTH has been established (Ribeiro and Ahne, 1982). Primary cultures of trout steroidogenic testicular cells are being used to study the regulation of steroidogenesis (Loir, 1990) and information about the local control of spermatogenesis is being obtained with primary cultures of trout Sertoli cells and germ cells (Loir, 1989). Ovarian follicle cultures have been used to study steroidogenesis (Van der Kraak, 1991) and sex steroid secretion (Carragher and Sumpter, 1990). The hormonal regulation and morphological changes of fish oocyte maturation can also be examined *in vitro* (Saat and Veersalu, 1990).

Purified viruses for use as vaccines are likely to be the first health product to be obtained from piscine cell cultures. Fish viral vaccines are still in the developmental phase (Sindermann, 1990) but large-scale viral production has been studied (De Kinkelin and Le Berre, 1979). This requires scaling up cell cultures, and for this purpose fish cell growth has been carried out on microcarriers (Nicholson, 1980), in a ceramic matrix (Lydersen *et al.* 1985) and in suspension (Lidgerding, 1981). Development of an effective vaccine is an impetus for further development of large-scale cell culture strategies such as those being worked out for mammalian cells (Arathoon and Birch, 1986; Bols *et al.* 1988).

Another potential product from fish cell cultures is proteins for use in fish reproduction, growth, and health care. These are likely to come from three polypeptide groups: gonadotropins, growth factors, and cytokines respectively. Generally, these polypeptides are too large to be synthesized chemically. Therefore, the two sources from which they can be purified are tissues and cell culture systems. With the development of efficient methods for transferring and expressing genes, fish cells are just one of a number of cell culture systems that could be used for this purpose. Fish genes for some potential products have been expressed in bacteria (Sekine *et al.* 1985; Peters *et al.* 1989) and yeast (Hayami *et al.* 1989).

Fish can be a source of proteins for applications in human health care and in the food industry. Currently at least two fish proteins are employed in medicine. Protamine is used to reverse anticoagulation during procedures such as vascular surgery and cardiac catheterization (Horrow, 1985). Salmon calcitonin is used to treat Paget's disease of bone and certain forms of osteoporosis, and, in this regard, is more effective than mammalian calcitonin (Wisneski, 1990). In future, other examples might be found of piscine hormones, enzymes, or cytokines being more efficacious in human medicine than their mammalian counterparts and as a result create a demand for fish pharmaceutical proteins. Potentially, fish antifreeze proteins can be used in medicine to cryopreserve human organs (Fahy, 1988) and in the food industry to preserve frozen foods (Knight *et al.* 1984). Different methods are

becoming available for producing these proteins, but fish cell cultures have yet to be used, although they would be expected to perform the appropriate post-translation modifications correctly.

The possibility of producing human pharmaceutical proteins from fish cell lines should be considered. An attribute of fish cells that could be exploited is their ability to grow at temperatures as low as 4°C (Plumb and Wolf, 1971; Mosser *et al.* 1986). A recombinant mammalian protein secreted into the medium at this temperature is likely to be less susceptible to proteolytic breakdown than at 37°C. This could aid downstream processing in batch systems. For this to be successful, fish expression vectors will have to be developed that allow human genes to be over expressed in fish cells at low temperatures. Only one attempt has been made at expressing mammalian genes in fish cells, none, in which the mammalian genes have been under the direction of fish sequences. Fish cell lines transfected with a human GH gene under control of the human metallothionein promoter expressed GH mRNA but not GH (Friedenerich and Scharlt, 1990).

Studies on fish cell lines carried out in India

The knowledge on the cell lines/culture from fish is fairly well developed and progressive in the advanced countries but the information in this field from India is limited as evidenced in the literature. Most of the works are based on primary cell cultures not on continuous cell lines, except recent works published by Sahul Hameed *et al.* (2006) and Parameswaran *et al.* (2006b). Singh *et al.* (1995) have developed primary cell culture from kidney of freshwater fish (*Heteropneustus fossilis*). Sathe *et al.* (1995) have established and characterized a new fish cell line (with 30 passages only), MG-3 from the gills of mrigal, (*Cirrhinus mrigala*). Sathe *et al.* (1997) have also developed a cell line from the gill tissues of Indian cyprinoid (*Labeo rohita*). Lakra and Bhonde (1996) derived primary culture from the caudal fin of an Indian major carp, (*Labeo rohita*). Rao *et al.* (1997) developed primary cell culture from the heart of Indian major carps. Prasanna *et al.* (2000) initiated cell culture from fin explant of mahseer (*Tor putitora*). Sunil Kumar *et al.* (2001) have developed primary cell culture system from the ovarian tissue of African catfish and passaged only 15 times after which they ceased to multiply and consequently perished. Lakra *et al.* (2005) developed two new primary cell culture systems from fry and fingerling of the (*Lates calcarifer*). Lakra *et al.* (2006) developed a new fibroblast like cell line from the fry of golden mahseer *Tor putitora* (Ham).

A continuous cell line (SISK) from kidney of sea bass, *Lates calcarifer*, has been established and characterized (Sahul Hameed *et al.* 2006). The cell line was maintained in Leibovitz' L-15 supplemented with 15% fetal bovine serum. This cell line has been sub-cultured more than 100 times over a period of 2 years. The SISK cell line consists

predominantly of epithelial-like cells. These cells showed strong positive reaction for epithelial markers such as cytokeratin 19 and pancytokeratin. The cells were able to grow at temperatures between 25 and 32 °C with optimum temperature of 28 °C. The growth rate of sea bass kidney cells increased as the FBS proportion increased from 2% to 20% at 28 °C with optimum growth at the concentrations of 15% or 20% FBS. The distribution of chromosome number was 30 to 56 with a modal peak at 48 chromosomes. Polymerase chain reaction products were obtained from SISK cells and tissues of sea bass with primer sets of microsatellite markers of sea bass. Five fish viruses were tested on this cell line to determine its susceptibility to these viruses and this cell line was found susceptible to MABV NC1 and nodavirus, and the infection was confirmed by RT-PCR and CPE. This suggested that the SISK cell line had good potential for the isolation of various fish viruses. This cell line has been shown to be susceptible to bacterial extracellular products. The SISK cell line is India's first marine fish cell line.

The development and characterization of a new tropical marine fish cell line (SISS), derived from the spleen of sea bass, *Lates calcarifer* has been described (Parameswaran *et al.* 2006a). The cell line was maintained in Leibovitz's L-15 supplemented with 15% fetal bovine serum. This cell line has been sub-cultured more than 70 times over a period of one and half years. The cells were able to grow at temperature between 25 and 32 °C with optimum temperature of 28 °C. The growth rate of sea bass spleen cells increased as the FBS proportion increased from 2 to 20% at 28 °C with optimum growth at the concentrations of 15 or 20% FBS. The SISS cell line consists predominantly of fibroblastic and epithelial-like cells. Polymerase chain reaction products were obtained from SISS cells and tissues of sea bass with primer sets of microsatellite markers of sea bass. Five fish viruses were tested on this cell line to determine its susceptibility to these viruses and this was found susceptible to IPNV VR-299 and nodavirus, and the infection was confirmed by RT-PCR and CPE. Further, this cell line is characterized by immunocytochemistry, endocytotic uptake of gold nanoparticles using confocal-laser-scanning microscopy (CFLSM), transfection with pEGFP-N1, proliferate marker.

A continuous cell line was established from blastula stage embryos of sea bass (*Lates calcarifer*) (Parameswaran *et al.* 2006b). The sea bass embryonic cells were maintained in Leibovitz's L-15 supplemented with 15% fetal bovine serum. The embryonic cell line was sub-cultured more than 70 passages over a period of 1.5 years and was designated as Sahul Indian sea bass embryonic (SISE) cell line. The cells were able to grow at temperatures between 25° and 32°C with an optimum temperature of 28 °C. The growth rate of sea bass embryonic cells increased as the FBS proportion increased from 2 to 20% at 28 °C with optimum growth at the concentration of 15 or 20%. Polymerase chain reaction products were obtained from embryonic cells and blastula

of sea bass with primer sets of microsatellite markers of sea bass. Four fish viruses were tested on this cell line to determine its susceptibility to these viruses and this cell line was found susceptible to IPNV VR-299 and nodavirus, and the infection was confirmed by cytopathic effect (CPE) and RT-PCR. Further, this cell line was characterized by immunocytochemistry using confocal-laser-scanning microscopy (CFLSM), transfection with pEGFP-N1, proliferate marker (BrdU).

We have established a pluripotent embryonic stem cell line SBES from blastula stage embryos of sea bass (*Lates calcarifer*) (Parameswaran *et al.* 2006b). The SBES cells were cultured at 28 °C in Leibovitz L-15 medium supplemented with 20% foetal bovine serum without using feeder layer. The ES cells were round or polygonal and grew exponentially in culture. The SBES cells exhibited an intense alkaline phosphatase activity. The undifferentiated state of this cell was maintained in the presence of LIF and the cells expressed Oct4. On treatment with all-trans retinoic acid, these cells differentiated into neuron-like cells, muscle cells and beating cardiomyocytes indicating their pluripotency. The cell line could be transfected with GFP reporter gene. SBES cell line was found to be susceptible to nodavirus and IPNV VR299 fish viruses as evidenced by cytopathic effect and RT-PCR, suggesting its potential for the isolation of fish viruses. This embryonic stem cell line derived from an oviparous fish blastula exhibits several peculiar features of viviparous mammalian embryonic stem cell lines making it an economically viable substitute for later. The present study highlights the importance and potential of piscine ES cell lines for stem cell research without evoking ethical issues and invasive interventions, sparing thus the mammalian embryos.

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