A new epithelial cell line, HBF from caudal fin of endangered yellow catfish, *Horabagrus brachysoma* (Gunther, 1864)

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Abstract A new epithelial cell line, *Horabagrus brachysoma* fin (HBF), was established from the caudal fin tissue of yellow catfish, *H. brachysoma* and characterized. This HBF cell line was maintained in Leibovitz's-15 medium supplemented with 15 % fetal bovine serum (FBS) and subcultured more than 62 times over a period of 20 months. The HBF cell line consists predominantly of epithelial cells and is able to grow at temperatures between 20 and 35 °C with an optimum temperature of 28 °C. The growth rate of these cells increased as the proportion of FBS increased from 5 to 20 % at 28 °C with optimum growth at the concentrations of 15 % FBS. Partial amplification and sequencing of fragments of two mitochondrial genes 16S rRNA and COI confirmed that HBF cell line originated from yellow catfish. The HBF cells showed strong positive reaction to the

cytokeratin marker, indicating that it was epithelial in nature. HBF cell line was inoculated with tissue homogenate from juveniles of Sea bass, *Lates calcarifer* infected with viral nervous necrosis virus (VNNV) and found not susceptible to VNNV. The extracellular products of *Vibrio cholerae* MTCC 3904 were toxic to the HBF cells. These cells were confirmed for the absence of *Mycoplasma* sp by PCR.

Keywords Ornamental fish · Fish virus · Yellow cat fish · *Horabagrus brachysoma* · Fish cell line

Introduction

Yellow catfish or Sun catfish, *Horabagrus brachysoma* (Gunther, 1864) is a cultivable catfish, endemic to the rivers originating from southern part of the Western Ghats, India. The fish is also reported from brackish-water, especially in the Vembanad Lake, Kerala during south-west monsoon and has a good market value as food fish (Kurup and Samuel 1985). *H. brachysoma* has aquaculture potential in both the food and ornamental trades. It is already established on the international market as a highly desired ornamental fish (Gopalakrishnan and Ponniah 2000). Yellow catfish has been considered to be an important fish from researchers, consumers and ornamental fish hobbyists and a potential candidate species for commercial aquaculture due to its high consumer demand in Southeast Asian continental markets (Beevi and Ramachandran 2002).

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In recent years, cell lines from aquatic animals have fascinated awareness as a means of expediting viral disease diagnosis in India. Fish viruses are host-specific that makes a cell line derived from particular fish more appropriate for investigating the viruses reported from these fish. In fish, cell lines have been used to study important topics such as epidemiology, molecular carcinogenesis, toxicology and functional genomics (Bols et al. 2011) and fish cell lines are particularly useful in detecting viruses and powerful tools to isolate and study fish viruses that cause epizootic outbreaks and massive mortality (Chen et al. 2004). Worldwide, more than 283 fish cell lines have been established and characterized from different freshwater and marine fishes (Lakra et al. 2011) and the number is increasing. Recently, cell lines from eye of Rohu (Ahmed et al. 2009), gill tissue of air breathing fish *Channa striatus* (Hamilton) (Majeed et al. 2014), and *Catla catla* (Taju et al. 2013), have been established in India.

The focus of the present study was the establishment and characterization of a new cell line from yellow catfish. The cell line was designated HBF (*H. brachysoma* fin). The subcultured cells were evaluated for optimal growth conditions, as well as their stability in liquid nitrogen, karyology, etc. The susceptibility of this cell line to a marine fish virus (no viruses affecting freshwater fish available in India) and bacterial toxins was investigated. This newly established HBF cell line will help in developing effective diagnostic methods for detecting viral infection in this commercially important aquatic species.

Materials and methods

Development of primary cell culture from caudal fin of yellow catfish

Live yellow catfish, *H. brachysoma* (70 specimens, 15–20 cm in total length) were collected from Chalakkudy River along the Western Ghats. To begin primary cell culture, after thoroughly removing the surface mucus on the body of the fish, the caudal fin was clipped and washed twice with phosphate buffered saline containing streptomycin (200 µg per ml), penicillin (200 IU per ml), and amphotericin B (5 µg per ml) (Sigma-Aldrich, St. Louis, MO, USA). Thereafter, the fin was minced into pieces (1 mm³),

using sterile scissors and then washed twice with PBS. Approximately 25 fin clips were seeded uniformly into 25 cm² tissue culture flasks (Nunc, Roskilde, Denmark) and 100 µl of heat-inactivated fetal bovine serum (FBS) (GIBCO, Grand Island, NY, USA) was added to facilitate the attachment of fin explants to the surface of the flask. After 30 min, excess FBS was drained from the flasks and incubated in a 28 °C incubator with growth medium, Leibovitz's-15 (L-15) (GIBCO, USA) medium supplemented with 15 % FBS and antibiotics (200 IU penicillin per ml, 200 µg streptomycin per ml and 5 µg amphotericin B per ml).

Fifty percent of the growth medium was changed every 3 days with new growth medium. After obtention of confluence of the primary cell culture, the medium was removed and the cells were trypsinized with commercial trypsin–EDTA solution (GIBCO) and subcultured at a split ratio of 1:2. After 15 passages, the amount of FBS in the growth medium was decreased from 15 to 10 %, and the antibiotics were decreased to normal concentrations (100 IU penicillin per ml, 100 µg streptomycin per ml and 2.5 µg amphotericin B per ml).

Growth studies

The effect of different temperatures and FBS concentration on cell growth was determined with the fin cell line at 25th passage and 56th passage as per the method described by Huang et al. (2011). Six-well tissue culture plates (Nunc) were used for these tests, and each well was seeded with 7×10^4 cells and incubated at 28 °C for 24 h to allow for cell attachment. Batches of plates were incubated at selected temperatures of 20, 24, 28, 32 and 35 °C over 5 days for the growth test. Every other day, duplicate wells at each temperature were trypsinized and the cell densities, morphology of the cells, etc. were noted. Similar procedures were performed for the effects of 5, 7.5, 10, 15 and 20 % of FBS at 28 °C.

Cell cryopreservation

Cells at various passage levels (10, 20, 25 and 35) were used for cryopreservation in liquid nitrogen. The cell in an actively growing state (log phase) was selected for cryopreservation to ensure optimal health and good recovery. The culture medium was changed 24 h prior

to harvesting. The cells were harvested using the subculture protocol described above. The enumeration of the cells was done with a hemocytometer, and cell viability was checked by trypan blue staining. The cells were subsequently centrifuged to form a pellet at $200\times g$ for 8 min, the supernatant was removed, and then the cell pellet was re-suspended in freezing media (10 % DMSO + 50 % fetal bovine serum + 40 % L-15) to reach a final cell concentration of 5×10^6 viable cells per ml. Subsequently, 1 ml of the cell suspension was allocated into one sterile cryovial and vials were capped and placed into boxes filled with the proper amount of isopropyl alcohol. Then, these boxes were placed in a $-20\text{ }^{\circ}\text{C}$ for 4 h and then in $-80\text{ }^{\circ}\text{C}$ freezer overnight. 16S rRNA using primers (F 5' CGC CTG TTT ATC AAA AAC AT 3' and H 5' CCG GTC TGA ACT CAG ATC ACG T 3') (Ward et al. 2005) and COI using primer (F 5' TCA ACC AAC CAC AAA GAC ATT GGC AC 3' and R 5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3') (Palumbi et al. 1991). Subsequently, the vials were transferred to a liquid nitrogen can (Werners et al. 2004). To recover the cells, the frozen vials were removed from liquid nitrogen and quickly thawed in $37\text{ }^{\circ}\text{C}$ water bath, and then the cells were transferred into a flask with complete medium and incubated at $28\text{ }^{\circ}\text{C}$. The medium was renewed 24 h later (Freshney 2005).

Chromosome preparation

Chromosome spreads were prepared from cells at passage 25 and 56. About 2×10^6 cells were seeded into a 75 cm^2 flask and grown in L-15 medium supplemented with 15 % FBS and antibiotics. After 1 day incubation at $28\text{ }^{\circ}\text{C}$, $0.2\text{ }\mu\text{g}$ per ml of colcemid (Sigma-Aldrich) was added to the medium for 3 h to arrest the cells in metaphase. The majority of cells (70 %) were dislodged from the flask by gentle tapping and harvested by centrifugation ($200\times g$, 8 min). Cell pellets were suspended in a hypotonic solution consisting of 0.5 % KCl for 10 min, and thereafter fixed in a 3:1 mixture of methanol: acetic acid. Chromosomes spread on a glass slide were then prepared according to the conventional drop-splash technique (Freshney 2005), followed by staining with 5 % Giemsa for 10 min. Finally, the slides were observed using a phase contrast microscope. A total of 100 chromosome spreads were counted.

Immuno-cytochemical characterization

Immuno-phenotyping of fin cells was carried out as per the method described by Mauger et al. (2008). Briefly, HBF cells at 45th passage were grown on sterile cover slips for 24 h. The cells were subsequently fixed and permeabilized with methanol at $-20\text{ }^{\circ}\text{C}$ for 30 min. For immunostaining, the cover slips were pre-incubated with PBS containing 1 % BSA for 1 h at $37\text{ }^{\circ}\text{C}$, and then incubated overnight at $4\text{ }^{\circ}\text{C}$ with mouse anticytokeratin (pan) clone AE1/AE3 antibodies (Invitrogen, Carlsbad, CA, USA) and mouse anti-vimentin antibodies (Invitrogen). In control cover slips, only PBS with 1 % BSA (HiMedia, India) was used in place of primary antibodies. After PBS washing, cells were incubated for 1 h with rabbit anti-mouse IgG FITC conjugate (diluted 1:50 in PBS containing 1 % BSA). The cover slips were washed again in PBS, mounted and images were documented by the fluorescent microscope.

Identification of cell origin

The origin of the HBF cell line was validated by partial amplification and sequencing of 16S rRNA using primers (F 5' CGC CTG TTT ATC AAA AAC AT 3' and H 5' CCG GTC TGA ACT CAG ATC ACG T 3') (Ward et al. 2005) and COI using primer (F 5' TCA ACC AAC CAC AAA GAC ATT GGC AC 3' and R 5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3') (Palumbi et al. 1991). Briefly, the muscle tissue and HBF (at 45th passage) cell pellet were homogenized separately in NTE buffer (0.2 mM NaCl, 0.02 mM Tris-HCl, 0.02 mM EDTA, pH 7.4) and centrifuged at $3,000\times g$ at $4\text{ }^{\circ}\text{C}$, after which the supernatant fluids were placed in fresh centrifuge tubes together with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5 % sodium dodecyl sulphate, 0.1 mg/ml, proteinase K). After incubation at $65\text{ }^{\circ}\text{C}$ for 2 h, the digests were deproteinized by successive phenol/chloroform/isoamyl alcohol extraction and DNA was recovered by ethanol precipitation, drying and re-suspension in TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0). PCR was carried out in VetriTM 96 well thermal cycler (Applied Biosystems, Singapore). Each PCR reaction was in a $25\text{ }\mu\text{L}$ volume containing both forward and reverse primers (10 μM , 0.5 μL each), MgCl_2 (25 mM, 1.5 μL) dNTPs (2 mM,

2.0 µl), PCR buffer (10×, 2.5 µl), Taq DNA polymerase (1U, 0.5 µl), template DNA (0.3–0.4 µg) and nucleic acid free water. PCR cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, annealing temperature of 50 °C (for CO1) and 58 °C (for 16S rRNA) for 30 s, 72 °C for 45 s and a final extension of 5 min at 72 °C. The PCR products were visualized on 1.2 % agarose gels and the amplified products were selected for sequencing. The cleaned up PCR products were sequenced in Applied Biosystems AB 3730 XL capillary sequencer. The raw DNA sequences were aligned against known sequences from the National Center for Biotechnology Information (NCBI) database and edited using BIOEDIT sequence alignment editor version 7.0.5.2.

Detection of Mycoplasma in the cell line

Mycoplasma contamination of HBF cells was detected at the 45th passage level. Cells were grown in L-15 medium without antibiotics for 5 days. One ml of harvested HBF cells was transferred into micro centrifuge tubes and centrifuged at 200×g for 10 min and supernatant was transferred into micro centrifuge tubes and centrifuged further at 250×g to remove debris. Finally, the supernatant was centrifuged at 15,000×g for 10 min and the supernatant was decanted and pellet was re-suspended using 50 µl of buffer solution followed by heating at 95 °C for 3 min. The solution was stored at 20 °C until use. *Mycoplasma* detection was done by the amplification of a fragment of 270 bp of the 16S rDNA of *Mycoplasma* sp. using the primers MSGO (5' TGC ACC ATC TGC CAC TCT GTT AAC CTC 3') and GPO1 (5' ACT CCT ACG GGA GGC AGC AGT 3') (van Kuppeveld et al. 1992). The amplification products were analyzed in 1 % agarose gel.

Susceptibility to bacterial extracellular products and nodavirus

The cytotoxicity of bacterial extracellular products (ECP) from *Vibrio cholerae* MTCC 3904 to HBF cells at the 45th passage was tested. The technique described by Liu (1957) was used. Briefly, sterilized cellophane sheets were placed on the surface of Brain Heart Infusion Agar (HiMedia, India) plates and inoculated by spreading 0.5 ml of a 24 h old broth culture of *V. cholerae* with a sterile swab and

incubated at 37 °C. After incubation for 48 h, cells were washed off the cellophane with a minimum volume of PBS. All cell suspensions were centrifuged at 10,000×g for 30 min at 4 °C. The supernatant was filtered through a 0.22 µm pore-size membrane (Millipore, Billerica, MA, USA), freeze-dried and reconstituted in PBS to a final volume of 10 ml. All ECP samples were stored at −20 °C until use. The HBF cells at 45th passage were grown as a monolayer in 24-well plates at 28 °C using L-15 medium supplemented with 5 % FBS. For the toxicity test, 0.1 ml serial twofold dilutions of ECP were added to wells. For negative controls, wells were inoculated with 0.1 ml of sterile saline. Plates were incubated at 28 °C and the effects of ECP on the cells were observed after 24 and 48 h.

To test the susceptibility of the cell line to a marine fish virus, i.e. nodavirus was used (no freshwater fish viruses have been reported from India yet). Cells were inoculated in wells of a 24-well plate and incubated for 12–24 h at 28 °C to give a confluence of 70–80 %. After removal of the medium, 0.1 ml of virus suspension at a dilution of 10^{−1}–10^{−3} was added and allowed to adsorb for 1 h. Then, 5 ml of maintenance medium (L-15 with 5 % FBS) was added. The cells were incubated at 28 °C and examined daily for 2 weeks for the appearance of cytopathic effect (CPE) up to 10 blind passages.

Results

Primary cell culture and subculture

Cell cultures were initiated from caudal fin of a yellow catfish and the cells migrated from the tissue fragments and grew well, forming a monolayer during the first month. *Horabagrus brachysoma* fin (HBF) cells (Fig. 1a, b) were subcultured over 62 times. In the subsequent subculture, HBF cells adhered well and achieved confluence within 5 days at 28 °C. Cells were subcultured in L-15 medium with 15 % FBS at a ratio of 1:2 every 5 days for the initial ten passages. After ten subcultures, cells were subcultured at a ratio of 1:2 at 4–6 day intervals, with FBS being reduced to 10 % in the L-15 culture medium. HBF cells consisted of both fibroblast-like and epithelial-like cells in the early cell passages; however, they became predominantly epithelial cells by 15 the passage.

Fig. 1 **a** Explant of caudal fin of *Horabagrus brachysoma* showing radiation of cells ($\times 100$); **b** HBF cell line at 15th passage ($\times 100$); **c** HBF cell at 30th passage ($\times 100$); **d** HBF cell

showing presence of cytokeratin marker; **e** HBF cells grown in L-15 medium with 15 % FBS at 35 °C ($\times 200$); **f** HBF cells at 1 day post inoculation to ECP of *Vibrio cholerae* ($\times 100$)

Fig. 2 Growth response of the HBF cell line at the 25th passage to selected temperature. Data are mean $\pm$ SD of three measurements

Fig. 3 Growth response of the HBF cell line at the 25th passage to selected fetal bovine serum (FBS) concentrations. Data are mean $\pm$ SD of three measurements

Growth characteristics

Optimal growth of the newly established cells was dependent on temperature and serum concentration. The cells were able to grow at temperatures from 24 to 32 °C, but no significant growth was observed at 20 and 35 °C. The maximum growth occurred at 28 °C (Fig. 2). The growth rate of HBF cells increased as the FBS proportion increased from 5 to 20 % at 28 °C. Cells exhibited poor growth when 5 % FBS was added to L-15 medium, with relatively slow growth at 7.5 % FBS, moderate growth at 10 % FBS and maximum growth at 20 % FBS (Fig. 3). The higher the

concentration of FBS in the medium was directly proportional to, the growth rate of cells. There was very minimal difference in the growth rate of the HBF cells at 15 and 20 % FBS. So in order to reduce the cost of the medium, 15 % FBS was used regularly to maintain the HBF cells. After 5 days of culture, cells cultured in medium containing 5 % FBS grew significantly slower than those cultured in medium containing higher FBS concentrations. HBF cells changed morphology at 35 °C (edges of the cells became serrated) (Fig. 1e). There were no differences observed in the growth curve of the HBF cells in both passages (the 25th and the 56th).

Fig. 4 Chromosome analysis of HBF cells. **a** Frequency distribution of chromosomes in 100 spreads; **b** karyotype of HBF cells (passage 35) indicates all 30 pairs of chromosomes; **c** cellular chromosomes of HBF cells arrested in metaphase

Cryopreservation

The HBF cell line was cryopreserved at different passages levels. Cell populations remained over 90 % viable when stored at -196°C for a period of 20 months. All viable cells attached and grew well following seeding at 28°C . No apparent morphological changes of these cells were observed following storage.

Chromosomal analysis

Chromosomal counts from 100 random metaphase spreads of HBF (at the 25th and the 56th passage) revealed that the chromosome number ranged from 32 to 70 in HBF cells, with a distinct peak at 60 chromosomes (Fig. 4a). The metaphase spread of HBF cells at the 25th passage with a normal diploid number revealed a normal karyotype morphology (Fig. 4b), consisting of 14 pairs of metacentrics, 10 pairs of submetacentrics, 4 pairs of subtelocentrics and 2 pairs of telocentrics ($2n = 28m + 20sm + 8st + 4t$) (Fig. 4c).

Immuno-cytochemical characterization

All HBF cells at the 45th passage gave strong positive fluorescence signals for cytokeratin, an epithelial cell marker (Fig. 1d). No reactivity was observed in

control and in cells incubated with anti-vimentin antibodies.

Identification of cell origin

Amplification from the 16S and COI mitochondrial DNA for cell line at the 45th passage level revealed the expected PCR products of 562 and 642 bp, respectively. DNA sequencing and comparative analysis demonstrated that both mitochondrial genes sequences amplified from fresh yellow catfish muscle tissue and from HBF cell line were identical. Subsequent comparative analysis of the identified sequences demonstrated a 98 % match for 16S and a 99 % match for COI to known yellow catfish mitochondrial DNA sequences. These data confirmed that HBF cell line is indeed derived from yellow catfish. These sequences have been submitted to GenBank and accession numbers are awaited.

Detection of mycoplasma contamination

No 270 bp DNA band (target amplified product size) was found on agarose gel when a PCR product from HBF cell line at the 45th passage was analysed using gel electrophoresis. This strongly suggests that the cell line was free of *Mycoplasma* contamination.

Susceptibility to bacterial extra cellular products and nodavirus

The *Vibrio cholerae* ECP were toxic to the HBF cell line. Cytotoxic effects were noticed on the HBF cells within 24–48 h after inoculation. The morphological changes like rounding, shrunken, detachment of cells and destruction of the monolayer were recorded in the HBF cells (Fig. 1f). Nodavirus, a marine fish virus (the only fish virus reported from India) was tested on the cells but they were not susceptible to the virus. No significant CPE was observed in the cells after 2 weeks even after 10 blind passages of the samples.

Discussion

The main purpose of this study was to develop and to characterize a new cell line from *H. brachysoma*. Particularly, a new cell line, HBF was established from yellow fish tail fin using the explant method. The HBF cell line has been established using the explant technique instead of enzymatic digestion methods as it has many advantages such as speed, ease, lesser damage to the cell surface and maintenance of cell interactions when compared to cell suspensions (Avella et al. 1994). The explant culture technique was found to be the suitable method for developing primary cell cultures from *H. brachysoma* as observed in previous works on development of cell lines from *L. calcarifer*, *Cyprinus carpio*, *Etroplus suratensis*, *Puntius denisonii* and *Puntius fasciatus*, (Lakra et al. 2010a, b; Swaminathan et al. 2010, 2012, 2013; Chaudhary et al. 2013).

The HBF cell line maintained stable growth for more than 62 passages, over a period of 2 years. The FBS was used for attachment of the explants to the flask surface and was adequate in terms of its efficiency to support explant attachment and initial cell radiation. L-15 medium has also been used by other investigators to establish other fish cell cultures (Lakra et al. 2010a, b; Chaudhary et al. 2013; Zhu et al. 2013). The shaking or agitation of the flasks of HBF cells led to cell shrinking and the breaking down of the cell monolayer during initial passage levels as documented in other cell lines (Lio-Po et al. 1999). However, this occurrence reduced gradually and completely disappeared by the 20th passage. This

might be due to the fact that the HBF cells did not completely adapt to the culture conditions during the initial passages. The growth temperature range for these cells was 20–35 °C, with optimum growth at 28 °C and this would be an advantage of HBF cell line which is a potential cell line suitable for isolating both warm- and cold-water fish viruses (Nicholson et al. 1987). But cells from poikilothermic teleosts are not able to grow at their sub-lethal temperatures (Hightower and Renfro 1988). For example, the rainbow trout, *Oncorhynchus mykiss*, cell line RTG-2 cannot grow at 26–28 °C and cannot survive at 30 °C (Mosser et al. 1986). It has been also reported that goldfish, *Carassius auratus*, cells easily change their growth rate in association with temperature shifts (Sato et al. 1990). As expected, the growth rate of the cells increased as the FBS concentration increased from 5 to 20 %, which stimulates the cell growth and division. It is important to note that less FBS concentration i.e. 5 % concentration of FBS also provided growth, which is advantageous for maintaining this cell line at low cost.

Cryopreservation of cell line is essential for long-term storage and also is essential to avoid loss by contamination, to minimize genetic change in continuous cell lines and to avoid ageing and transformation in cells. The viability of cells preserved in liquid nitrogen demonstrated that these cells were stable and able to survive low temperature storage for 18 months. The viability of cryopreservation of cells was demonstrated, with recovery of up to 90 % after thawing. These results coincide with other cell lines such as GF-1 with 73 % (Chi et al. 1999), SF cell line with 80–85 % viability (Chang et al. 2001), RE with 80 % (Ahmed et al. 2009) and AGF II with 70 % (Wang et al. 2012). The cell line described here exhibited a modal chromosome number of $2n = 60$, which has been documented in the literature for this aquatic species (Nagpure et al. 2003).

Mycoplasma contamination in the cell culture may alter host cellular characteristics and cell lines may be lost since mycoplasmas are hard to eradicate. These drawbacks emphasize the need to regularly screen cell cultures for mycoplasmal contamination. Routine screening is essential since contaminated cell cultures are the most important source of cross contamination (Stanbridge 1971). The prevalence of *Mycoplasma* contamination in cell culture is 1 % for primary cultures, 5 % for early passage cultures, and 15–35 %

for continuous cell cultures (Uphoff and Drexler 2002) and it is indicating that these usually do not originate from the donor of the cells, but are introduced during cell propagation. The HBF cell line was not have *Mycoplasma* contamination at the current passage level and confirmed by PCR. In earlier studies, Frerichs (1996) found 11 cell lines derived from coldwater and warm-water fish using DNA staining, microbiological culture and ELISA positive for *Acholeplasma laidlawii* and *Mycoplasma arginini*. In another study, CSK cell line was characterized by chromosome number, transfection and *Mycoplasma* detection (Majeed et al. 2013).

PCR produced products of expected size and their sequencing, indicated that cells were of yellow catfish origin. Mitochondrial DNA, particularly 16S rRNA, and COI genes (Lakra et al. 2010a, b; Swaminathan et al. 2012; Chaudhary et al. 2013) and 18S rRNA (Gong et al. 2011) have frequently been used in species identification, classification and in establishing phylogenetic relationships among aquatic and mammalian species.

Antibodies of fibroblastic and epithelial markers were applied to confirm the phenotype of cells. The cells showed strong positivity to pancytokeratin, indicating that HBF is epithelial in nature. Chaudhary et al. (2013) demonstrated that the CTE cells showed strong positivity for cytokeratin, indicating that cell line was epithelial in nature. EAGK cells gave fluorescence signal when reacted with the antibodies to vimentin and fibronectin suggested that these cells are fibroblast cells (Gong et al. 2011). The HBF cell line was very sensitive to the ECP of *Vibrio cholerae* and morphological changes were similar to those described in the SAF cell line (Bejar et al. 1997) due to the toxicity of *Vibrio* ECP.

With the development of science and technology, the role of limited cell lines will become increasingly prominent, and there will be currently unforeseen applications. It is important to maintain the genetic diversity of livestock and poultry particularly, the genetic resources of endangered animals. The preservation of individual animals, semen, embryos, genome, and complementary DNA libraries are all practical options in this regard. In addition to these methods, modern somatic cell cloning techniques can be used to conserve animal genetic materials (Wu 1999). Because of the significant economic, scientific and ecological importance of yellow catfish, more

studies on the biology and disease control in this fish species are essential. Therefore, the establishment of an in vitro cell line from yellow catfish tail fin will greatly enhance the development of an effective programme on the biological conservation of this important fish species.

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