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Establishment and characterization of India's first marine fish cell line (SISK) from the kidney of sea bass (*Lates calcarifer*)

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Received 13 July 2005; received in revised form 31 December 2005; accepted 8 January 2006

Abstract

A continuous cell line (SISK) from kidney of sea bass, *Lates calcarifer*, has been established and characterized. The cell line was maintained in Leibovitz' L-15 supplemented with 15% fetal bovine serum. This cell line has been subcultured more than 100 times over a period of 2 years. The SISK cell line consists of predominantly of epithelial-like cells. These cells showed strong positive for epithelial markers such as cytokeratin 19 and pancytokeratin. 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 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 was found to be susceptible to MABV NC1 and nodavirus, and the infection was confirmed by RT-PCR and CPE. This suggests that the SISK cell line has 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 the India's first marine fish cell line.
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Keywords: *Lates calcarifer*; Cell line; Characterization; Viruses; Susceptibility

1. Introduction

Over the last two decades, intense interest has been generated in India, to develop mariculture in the coastal belt and recently mariculture has been turning to fin fish from penaeid culture because of serious viral diseases in shrimp and caused substantial economic loss each year. Sea bass, *Lates calcarifer*, is a potential candidate species for farming in India, because of its fast growth rate, tolerance to wide environmental conditions and its demand in domestic and export markets. It is extremely important to establish a continuous cell line for monitoring the viral and rickettsial diseases of fish. The cell lines also provide an important tool for studying toxicology, carcinogenesis, cellular physiology and

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doi:10.1016/j.aquaculture.2006.01.011

genetic regulation and expression. A common method for determining whether a virus is present in a healthy fish population is to attempt to isolate it in an appropriate cell line. A cell line will also allow further study of viruses isolated in disease outbreaks. A large number of cell lines have been established in fresh water fishes (Fryer and Lannon, 1994; Hong et al., 2004), but relatively only a few cell lines were developed in marine fish (Tocher et al., 1989; Fernandez-Puentes et al., 1993a,b; Bejar et al., 1997; Tong et al., 1997; Chi et al., 1999, 2005; Chang et al., 2001; Chen et al., 2003a,b, 2004, 2005; Kang et al., 2003; Qin et al., 2006). It is very essential to develop species-specific cell lines from marine fish for use in viral diagnostics.

Breeding and larval rearing of sea bass have been standardized in Central Institute of Brackishwater Aquaculture, Chennai, India (CIBA, 2003) and sea bass hatcheries have been established in different parts of India to produce seed. A disease of suspecting of viral origin has been observed frequently in the hatcheries and little is known about this viral infection (Azad et al., 2005). A detail study on this viral infection has not been carried out due to lack of cell lines. Thus cell line is urgently desired in sea bass for isolating and identifying viruses that cause viral diseases in this species. So far, only one cell line has been developed from *L. calcarifer* fry (Chang et al., 2001). The present study describes the development and characterization of a continuous cell line from the kidney of sea bass.

2. Materials and methods

2.1. Initiation of primary cell culture and routine maintenance

Normal and apparently healthy juvenile sea bass (*L. calcarifer*) (5–10 g in weight) were collected from grow-out ponds of CIBA, Chennai and transported live to the laboratory. In laboratory, the animals were maintained in sterile, aerated seawater containing 1000 IU/ml penicillin and 1000 μg/ml streptomycin for 24 h at room temperature (25–28 °C). The fish were anaesthetized in iced water, dipped in 5% chlorex for 5 min and wiped with 70% alcohol, and operated in vivo. The gill, heart, spleen, kidney and liver tissues of the fish were taken aseptically and washed three times in antibiotic medium containing Leibovitz' L-15 (GIBCO), 500 IU/ml penicillin and 500 μg/ml streptomycin. The tissues were minced into small pieces (approximately 1 mm^3 in size) in Leibovitz' L-15 with 500 IU/ml penicillin, 500 μg/ml streptomycin and 2.5 μg/ml Fungizone. The tissue fragments were inoculated into 25 cm^2 cell culture flasks and 5 ml of growth medium was added in each flask. The medium was L-15 supplemented with 20% fetal bovine serum (FBS), 100 IU/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml Fungizone. The flasks were incubated at 28 °C and the medium was replaced every 5 days.

When the cells formed a monolayer, the old medium was removed and the cell sheets were washed with phosphate-buffered saline (PBS) twice and dispersed with 0.25% trypsin/EDTA solution [0.25% trypsin and 0.2% ethylenediaminetetraacetic acid (EDTA) in PBS]. The cells were resuspended in 10 ml of growth medium and were distributed into two flasks. The concentration of FBS in the L-15 medium was reduced to 15% for subcultures 20–60 and then to 10%.

2.2. Growth studies

The effect of temperature and FBS concentration on cell growth was carried out with kidney cell line at the 60th passage level. A number of 25 cm^2 cell culture flasks were each seeded with 10^5 cells and incubated at 28 °C for 24 h to allow for cell attachment. Then batches of flasks were incubated at selected temperatures of 20, 28, 32, 37 and 40 °C for growth test. Every other day, duplicate flasks at each temperature were washed with PBS twice after which 0.2 ml of 0.25% trypsin/EDTA were added to each flask. When the cells rounded up, the cell density was measured microscopically by using a haemocytometer and the numbers are expressed as cells/mm^2. The experiment was carried out for 5 days.

Table 1
Microsatellite loci and primers used for detection of sea bass DNA from SISK Cells

Primer no.	Locus	Annealing temperature (°C)	PCR product size in bp	Sequences	Orientation
(1)				TCAAATCAGTTTGTGACACG	Upstream primer
	LCAM03	55	231	GTCTTGGCTCTGGATCAGTG	Downstream primer
				AGTTTTTCATGTCAGCACTTCACA	Upstream primer
(2)	LCAM38	55	187	CACTCGTTCTCGCTGTTACACC	Downstream primer
				GTGCCACCTGCCTGACC	Upstream primer
(3)	LCAM21	55	193	GCCATGACTGATTGCTGAGA	Downstream primer

Primers from Yue et al. (2001, 2002).

The growth response to different concentrations of FBS was carried out using the same procedure as mentioned above at 28 °C.

2.3. Storage in liquid nitrogen

Two-day-old subcultures of sea bass kidney cells at the 30th and 60th subcultures were harvested by centrifugation and suspended in culture medium containing 10% FBS and 10% dimethyl sulphoxide at a density of 10^6 cells ml^{-1}. The cell suspensions were dispensed into 2 ml plastic ampoules and kept initially at −20 °C for 4 h and then at −75 °C overnight and finally transferred into liquid nitrogen (−196 °C). The frozen cells were recovered from storage 1 and 6 month post-storage by thawing in running water at 28 °C. Following removal of the freezing medium by centrifugation, the cells were suspended in L-15 with 10% FBS and tested for viability by haemocytometer counting after trypan blue staining. The viable cells were seeded into 25 cm^2 cell culture flask and observed.

2.4. Chromosome analysis

Kidney cells at passages 37 and 60 were used for chromosome analysis. The cells were inoculated in a 25 cm^2 culture flask and incubated for 24 to 36 h. Colchicine (0.04%) (Sigma) was added to the cells and incubated for 2 h in culture flasks. Cells were removed from the flask surface and centrifuged at 500×*g* for 5 min. The pellet was gently resuspended in 0.027 M KCl and incubated at 25 °C for 30 min. Cells were then centrifuged again at 500×*g* for 5 min. Supernatants were discarded and cells resuspended. Freshly mixed, cold 3:1 methanol–acetic acid fixative was added slowly while aspirating the cell suspension gently. These fixed cells were then washed three times with fresh fixative, and then resuspended in a small amount of fixative. The suspension was dropped onto glass slides, air dried and stained with 5% Giemsa (pH 6.8) for 15–20 min. Chromosome counts were performed in more than 100 metaphase plates from both passages.

2.5. Viral susceptibility and confirmation by RT-PCR

Five viruses namely Nodavirus (VNN), Marine birnavirus-NC1 (Mabv Nc-1), Chum Salmon Virus (CSV), Infectious Hematopoietic Necrocis Virus (IHNV) and Infectious Pancreatic Necrosis Virus-Sp (IPNV-SP) were selected to inoculate in sea bass kidney cell lines at the 60th subcultures to determine the virus susceptibility and cytopathic effect (CPE). The preparation of these viruses was performed as described in Kang et al. (2003). For infection, kidney cells were inoculated in a well of a 24-well plate to give a confluence of 60–70%, and incubated for 12 to 24 h at 28 °C. After removal of the medium, 0.1 or 1 ml of virus suspension at a dilution of 10^{-1} to 10^{-3} was inoculated into the cell culture in a 24-well plate or flask, and allowed to adsorb for 1 h. Then 0.5 or 5 ml maintenance medium

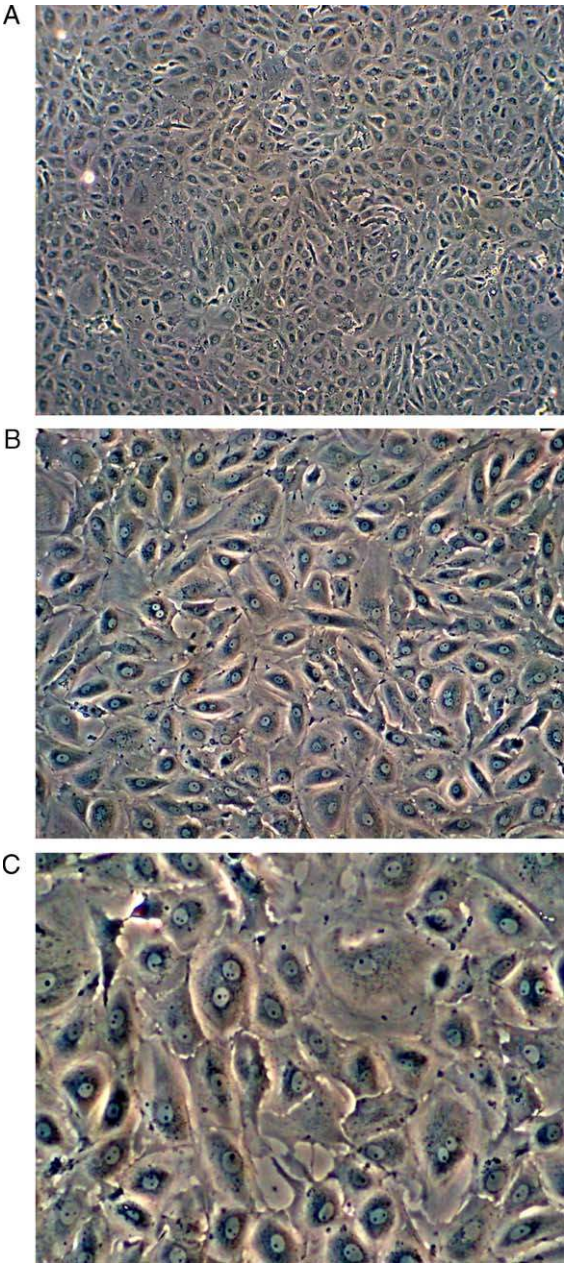

Fig. 1. Phase-contrast photomicrograph of the SISK cell line at (A) passage 30 (×100), (B) passage 100 (×200), (C) cell morphology (×400).

containing 5% FBS was added. The cells were incubated at 23 °C and examined daily for the appearance of CPE.

The virus infected cells were freeze and thawed thrice and centrifuged at 5000×*g* for 7 min at 4 °C. To extract RNA, 0.5 ml of supernatant was mixed with 1 ml of TRIzol reagent (GIBCO-BRL) and RNA was extracted according to the protocol of the manufacturer. Briefly, 1 ml of TRIzol reagent was added to 0.5 ml of sample and incubated for 5 min at room temperature, and then 0.2 ml of chloroform was added. The sample was vigorously shaken for 2 to 3 min at room temperature then centrifuged at 12,000×*g* for 15 min at room temperature. Then the RNA was precipitated from the aqueous phase with isopropanol, washed with 75% ethanol and dissolved in 20 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). The amount of nucleic acid in the sample was quantified by measuring the absorbance at 260 nm. The purity of the preparation was checked by measuring the ratio of $OD_{260\ nm}/OD_{280\ nm}$.

RT-PCR was carried out using the Reverse-IT TM 1-step RT-PCR kit (ABgene), allowing reverse transcription (RT) and amplification to be performed in a single reaction tube. One pair of primers specific to MABV NC1 was designed from sequence data of the MABV NC1 genome (GenBank accession no. AY129666). The sequences were 5′ AGC TTA AGG ACA CGG TAA GC 3′ (forward) and 5′ GTC TGT TTA GCT GTC CTG AG 3′ (reverse). The size of the DNA amplicon was 249 bp.

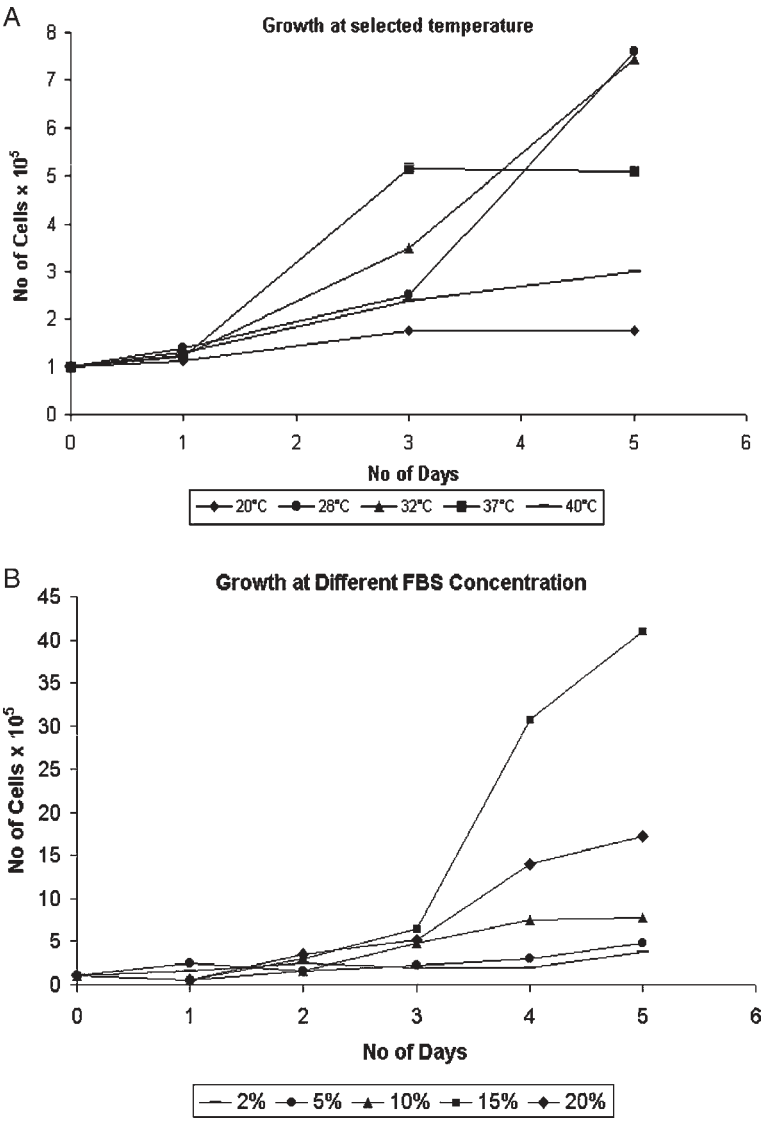

Fig. 2. Growth responses of the SISK cell line at the 60th passage to selected temperature (A) and fetal bovine serum (FBS) concentrations (B).

Reactions were performed in 50 μl RT-PCR buffer containing 20 pmol of each primer and RNA template, using the following steps: RT at 52 °C for 30 min; denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s and elongation at 72 °C for 1 min, ending with an additional elongation step of 10 min at 72 °C. For nodavirus detection, the primers designed by Thiery et al. (1999) were used and the sequences were 5′ GTT CCC TGT ACA ACG ATT CC 3′ (forward) and 5′ GGA TTT GAC GGG GCT GCT CA 3′ (reverse). The size of the DNA amplicon was 294 bp. The reaction conditions were similar to that described as above for MABV NC1 but the annealing temperature was 50 °C. The RT-PCR products (10 μl) were then analyzed by electrophoresis on a 1.2% agarose gel.

2.6. Cytotoxicity test of bacterial extracellular products

Bacterial extracellular products (ECP) of fish and shellfish pathogenic *Vibrio* strains, such as *Vibrio anguillarum*, *Vibrio alginolyticus* and *Vibrio harveyii*

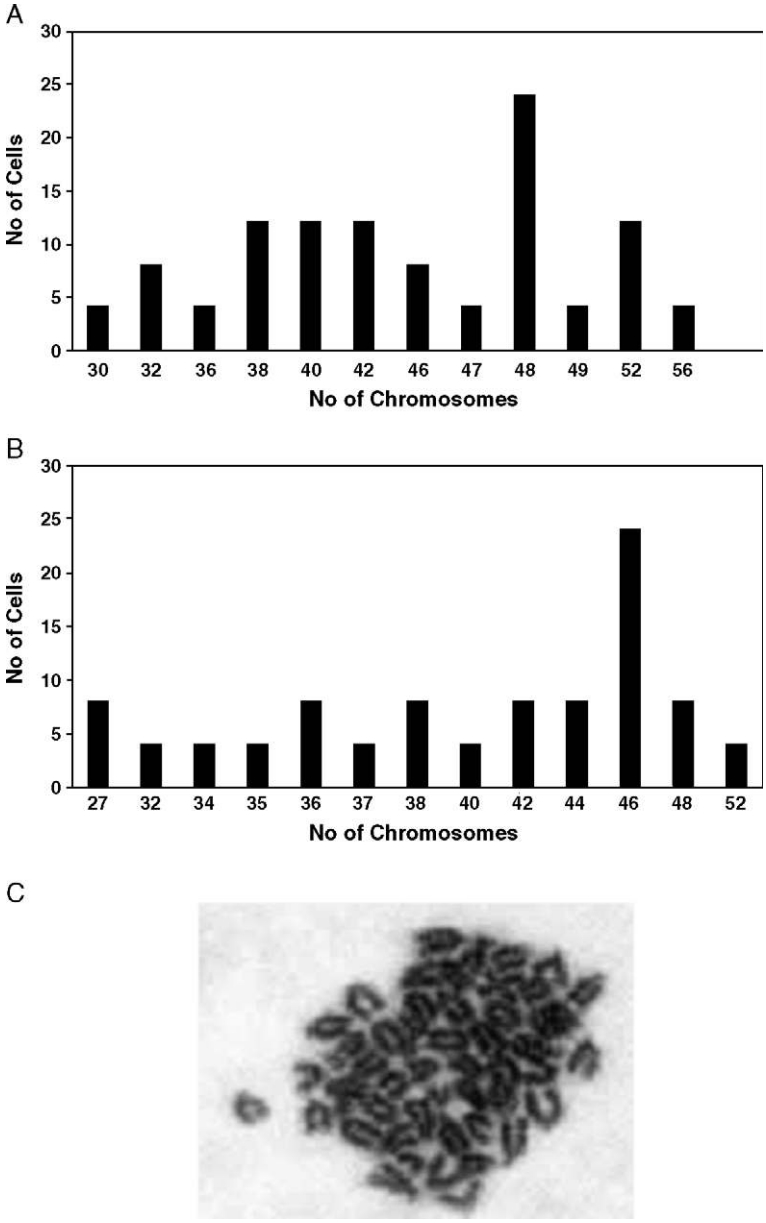

Fig. 3. Chromosome number distribution, at passages 37 (A) and 60 (B), and metaphase (C). In total, 100 metaphases were counted.

were obtained following the protocol described by Balebona et al. (1995). Kidney cells were grown as a monolayer in 24-well plates at 28 °C using L-15 medium supplemented with 5% fetal bovine serum. For the toxicity test, the cell line was inoculated with 0.1 ml serial dilutions of ECP sample. For negative controls, plates inoculated with sterile saline were used. Plates were incubated at 28 °C and the effects of ECP on the cells were observed after 24 and 48 h.

2.7. Polymerase chain reaction

Template DNA for PCR assays was prepared by extraction from tissues of sea bass and kidney cells following the method described by Lo et al. (1996). Briefly, the samples were homogenized separately in NTE buffer [0.2 m NaCl, 0.02 m Tris–HCl and 0.02 m ethylenediaminetetraacetic acid (EDTA), pH 7.4] and centrifuged at 3000×*g* at 4 °C, after which the supernatant fluids were placed in other 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^{-1} proteinase K). After incubation at 65 °C for 2 h, the digests were deproteinized by successive phenol/chloroform/iso-amyl alcohol extraction and DNA was recovered by ethanol precipitation, drying and resuspension in TE buffer. Three primer pair sequences of the microsatellite markers of *L. calcarifer* were randomly selected (Table 1) and polymerase chain reaction (PCR) was performed as described by Yue et al. (2002) and Coimbara et al. (2001). Thermal cycling was carried out in a Eppendorf thermal cycler (Germany) and consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, annealing temperature of 55 °C for 1 min, 72 °C for 30 s, and a final extension of 10 min at 72 °C. Amplified products were analyzed in 1.2% agarose gel containing ethidium bromide and visualized with a UV transilluminator.

2.8. Immunofluorescence staining and confocal laser scanning microscopy

For immunophenotyping of the SISK cell line, the cells were grown on coverslips for 24 h, fixed with 3.7% *p*-formaldehyde for 10 min at 4 °C, washed with PBS,

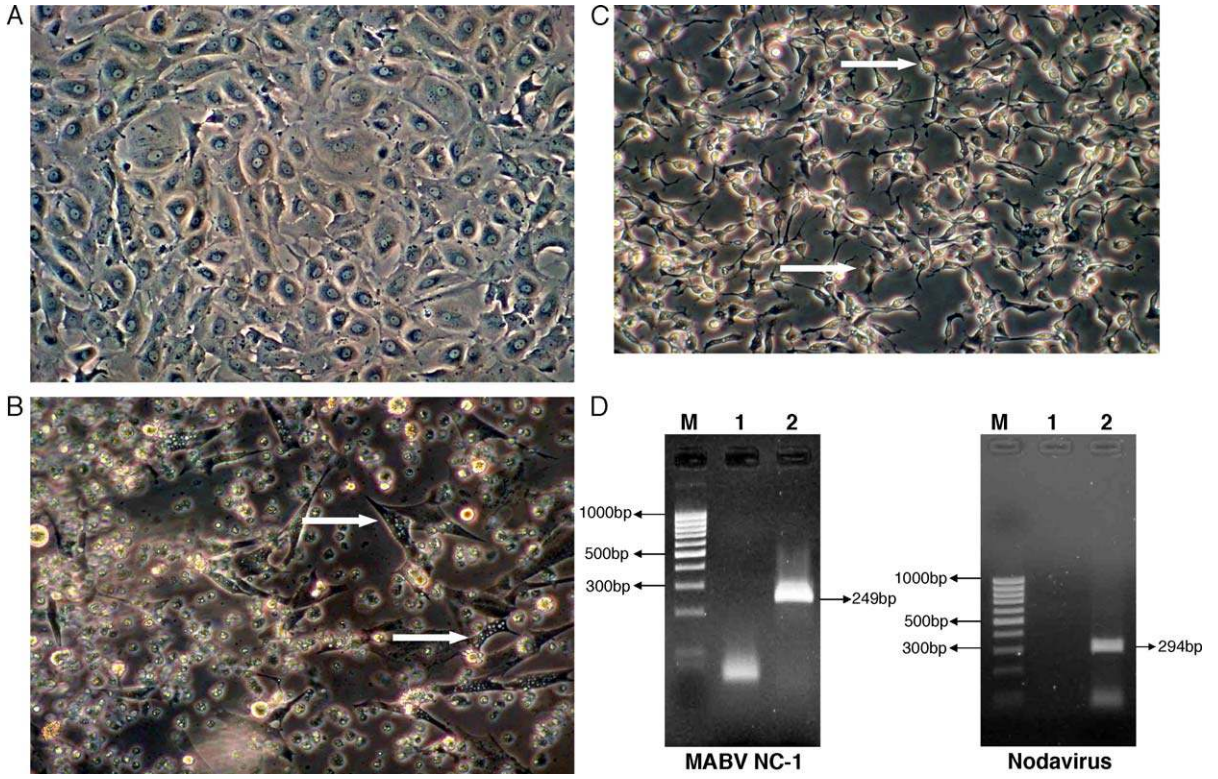

Fig. 4. Susceptibility of SISK cells at the 60th passage to MABV NC1 and nodavirus. (A) Confluent uninfected SISK cells, (B) extensive CPE with multiple vacuolation (arrow) in SISK cells infected with nodavirus, (C) extensive CPE and syncytia formation (arrow) in SISK cells infected with MABV NC-1. (D) Confirmation of viral infection in SISK cells by RT-PCR (M—100 bp marker, lane 1—uninfected cells, lane 2—infected cells).

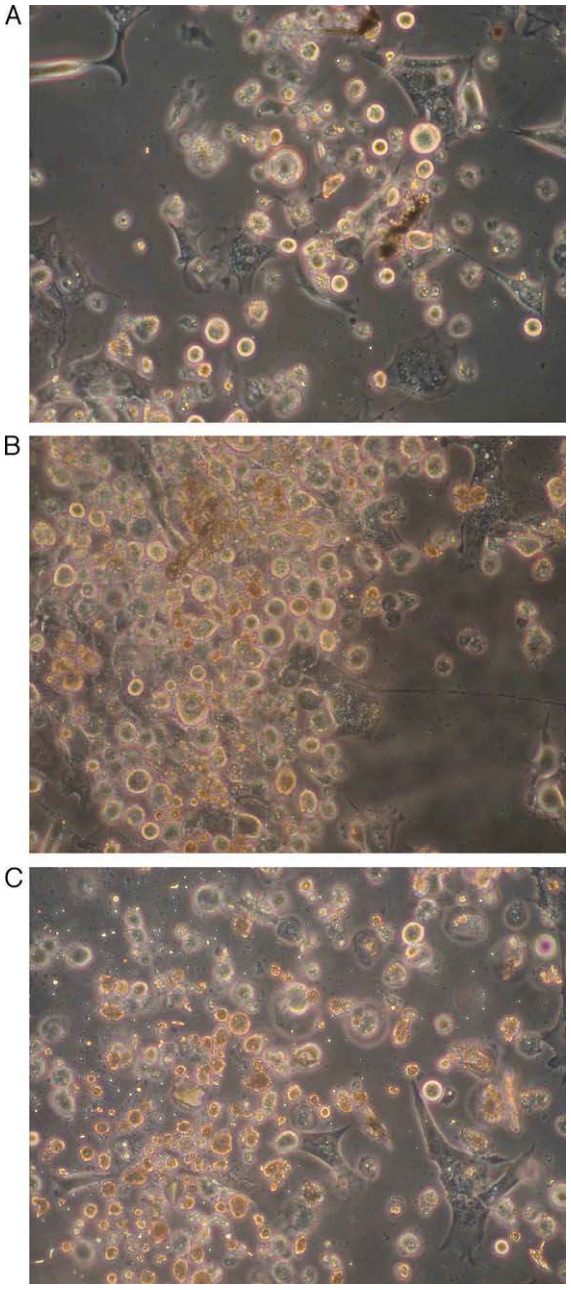

Fig. 5. Cytotoxic effects of extracellular products of Vibrio strains on the SISK cell line. (A) *V. anguillarum*, (B) *V. alginolyticus*, (C) *V. harveyii*.

permeabilized with 0.1% Triton X-100, and blocked in PBS containing 1% bovine serum albumin (BSA). Mouse monoclonal antibodies antihuman nestin, vimentin, glut 4, ki67, cytokeratin 7/17, pancytokeratin or cytokeratin 19 (Sigma, St. Louis) were diluted 1:75 in PBS with 1% BSA and directly added to the fixed cells and kept for 2 h at RT. Then the cells were washed with wash buffer, followed by addition of the appropriate second antibody. Secondary antibodies, anti-mouse IgG FITC and anti-rabbit IgG FITC at the dilution of 1:50 in the same solution as the primary antibody were applied for 45 min at RT. Then the cells were washed with wash buffer and the coverslips were mounted with antifade 1, 4-diazobicyclo-2, 2, 2-octanex (DABCO) in mounting medium (Sigma). The coverslips were observed using a pinhole setting of 100 μm with CFLSM (Carl Zeiss, Jena Germany). Images were captured by the CCD-4230 camera coupled with the microscope and processed using the computer-based programmable image analyzer KS300 (Carl Zeiss) (Anjali et al., 2003).

2.9. Determination of cell proliferation by MTT assay

Cell proliferation in the sea bass kidney cell line was determined by 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-tetrazdium bromide (MTT) assay for different periods up to 60 h at 40, 50 and 70th passages (Hansen et al., 1989). The growth potential of kidney cell line was assessed by its reactivity to 5-bromo-2-deoxyuridine (BrdU), a proliferation marker (Erlanger and Beiser, 1964).

3. Results

Cell cultures were initiated from several tissues of sea bass, including heart, liver, kidney and gills. The cells migrated from the different tissue fragments and grew well and formed monolayer during the first month. However only the cells from the kidney tissue grew continuously when subcultured at intervals of 5 to 7 days. The cells were split at a ratio of 1:2 or 1:3. The initial subcultures of cell line consisted of both epithelial-like and fibroblast-like cells. After 20 subcultures, only epithelial type cells were observed. The cells were split at a ratio 1:2 every 2–3 days after 50th subculture. Rounded-up cells were frequently observed in the early subcultures. The kidney cell line has been subcultured more than 100 times since initiation in

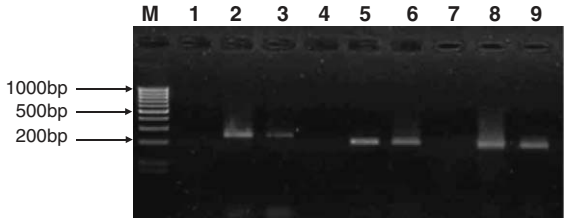

Fig. 6. Agarose gel electrophoresis of PCR products from SISK cells. M, molecular weight marker, lanes 1, 4 and 7—without template; lanes 2, 5 and 8—muscle tissue of sea bass; lanes 3, 6 and 9—SISK cell line.

September 2003 and is designated as Sahul India Sea bass kidney cell line (SISK). Morphologically, sea bass kidney cell line is composed of epithelioid cells with diameters of 15 μm (Fig. 1).

Sea bass kidney cells exhibited different growths at different temperatures (Fig. 2A). The cells were able to grow at temperature between 25 and 32 °C. However, maximum growth was obtained at 28 °C. No significant

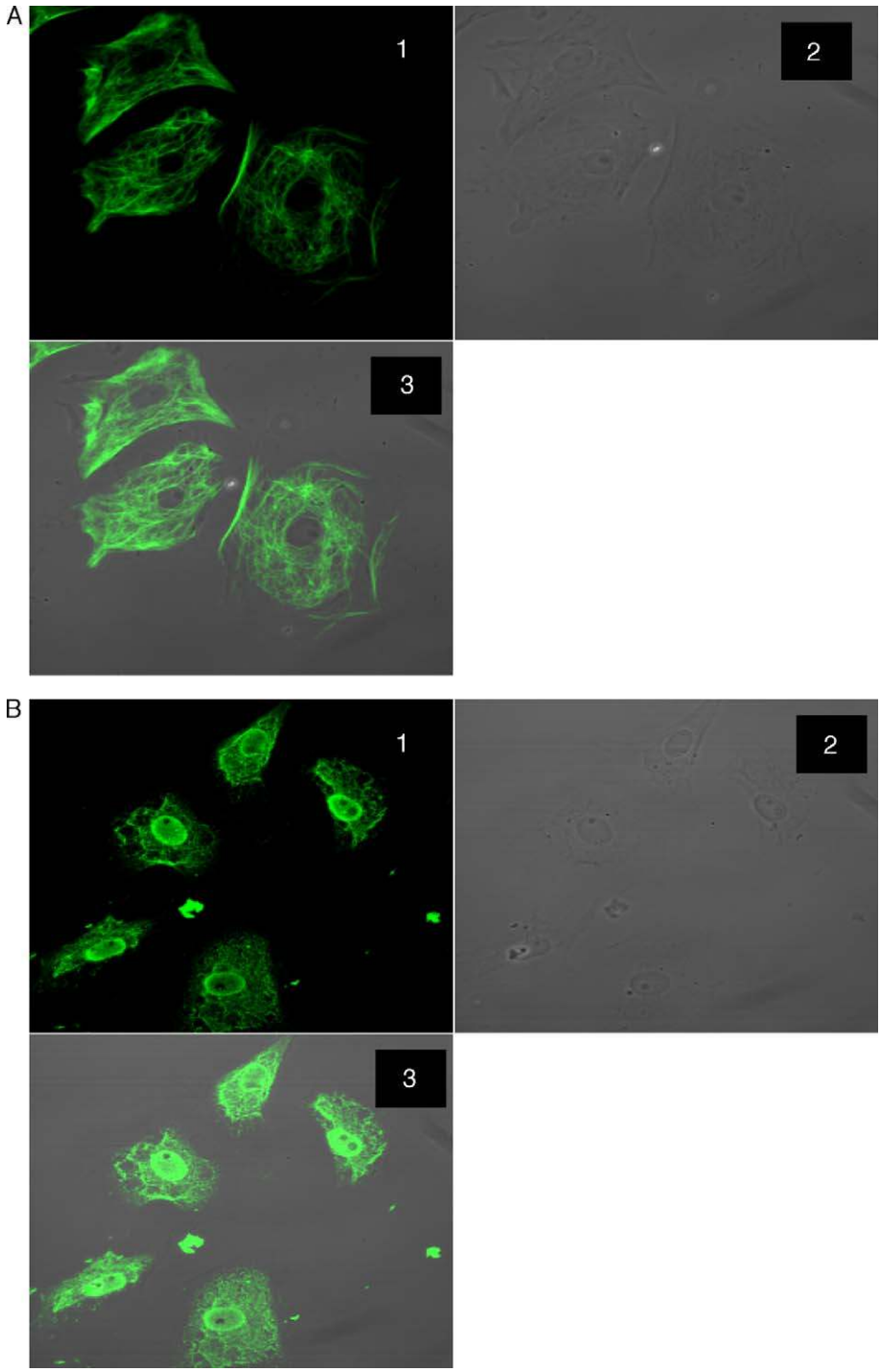

Fig. 7. Confocal microscopy for detection of pancytokeratin (A) and cytokeratin 19 (B) using anti-mouse IgG FITC. Panels 1, 2 and 3 show the fluorescent image, phase image, and fluorescent image overlapped with the phase image of the cells, respectively.

growth was observed at 20, 37 and 40 °C in the cells (Fig. 2A). The growth rate of sea bass kidney cells increased as the FBS proportion increased from 2% to 20% at 28 °C (Fig. 2B). Cells exhibited poor growth at 2% and 5% concentrations of FBS, relatively good growth at 10% but maximum growth occurred with the concentrations of 15% and 20% FBS (Fig. 2).

Sea bass kidney cells recovered from storage at the 30th and 60th subcultures grew to confluency in 2 days. The average viability for recovered kidney cells at the 30th and 60th passages was estimated about 80% to 90%.

The results of chromosome counts of 100 metaphase plates from sea bass kidney cells at passage 37 showed a diploid number ranging from 30 to 56 with a modal peak at 48 chromosomes (Fig. 3A). The chromosome number distribution at passage 61, based on 100 metaphase plates, displayed a 2*n* value ranging from 27 to 52 with the modal value at 46 chromosomes (Fig. 3B). The chromosome morphologies of sea bass kidney cells were similar, with all chromosomes being mostly telocentric (Fig. 3C).

The suitability of sea bass kidney cells for virus isolation was evaluated by its susceptibility to 5 fish viruses (Nodavirus, MABV NC1, CSV, IHNV and IPNV Sp). Significant CPE was observed in the cells 32 h post-infection (p.i) with nodavirus and MABV NC1 (Fig. 4B, C), but not with other 3 viruses. No CPE was observed in uninfected kidney cells (Fig. 4A). Initially, the specific CPE developed as localized areas of rounded and refractile cells which later spread over the monolayer in 48 h p.i. to form a network of degenerating cells. The monolayer was completely disintegrated after 3 days. The CPE with typical multiple vacuolation was observed in cells infected with nodavirus (Fig. 4B) and network degeneration with syncytial formation in cells infected with MABV NC1 (Fig. 4C). The infection in the cells by these viruses has been confirmed by RT-PCR. The experimentally infected sea bass cell line showed positive for Nodavirus and MABV NC1 by RT-PCR (Fig. 4D).

The ECP from different species of *Vibrio* proved to be cytotoxic for sea bass kidney cell line. Cytotoxic effects could be observed within 12 h after inoculation

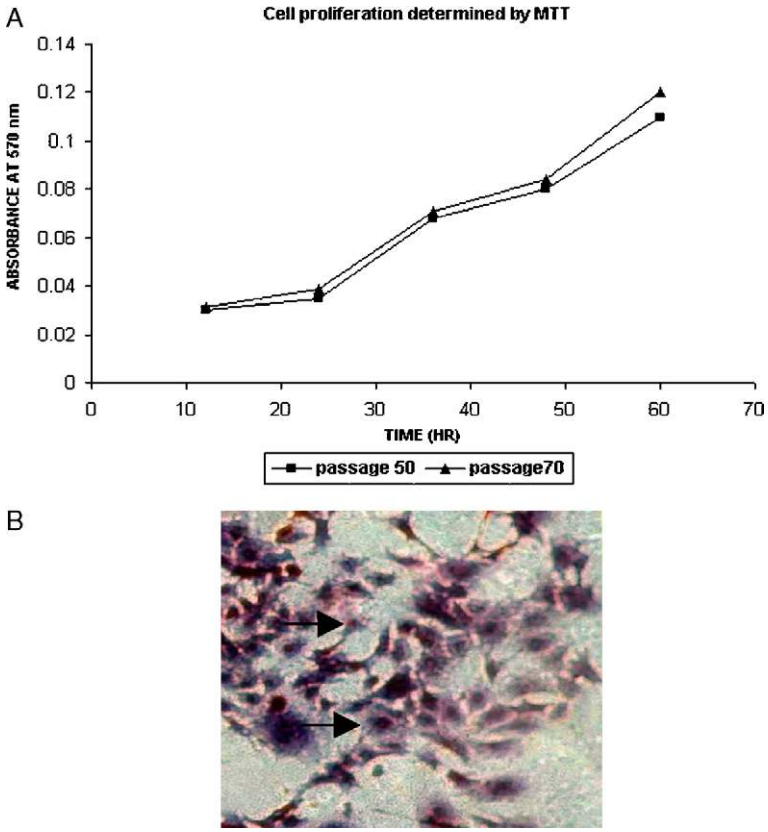

Fig. 8. Cell proliferation determined by MTT assay, at passages 50 and 70. (A) Graphical representation of growth of SISK cells over a period of 60 h. (B) Represents the growth potential of SISK cell line assessed by their reactivity to BrdU, a proliferation marker. Arrow indicates the incorporation of BrdU in the DNA of the nucleus.

of ECP. The morphological changes detected were similar to those described for viral cytopathic effects. While all ECP produced rounding, shrinking, detaching and finally monolayer destruction (Fig. 5A–C), numerous vesicles and dendritic formations were additionally observed in affected cells, due to the effect of ECP of some *Vibrio* species.

Three primer sets of microsatellite markers for sea bass (*L. calcarifer*) worked well against kidney cell line, suggesting that this cell line originated from sea bass (Fig. 6).

All cells of sea bass kidney cell line were strong positive for cytokeratin 19 and pancytokeratin (Fig. 7A, B) whereas these cells showed negative for all other proteins tested.

The growth potential assayed using MTT assay indicates that the cells of this cell line were fast growing with a population doubling time of 36 h at 40 and 70th passages (Fig. 8A). Because BrdU serves as an index of the proliferation activity of the cells, the proliferation of the cell line was assessed by BrdU immunoreactivity. As represented in Fig. 8B, more than 90% of the cells were expressing BrdU in the SISK cell line.

4. Discussion

The successful production in aquaculture industry has been increasingly hampered by many factors including diseases, especially caused by viruses. Susceptible cell lines are essential for the isolation, cultivation and characterization of fish viruses. Since the first fish cell line reported in the literature in 1962 (Wolf and Quimby, 1962), at least 157 fish cell lines have been established (Fryer and Lannon, 1994). Most of them were derived from freshwater or anadromous fish species. However, the studies on marine fish cell lines is growing rapidly, reflecting the increased interest in marine fish cultivation. In India, only a few freshwater fish cell lines and primary cell culture have been developed (Sathe et al., 1995; Lakra and Bhonde, 1996; Rao et al., 1997). No marine fish cell line is available in India. This paper describes the establishment and characterization of India's first marine fish cell line, designated as SISK, derived from kidney of sea bass (*L. calcarifer*). This cell line is well adapted to grow in Leibovitz' L-15 supplemented with fetal bovine serum. The suitability of Leibovitz' L-15 in supporting fish cell lines compare to that of other media has been documented by Fernandez et al. (1993) when they compared the growth of many fish cell lines in different culture media at different temperature and sodium chloride concentration. The SISK cell line has been maintained through more than 100 passages over a 2-year period. This cell line has good adaptation for growth in Leibovitz' L-15 without special requirements, such as NaCl addition which is needed for other marine fish cells (Clem et al., 1961; Law et al., 1978; Li et al., 1984; Fernandez et al., 1993).

The growth temperature range for SISK was 20 to 32 °C with optimum growth at 28 °C which was identical with other fish cell lines reported previously (Nicholson et al., 1987; Tong et al., 1997, 1998; Kang et al., 2003). One of the advantages of cell lines that grow over a wide temperature range is their potential suitability for isolating both warm water and cold water fish viruses (Nicholson et al., 1987). The ability of SISK cells to grow at temperatures between 25 and 32 °C should thus increase the spectrum of viruses that they can be used to isolate. The growth rate of SISK cells increased as the FBS concentration increased from 2% to 20%. However, a 5% concentration of FBS also provided relatively good growth and this is an advantage to maintain this cell line at low cost. Cryopreservation of cell lines is necessary for long-term storage. The feasibility of cryopreservation of this cell line was demonstrated, with appreciable recovery after thawing of up to 90%, while that of SAF-1 (gilt-head sea bream, fin), GF-1 and SF was 50% (Bejar et al., 1997), 73% (Chi et al., 1999) and 80–85% (Chang et al., 2001), respectively.

The modal values of $2n=48$ obtained at the passage 37 correspond to the standard $2n$ of this species. The diversity of the chromosome numbers of SISK cells at the 61st passage and the occurrence of aneuploidy and heteroploidy are indicators of cell transformation (Freshney, 1986), but the cells have not changed morphologically during their in vitro life. Fish cell lines do not always contain the same number of chromosomes as the intact host species (Hsu, 1973). To confirm the cell lines originated from sea bass, PCR reactions with three microsatellite markers (Yue et al., 2001, 2002) were performed. PCR products of expected size from tissues of sea bass and cell line were obtained from all the three markers used in this study. These results indicate that SISK cells are of sea bass origin. Various antibodies of epithelial markers were used to confirm the epithelial nature of this cell line and SISK cells showed strong positive to pancytokeratin and cytokeratin 19. These results show that SISK cell line is an epithelial type.

The growth potential of SISK cell line was assayed by MTT assay and the results indicate these cells were fast growing with a population doubling time of 36 h. Because of fast growing nature, this cell line may have various applications like toxicological studies

and various aspects related to genetic engineering such as evaluating the efficiency of the promoters in transgenic constructions, and mutagenesis in addition to virology. The incorporation of BrdU in the nucleus of SISK cells indicates that these cells were proliferative type.

Bejar et al. (1997) have proved the suitability of SAF cell line from gilt-head sea bream to demonstrate the cytotoxic factors of fish pathogenic bacteria, such as *Vibrio*. In the present study, cytotoxic effect of ECP of different species of *Vibrio* was tested using SISK cell line and morphological changes observed in cell line were similar to those described by Bejar et al. (1997). This indicates the suitability of SISK cell line to test the cytotoxic factors of fish and shrimp vibrios.

Susceptibility of cell lines to viral infection is the basis for isolating and characterizing fish viruses. In the present study, five fish viruses were tested on SISK cell line to determine its susceptibility to these viruses and the results showed that this cell line was susceptible to MABV NC1 and Nodavirus. This has been confirmed by RT-PCR using specific primers for these viruses. The results of CPE and RT-PCR indicate that nodavirus can be propagated in SISK cell line, and confirms the potential of the SISK cell line as a powerful tool for isolating and identifying infectious viruses from the sea bass. Previous attempts to isolate nodavirus in a variety of cell lines were largely unsuccessful (Breuil et al., 1991; Mori et al., 1991; Munday et al., 1992). However, successful isolation of nodaviruses have been reported in other cell lines, the SB cell line (Chua et al., 1995), the SSN-1 snakehead cell line (Frerichs et al., 1996), the GF-1 grouper cell line (Chi et al., 1999), the SF sea bass cell line (Chang et al., 2001), TF turbot cell line (Aranguren et al., 2002) and the BB cell line from sea bass (Chi et al., 2005). The present study shows that the SISK cell line can be used for the isolation of nodavirus from diseased fish in India. In the nodaviral infection, CPE was more apparent and consisted mainly of cells rounding up, multiple vacuolation and detaching from the monolayer. This CPE was similar to that reported by Chua et al. (1995) and Chang et al. (2001) in the SB cell line and SF cell line, respectively. The epithelial type cells have been found to be highly susceptible to the nodavirus (Chi et al., 1999; Chang et al., 2001) and the SISK cell line developed in the present study is epithelial type by morphology and confirmed by epithelial markers. The susceptibility of this cell line to nodavirus is also confirmatory to support the epithelial nature of this cell line as observed by Chi et al. (1999).

In summary, a marine fish cell line, SISK, was established from kidney of *L. calcarifer*, and potentially used for the study of infectious viruses in sea bass and for developing cell models for toxicological studies to replace whole animals and genetic engineering.

Acknowledgement

The authors thank the management of C. Abdul Hakeem College for providing the facilities to carry out this work. The authors also thank the Director, Central Institute of Brackishwater Aquaculture for providing the experimental animals. This work was funded by Department of Biotechnology, Government of India, New Delhi, India.

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