Development of two cell lines from *Epinephelus coioides* brain tissue for characterization of betanodavirus and megalocytivirus infectivity and propagation


a Department of Life Sciences, National University of Kaohsiung, Kaohsiung, 811, Taiwan, ROC
b Department of Animal Science, National I-Lan University, I-Lan, 260, Taiwan, ROC
c Institute of Biotechnology, National University of Kaohsiung, Kaohsiung, 811, Taiwan, ROC

**ABSTRACT**

Betanodaviruses and megalocytiviruses are the causative agents of viral nervous necrosis and iridoviral disease, respectively, among marine species farmed in Taiwan. Because there are few cell lines susceptible to these viruses, we wished to identify additional lines with which to study viral pathology and epidemiology. Thus, we established two clonal cell lines designated GBC1 and GBC4 from the brain of an orange-spotted grouper, *Epinephelus coioides*. Both cell lines grew well in Leibovitz’s L-15 medium supplemented with 5% (GBC1) or 10% (GBC4) fetal bovine serum at temperatures between 20 °C and 35 °C. Cytokeratin immunofluorescence staining revealed that both cell lines were of epithelial origin. GBC4 cells expressed glial fibril acidic protein suggesting that they are astroglial lineage cells. The modal diploid chromosome number was 44 and 48 for GBC1 and GBC4, respectively. GBC1 cells were highly susceptible to grouper nerve necrosis virus (GNNV) and yielded titers of 1010 TCID50 ml−1 but were non-susceptible to the giant seaperch iridovirus (GSIV). By contrast, GBC4 cells were susceptible to GSIV with titers approaching 109 TCID50 ml−1 whereas GNNV infection only yielded titers of 106 TCID50 ml−1. GNNV propagated in GBC1 across a wide range of temperatures (15–37 °C) whereas in GBC4, GSIV propagated over 15–30 °C. Induction of the Mx protein upon GNNV infection occurred in GBC4 but not in GBC1, suggesting that the Mx protein inhibits virus production. Our results suggest that these two cell lines provide a valuable tool for the isolation and investigation of betanodavirus and megalocytivirus.

© 2008 Elsevier B.V. All rights reserved.

**1. Introduction**

Piscine nervous necrosis viruses (NNVs) and iridoviruses cause severe epizootic outbreaks resulting in mass mortality and large economic losses in farmed marine fishes in Taiwan as well as other Southeast Asian countries. Piscine NNVs are icosahedral, non-enveloped, single-stranded RNA viruses 25–30 nm in diameter and are classified in the genus Betanodavirus of Nodaviridae (ICTVdB Management, 2006b; http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/). They are the causative agents of viral nervous necrosis (VNN) disease in marine species in many countries (Renault et al., 1991; Mori et al., 1992; Chua et al., 1994; Chi et al., 1997; Grotmol et al., 1997; Bovo et al., 1999; Cutrín et al., 2007). The diseased fish commonly display vacuolating encephalopathy and retinopathy that frequently lead to death, especially at the larval and juvenile stages. More than 30 susceptible fish host species have been reported from more than ten families (Munday et al., 2002). All the NNVs can be classified into one of the four following genotypes based on partial sequences of the coat protein genes: SJNNV (striped jack NNV), RGNNV (red spotted grouper NNV), TPNNV (tiger puffer NNV), and BFNNV (barfin flounder NNV) (Nishizawa et al., 1997). The NNVs discovered in Taiwan, including grouper NNV (GNNV) and yellow grouper NNV (YGNNV), have the RGNNV genotype (Lai et al., 2001; Chi et al., 2003).

Iridoviruses have been isolated and identified from at least 50 fish species collected in the South China Sea (Wang et al., 2007). The distinctive symptom of iridovirus infection is the formation of inclusion body-cells in infected organs. They are icosahedral double-stranded DNA viruses and the virions consist of an envelope, a capsid, and an internal lipid membrane. The capsid is isometric and has a diameter of 125–300 nm and consists of probably 72 capsomers (ICTVdB Management, 2006a; http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/). Piscine iridoviruses are classified into either genus *Lymphocystivirus, Ranavirus,* or *Megalocytivirus*. Nakajima and Kurita (2005) further classified megalocytiviruses into three major groups by phylogenetic analysis of the major capsid protein (MCP): red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis iridoviruses (ISKNV), and turbot reddish body iridoviruses (TRBIV). In Taiwan, epizootic iridovirus outbreaks have occurred in several important farmed fish such as grouper (*Epinephelus spp.*), giant...
seaperch (*Lates calcarifer*), and red sea bream (*Pagrus major*) resulting in significant economic losses (Chou et al., 1998; Lai et al., 2000; Chao et al., 2001; Lai et al., 2000; Chao et al., 2002; Tsai et al., 2005). Although many fish cell lines have been established, relatively few have the ability to propagate betanodavirus or iridoviruses. The few cell lines (Chew-Lim et al., 1994; Nakajima et al., 1997; Chi et al., 1999a; Chang et al., 2001; Lai et al., 2001; Qin et al., 2006; Imajoh et al., 2007) capable of viral propagation but produce the viruses slowly and give low viral titers. It thus has become crucial to develop suitable cell lines not only for vaccine development but also for isolation and study of the viruses.

### 2. Materials and methods

#### 2.1. Fish and primary culture preparation

Young orange-spotted groupers (*Epinephelus coioides*), approximately 10 cm in length, were obtained from a commercial fish farm (Yeong-An, Kaohsiung, Taiwan). The fish were anesthetized by MS-222 (Sigma) and decapitated aseptically as described previously (Wen et al., 2008). The brain was removed and finely chopped with scissors in phosphate-buffered saline (PBS) (Ca²⁺- and Mg²⁺-free). The tissue fragments were then washed several times in an antibiotic solution (PBS containing 500 μg/ml streptomycin and 500 IU/ml penicillin) and were subsequently placed into a 25 cm² tissue culture flask (Nunc) containing 2 ml Leibovitz’s L-15 growth medium supplemented with 15% fetal bovine serum (FBS) (Gibco) and incubated at 25 °C. Every 4–5 days, half of the growth medium was replaced and replaced with fresh medium.

#### 2.2. Subculture and clone isolation

Cells were subcultured when confluent monolayers developed. Cells were washed twice with PBS and were dislodged from the flask surface by treatment with a 0.1% trypsin solution containing 0.2% EDTA and subcultured at a split ratio of 1:2. At the 10th passage, cells were dislodged as before and plated in 3-cm tissue culture dishes at 1000 cells per dish. Clones were allowed to expand for up to 14 days, and single colonies of cells were isolated using cloning rings (Nunc). Cells therein were suspended and plated on 4-well plates, and gradually expanded into cohesive, relatively monovoluminous monolayers. Nuclei and nucleoli (arrows) are also apparent. Bar=30 μm.

#### 2.3. Expression of antigenic markers

Cells grown on coverslips were washed three times with PBS and fixed for 10 min in methanol at room temperature. Mouse anti-human keratin (Clone C-11), mouse anti-porcine glial fibrillary acidic protein (GFAP) (Clone GA-5), and mouse anti-porcine vimentin (Clone V9) monoclonal antibodies (all from NeoMarkers and diluted 1:200) were used to identify epithelial cells, astroglia, and mesenchymal cells, respectively. For fluorescence immunocytochemistry, FITC-conjugated goat anti-mouse IgG (Cappel; 1:100) was used. Otherwise, the cells were labelled with horseradish peroxidase-conjugated goat anti-mouse IgG (Cappel; 1:10,000) and visualized using TrueBlue peroxidase substrate (KPL). Orcein (KPL) was used as a counter stain. Negative controls (omission of the primary antibody) were included in each experiment.

#### 2.4. Scanning electron microscopy (SEM)

Cells grown on coverslips were washed three times with PBS, fixed in 2.5% glutaraldehyde (Sigma) and post-fixed in 1% OsO₄ (Sigma) for 30 min. The fixed cells were dehydrated using serial alcohol and critical point drying (Hitachi CP-2). The specimens were coated with a layer of platinum-palladium and observed under a Hitachi S-520 SEM.

#### 2.5. Growth study

To determine the optimum incubation temperature for cell growth, 5×10³ cells (GBC1 and GBC4 cells at passage 180 and 95, respectively) were seeded in duplicate in 25 cm² culture flasks and incubated at 15, 20, 25, 30, or 35 °C. The number of cells from duplicate flasks at each temperature was recorded every day for 5 days. Similarly, the effect of various FBS concentrations (2, 5, 10, or 15%) on cell growth was assessed at 25 °C.

#### 2.6. Chromosome analysis

Chromosome preparations were made from GBC1 and GBC4 cells after 135 and 90 passages, respectively. One-day-old cultures (70–80% confluency) were treated with 0.1 μg ml⁻¹ colcemid (Gibco) for 6 h at 25 °C and dislodged using 0.1% trypsin solution. After centrifugation at 150 ×g for 10 min, the cells were suspended in a hypotonic solution (0.04 M KCl) for 30 min at room temperature. After swelling, the cells were fixed with methanol and acetic acid (3:1) fixative. The...
suspension of fixed cells was dropped onto cold slides that then were
dried rapidly using a hair dryer. The cells were stained with Giemsa
solution (Merck), and the chromosome numbers were counted under
an Olympus XK microscope.

2.7. Iridovirus isolation and identification

Diseased giant seaperch (*Lates calcarifer*) collected from Yeong-An,
Kaohsiung, Taiwan were confirmed to have iridovirus infection using
PCR analysis as described (Wang et al., 2003). A piece of liver (about
0.5 g) from the diseased fish was homogenized in 5 ml L-15 medium.
The homogenate was centrifuged at 2000 × g for 10 min, and the
supernatant fluid filtered through a sterile 0.45-μm syringe-filter. The
filtrate (100 μl) was added to a monolayer of GBC4 cell culture and
incubated at 25 °C until the cytopathic effect (CPE) was observed.

After appearance of advanced CPE, the infected cells were
harvested and pelleted by centrifuged at 1000 × g. The cell pellet was
suspended in 500 μl lysis buffer (50 mM Tris–HCl pH 8, 20 mM NaCl,
2% SDS, 10 mM EDTA, proteinase K 100 μg ml⁻¹) and incubated at 58 °C
for 30 min. The giant seaperch iridovirus (GSIV-K1) genomic DNA was
extracted using phenol/chloroform as described (Wang et al., 2003).
DNA fragments of MCP gene were amplified by PCR from the GSIV
genomic DNA using the primers 5′-gaaaaacgaggccgatcata-3′
(forward) and 5′-tacgctatggccacaattca-3′ (reverse). PCR was performed using
Taq DNA Pol MasterMix (Ampliqon) according to the manufacturer’s
instructions. PCR amplification was performed as follows: 95 °C for
120 s and 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s.
After PCR, electrophoresis was performed on 1.5% agarose gel. The
amplified DNA fragment (∼1470 bp, data not shown) was cut from the
agarose and then purified with Gene-Spin 1-4-3 DNA Extraction kit
(Protech Tech Co.) and ligated with a TA TOPO PCR Cloning kit
(Invitrogen) and transformed into E. coli. Sequencing of the DNA
fragment was performed commercially (Genomics BioSci & Tech
Corp.). BLAST (NCBI) search indicated 99% sequence identity between
the MCP genes of GSIV-K1 (GenBank accession no. EU315313) and
RSIV (accession no. AY310918). Therefore, GSIV-K1 belongs to the RSIV
group of Megalocytiviruses.

2.8. Viral susceptibility and the CPE

Confluent GBC1 and GBC4 cells in 25 cm² culture flasks were
infected with GSIV-K1 or GNNV (Chi et al., 1999a) at 0.1–1000
multiplicities of infection (MOI) by adsorption for 1 h at room
temperature. The inoculum was removed and fresh L-15 medium was
added to the cells. Infected monolayers were incubated at 20, 25, 30,
or 35 °C and examined daily for the presence of the CPE.

2.9. Viral replication efficiency

The efficiency of GNNV and GSIV replication in GBC1 and GBC4
cells was also examined. GBC1 (1 × 10⁷ cells) and GBC4 (4 × 10⁶ cells) in
75 cm² flasks were infected with each virus at an MOI of 10 or 100.

![Fig. 2. Cytokeratin and vimentin immunofluorescence of *Epinephelus coioides*-derived cell lines. GBC1 (A and C, passage 133) and GBC4 (B and D, passage 85) cells grown on coverslips were incubated with mouse monoclonal anti-human keratin (A and B) or monoclonal anti-porcine vimentin (C and D) followed by FITC-conjugated anti-mouse IgG labeling. Bars=50 μm.](image-url)
Virus solution was adsorbed for 1 h at room temperature, and then the inoculum was removed. The monolayer was rinsed twice with L-15 medium, and 20 ml fresh L-15 medium was added and incubated at 15, 20, 25, 30, or 35 °C. A 37 °C incubation was also included for GNNV-infected GBC1 cells. Duplicate flasks of infected GCB1 or GBC4 cells from each temperature were harvested at 1, 2, 3, 4, 5, 10, and 15 days after viral inoculation, and the titrations were performed in 96-well plates and TCID50 ml−1 was calculated according to Reed and Muench (1938).

3.2. Effect of temperature and FBS on growth

Like other cell lines from tropical marine fish, GBC1 and GBC4 were able to grow at temperatures between 20 and 35 °C. Nevertheless, optimal growth was at 30 °C for GBC1 and at 35 °C for GBC4 (Fig. 4). GBC1 cells grew in 5% FBS at 30 °C with population doubling times less than 24 h, whereas GBC4 cells grew in 10% FBS at 35 °C with doubling times longer than 2 days. The saturation density for GBC4 cells approached 3 × 106 cells per 25 cm2 flask, whereas it was possible for GBC1 cultures to reach 1 × 107 cells per 25 cm2 flask (or greater if the culture medium was refreshed; data not shown).

Growth curves in the presence of varying concentrations of FBS are shown in Fig. 5 and indicate that both cell lines respond positively to FBS but that GBC1 cells require significantly less FBS than GBC4 cells. Adherence and spreading of GBC1 cells grown in high-serum medium was higher compared with cells grown in low serum (data not shown).

3. Results

3.1. Cell morphology and expression of antigenic markers

GBC1 and GBC4 cells were cloned and selected due to distinct morphology. SEM indicated that GBC1 cells were smaller and round and exhibited numerous microvilli (Fig. 1A), whereas GBC4 cells were larger, flat and polygonal, with multiple processes, a smooth surface, and growth in patch (Fig. 1B). Both cell lines exhibited epithelial characteristics as evidenced by cytoplasmic cytokeratin immunofluorescence (Fig. 2A,B). However, the expression of cytokeratin was lower in GBC1 than in GBC4. Strong vimentin labeling in the cytoplasm of the both lines indicated that they were immature cells (Fig. 2C,D). Many of the GBC4 cells were labeled with an antibody against GFAP (Fig. 3), suggesting that they were astroglial lineage cells. GBC1 cells were GFAP-negative (data not shown). GFAP expression in GBC4 cells was likely associated with cell contact because GFAP immunostaining was more prevalent in highly confluent cells. In keeping with this hypothesis, GBC1 cells, which were GFAP-negative, lacked contact inhibition and required less FBS for proliferation, suggesting that they were transformed cells. After a series of subcultures, spindle-shape epithelial cells appeared in both GBC4 and GBC1 culture.
3.3. Chromosomal analysis

The diploid karyotype of GBC4 cells is shown in Fig. 6A and consists of a single acrocentric pair of chromosomes and 23 pairs of telocentric (2n=2ac+46t) chromosomes. The results of chromosome counts of 100 metaphase plates from GBC4 cells at passage 90 revealed that the chromosome numbers varied from 24 to 50, with a mode of 48 (Fig. 6B). Karyotype analysis revealed that only about 20% of the cells had a diploid chromosome number of 2n=48, similar to the modal chromosome numbers of the orange-spotted grouper (Wang et al., 2004). On the other hand, chromosome numbers in the GBC1 cells at passage 135 varied from 22 to 58, with a modal value of 44 (Fig. 6C). A high proportion of cells in both cell lines displayed aneuploidy and heteroploidy, but these phenomena were more pronounced in the GBC1 cell line.

3.4. Virus susceptibility and the cytopathic effect

Vacuolization was observed in GBC1 cells within 2 days of inoculation with GNNV at an MOI of ≥1 and with growth temperatures above 25 °C. The appearance and advancement of this effect was more dependent on incubation temperature than on MOI. Monolayers of GBC1 cells incubated at 35 °C usually completely disintegrated within 4 days of infection (Fig. 7A), whereas only a few vacuolated cells were observed 4 days post-infection among cultures grown at 20 °C, even with an MOI ≥100. By contrast, the CPE in GBC4 cells was unobtrusively as compared with GBC1. A few apoptotic-like cells and evidence of burst cells were observed with an MOI ≥100 (Fig. 7C). Thus, GNNV-infection in GBC4 is latent compared with GBC1.

In contrast to GNNV infection, GSIV infection of GBC1 cells produced no CPE regardless of the MOI or incubation temperature (data not shown). On the other hand, numerous rounded cells and dark cells with a bright halo were observed among GBC4 monolayers inoculated with GSIV (Fig. 7D), indicating a CPE as previously defined (Imajoh et al., 2007). The appearance of the CPE correlated strongly with the MOI, and no CPE was observed at an MOI below 1. The day after inoculation, some of the GBC4 cells incubated at 35 °C became rounded, and were dislodged from the surface and lysed following the incubation time increase, but further extensive CPE was not observed. Nevertheless, progression of extensive CPE was noted at temperatures between 15 °C and 30 °C and increased with temperature. The monolayers when infected with an MOI of 100 usually disintegrated completely within 6 days post-infection at 30 °C but needed more than 10 days to disintegrate at 20 °C.
3.5. Viral propagation efficiency

The results of GNNV propagation at selected temperatures are shown in Fig. 8. GBC1 produced GNNV efficiently between 25 °C and 35 °C with yields above $10^{10}$ TCID$_{50}$ ml$^{-1}$ (Fig. 8A). When GBC1 cells were incubated between 30 °C and 37 °C, the highest progeny virus yields occurred 2 days post-infection, whereas optimal viral yield required 5 days when cells were incubated at 25 °C. Viral yields accompanied progression of the CPE and depended on incubation temperature; low yields occurred below 20 °C. Decrease of the viral infectivity was noted following the increase of incubation temperature and time. Inactivation of the virus was notable at 10 days post-infection at 35 °C and 37 °C. Therefore, the highest titer at 37 °C was lower than that at temperatures between 25 °C and 35 °C even though GNNV at 37 °C showed more apparent CPE.

GNNV replication in GBC4 cells at different temperatures was distinct from that seen in GBC1 cells (Fig. 9B). The highest virus yields were no more than $10^{6}$ TCID$_{50}$ ml$^{-1}$ and appeared the day after inoculation. The low viral yields presumably reflected the superior antiviral potential of GBC4.

The replication of GSIV in GBC4 cells at different temperatures was shown in Fig. 9. In contrast to GNNV, GSIV replicated well in GBC4 cells between 15 °C and 30 °C. Under these conditions, viral yields increased with increasing incubation temperature and time up to 5 days post-inoculation. Inactivation of the virus was most notable at 35 °C. Decreased infectivity of progeny virus was noted by 10 days post-inoculation when cells were incubated at 25 °C or 30 °C.

3.6. Mx gene expression induced by GNNV

Previous studies have shown that expression of Mx corresponds with resistance to NNV. The resistance of GBC4 cells to GNNV may therefore be due to the induction of Mx expression. The results of RT-PCR analysis of Mx mRNA and GNNV coat protein RNA in GBC1 and GBC4 cells are shown in Fig. 10. GNNV coat protein amplicons were observed in both GNNV-infected cell lines but not in the mock-infected control cells. However, an amplicon of about 630-bp was only produced from GNNV-infected GBC4 cells. Sequence analysis indicated 100% sequence identity between the 630-bp product and the Mx mRNA of the orange-spotted grouper. The sequence has been submitted to GenBank (accession no. EU417821).

4. Discussion

In this study, we generated two cell lines, GBC1 and GBC4, from orange-spotted grouper brain tissue. Immunostaining revealed that the cell lines express keratin and vimentin, suggesting they are neuroepithelial origin. Like other grouper cell lines (Chi et al., 1999a; Lai et al., 2003; Qin et al., 2006), GBC1 and GBC4 exhibited growth over a wide range of temperatures. Examination of morphology, karyotype, and growth characteristics indicated that GBC1 is a transformed cell line whereas GBC4 cells are non-transformed. Even though both of the cell lines were derived from brain tissue, immunostaining indicated that only GBC4 expressed GFAP, a specific...
marker of astroglia, the primary cell type in the brain. GFAP deficiency suggests that GBC1 is not an astroglial cell line; however, loss of GFAP expression may have occurred during transformation.

The snakehead cell line SSN-1 was the first fish cell line used to isolate, cultivate, and characterize betanodaviruses (Frerichs et al., 1996). However, SSN-1 is persistently infected with a C-type retrovirus. To study VNN disease, the NNV-permissive cell lines GF-1, GB3, GS, and GB were established from the fin, brain, and spleen of orange-spotted grouper and from the brain of yellow grouper, respectively (Chi et al., 1999a; Lai et al., 2001; Lin et al., 2006). Unfortunately, all of these cell lines produced low viral titers (no more than $10^9$ TCID$_{50}$ ml$^{-1}$), with the exception of the E-11 clone of SSN-1 that has been shown to produce titers of up to $10^{10.9}$ TCID$_{50}$ ml$^{-1}$. Therefore, the GBC1 cell line reported here, which yielded a CNVN titer above $10^{10}$ TCID$_{50}$ ml$^{-1}$, provides improved efficiency and economy for viral production.

Propagation of GNNV in GBC4 cells was poor and typically generated persistent infection without any obvious CPE. Persistent infection with NNV results from the induction of both interferon I and Mx (Lin et al., 2006; Lu et al., 2008; Wu and Chi, 2006). Wu and Chi (2006) revealed that Mx is expressed in the cell line isolated from barramundi brain (BB), which displays persistent NNV infection; whereas the NNV-free cell line CB lacks Mx expression. However, when cBB cells were infected with NNV, Mx was expressed. In this study, induction of Mx expression by GNNV was confirmed in GBC4 cells but not in the transformed GBC1 cells. Lin et al. (2006) showed that resistance to NNV was partially recovered after transfection of Mx gene into GB3 cells, which are NNV permissive and express low levels of Mx. In addition, Chi et al. (1999a) demonstrated that GF-1 cells were GNNV resistant until transformed. It is possible that these cells lose the ability to express Mx upon transformation. In this regard, Chen et al. (2008) demonstrated that Mx gene-transfected GF-1 cells have limited viral propagation, due to the observed binding of Mx to the viral coat protein. Mx expression was shown to decrease viral titers by approximately 2 log TCID$_{50}$ (Lin et al., 2006; Chen et al., 2008). However, it is possible that antiviral mechanisms other than Mx expression may function in GBC4 cells because viral propagation in these cells is even lower than in those of Mx constitutively expressing cells.

Previous reports have revealed that temperature plays an important role in NNV efficiency and NNV-induced fish mortality (Chi et al., 1999b; Lai et al., 2001, 2003; Aranguren et al., 2002; Ciulli et al., 2006). In addition, different NNV genotypes have been shown to require different temperatures for optimal proliferation in vitro (Iwamoto et al., 2000). The studies published to date indicate that NNV viral production in vitro is poor or ablated above 36°C or below 20°C. Nevertheless, in this study, GNNV propagated in GBC1 cells between 15°C and 37°C. Thus, both host cell type and viral genotype appear to influence the optimal temperature for viral propagation.

Temperature is also important for iridovirus infection. Oh et al. (2006) reported that fatal iridovirus infection occurred among turbots (Psetta maximus) reared between 22°C and 25°C, whereas no infection was observed among turbots reared between 17°C and 20°C. GSIV-K1 propagate at the temperatures, between 15 and 30°C in GBC4, lower than GIV which proliferate in GB at the temperatures between 24 and 32°C (Lai et al., 2003). Although there is some variation in temperature requirement (Jung and Oh, 2000; Lai et al., 2003; Wang et al., 2003), iridovirus propagation and disease outbreak appear optimal between 25°C and 30°C.

In addition to temperature, MOI appears to be important for optimal NNV propagation. Wu and Chi (2006) showed that MOI can
influence the onset of the CPE. In fact, acute CPE typically corresponds with high MOI and elevated temperatures as shown in GBC1. However, in GBC4 cells, viral production and CPE are more apparent at 20 °C than at those at high temperature, especially when an MOI of more than 100 is used. This may result from a reduction in Mx, the expression of which has been positively correlated with temperature. 

Susceptible cell lines for megacellviruses and betanodaviruses are scarce. BF-2 (bluegill fry-2) (Wolf and Quimby, 1966), CRF-1 (clonal red sea bream fin-1) (Imajoh et al., 2007), GF (grunt fin) (Clem et al., 1961), KRE (groupers) (Chou et al., 1998), and SK (seabass kidney) (Khongpradit et al., 2003) are the only cell lines reported to be susceptible to RSIV. However, BF-2 and KRE cannot support viral replication through serial passages, whereas GF cells require human serum, which is difficult to obtain. Nevertheless, all currently available cell lines yield low viral titers (no more than 10^8 TCID50 ml ^{-1}) 

Here, we report that GBC4 cells yield GSIV-K1 titers of up to 10^9 TCID50 ml ^{-1}, suggesting their potential for the isolation of fish iridoviruses. Several piscine cell lines are reportedly permissive to ranaviruses (Chew-Lim et al., 1994; Lai et al., 2000; Chang et al., 2001; Khongpradit et al., 2003), but their susceptibility to RSIV remains unclear. Similarly, the susceptibility of GBC4 to ranavirus needs further study. Even though the susceptibility of GBC1 and GBC4 cell lines to other genotypes of betanodaviruses and iridoviruses is uncertain, the availability of these two cell lines will improve future studies on viral infection in tropical marine fish and will further the subsequent development of epidemiological control of disease outbreaks.

References


