Establishment and characterization of two cell lines from bluefin trevally *Caranx melampygus*

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ABSTRACT: Bluefin trevally *Caranx melampygus* Cuvier is a popular game fish and highly valued sea food with potential importance for aquaculture. To help establish this marine animal as an important aquacultural species in Hawaii and the Pacific and develop *in vitro* cell culture systems for long-term management and control of potential viral diseases 2 cell lines were established from body muscle (bluefin trevally muscles, BTMS) and fins (bluefin trevally fins, BTF). Primary culture of these cells was conducted at 25°C using L-15 medium supplemented with 20 % fetal bovine serum and various antibiotics. These cells have been serially subcultured 37 to 41 times since their initiation in June 2002. Growth of the bluefin trevally cells was serum-dependent at the time of the experiments and their plating efficiencies ranged from 11 to 28.2%. Comparative analysis showed that these bluefin trevally cells grew equally well in the media L-15 (Leibovitz medium), RPMI 1640, M199 and MEM (minimum essential medium), which are commonly used for cell cultures derived from aquatic animals and mammalian species. Examination of the early passage cells stored at –196°C revealed a large percent (nearly 98 %) of cell viability following a 6 mo storage in liquid nitrogen. Karyotyping analysis indicated that these bluefin trevally derived cell lines remained diploid with a chromosome count of 48 at passage 7 and 12. These 2 cell lines shared a same pattern of viral susceptibility and they were sensitive to infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis (IPN), spring viremia carp virus (SVCV), viral hemorrhagic septicemia virus (VHSV), and snakehead rhabdovirus (SHRV) but refractory to channel catfish virus (CCV) infection. These newly established cell lines are currently being used to facilitate the diagnosis of viral disease affecting marine fish aquaculture in Hawaii, and will be available for future isolation and study of bluefin trevally fish viruses.

KEY WORDS: Cell lines · *Caranx melampygus* · Bluefin trevally · Omilu

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INTRODUCTION

Bluefin trevally *Caranx melampygus* Cuvier, which is also known as 'Omilu' in Hawaii, is a member of the jack or Carangidae family (Meyer et al. 2001). There are a total of over 200 jack species and blue jack *C. melampygus* is the most common one in Hawaii and the Pacific (Meyer et al. 2001). As a coastal and oceanic species, bluefin trevally is found widely throughout the Indo-pacific and is associated with reefs (Smith-Vaniz 1995). The rich environment of the coral reef supports a complex food web and provides rich hunting grounds for bluefin trevally species (Anonymous 2004). The juveniles appear seasonally in large numbers in shallow,

sandy inner reef flats in shore waters while the adults range widely throughout deeper lagoon and outer reef waters, and they usually enter channels and shallow reef areas (Lieske & Myers 1994, Smith-Vaniz 1995). Blue jack and other predators also play a key role in the community, contributing to the balance of life in the reef habitat (Anonymous 2004). Adult bluefin trevally usually occur singly or in small schools, except at the south tip of Peleliu in Palau (Republic of Palau), where an enormous aggregation of over 1000 individuals was documented (Smith-Vaniz 1995). Omilu, like other jacks, are large, active reef-associated predators. Trevally larvae are pelagic opportunistic feeders, feeding on zooplankton (Kim et al. 2001), while adult trevally prey mostly on reef

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fish and also crustaceans (Potts 1980, Holland et al. 1996). This warm-water marine finfish can grow to 1 m in body length (Lieske & Myers 1994).

Bluefin trevally is an important source of food and also a prize catch for recreational fisherman. Because of its relatively high market value, it is currently being developed for aquaculture and stock enhancement in the Pacific, particularly in Hawaii (Leber 1994). Recent studies have shown that bluefin trevally reaches sexual maturity between 30 and 40 cm standard length (SL) or at about 2 yr of age (Sudekum et al. 1991, Moriwake et al. 2001). This species is a multiple and serial spawner that appears to spawn more than once a year. Most importantly, bluefin trevally adapt well to captive conditions and are able to breed in confined conditions (Moriwake et al. 2001). This means that they can provide a reliable supply of eggs and larvae, enhancing the success of blue jack aquaculture.

Despite the popularity of blue jack as a game fish and highly valued food with potential importance for aquaculture (Sudekum et al. 1991, Holland et al. 1996), little is known about their sensitivity/resistance to infectious agents, particularly the infections caused by pathogenic viruses. Viruses have been frequently reported from fish, crustacean species and other marine animals; some viruses are highly lethal and are responsible for many significant losses of farmed marine animals (Wolf et al. 1960, Amend et al. 1969, Wolf & Darlington 1971, Plumb 1978, Lightner & Redmann 1981, Sano et al. 1981, Boon-yaratpalin et al. 1992, Chantanachookin et al. 1993, Chen 1995, Loh et al. 1997, Lu et al. 1997, Osterhaus et al. 1997). The lack of cell lines derived from bluefin trevally currently hinders the study and diagnosis of viral diseases of this fish. Successful development of blue jack farming in Hawaii and also worldwide is dependent partly on the establishment of permissive cell lines from this marine species for the isolation, cultivation, study, and control of pathogenic viruses.

In the present paper, we describe methods for the establishment of 2 cell lines from trevally and provide a characterization of their optimum growth, karyological typing and susceptibility to several fish viruses. These newly characterized trevally cell lines (bluefin trevally muscle [BTMS] and bluefin trevally fins [BTF], derived from omilu body muscle and fins, respectively) represent the first report of an *in vitro* cell culture system from this species. These cell lines may provide an important biological tool for future detection and diagnosis of trevally viruses.

MATERIALS AND METHODS

Primary cultures. One apparently healthy bluefin trevally with a body weight of 148 g and a body length

of 18 cm was used in this study. To initiate primary cell culture, 1 to 2 g of the following tissues were aseptically collected from the fish into antibiotic-incubation medium (AIM): heart, spleen, muscle (under mouth), snout, kidney and swim bladder. Tissue samples were incubated at room temperature ($25 \pm 2^\circ\text{C}$) for 2 h in AIM consisting of $1 \times$ Leibovitz medium (L-15), $1000 \mu\text{g ml}^{-1}$ streptomycin, 1000 U ml^{-1} penicillin, $25 \mu\text{g ml}^{-1}$ amphotericin B and $250 \mu\text{g ml}^{-1}$ gentamicin. Following 2 additional washes with AIM (15 min per wash), tissue specimens were minced with sterile instruments, and 20 to 30 tissue fragments (1 to 2 mm^3) were explanted in each 25 cm^2 Primaria-brand tissue culture flask (Falcon Primaria, Fisher Scientific).

Primary cell cultures were initially maintained in L-15 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Hyclone Laboratories) with various antibiotics (200 U penicillin , $200 \mu\text{g streptomycin}$, $50 \mu\text{g gentamicin}$, and $5 \mu\text{g amphotericin B ml}^{-1}$), according to previously described methods (Lu et al. 1999), and incubated at 25°C . When cells grew and became confluent, cells were subcultivated at a ratio of 1:2–3 every 5 to 7 d using a 0.25% trypsin-versene solution (Sigma Chemical).

Optimal growth studies. Growth characteristics of the newly established trevally cell lines were evaluated by growing cells in selected media, at different incubation temperatures, and using or excluding FBS. To determine the incubation temperature for optimal cell growth, approximately 4.5×10^5 bluefin trevally cells at the 12th passage were seeded in 25 cm^2 tissue culture flasks (Corning Labware). These cultures were grown in L-15 medium containing 10% FBS and incubated at 15, 20, 25, 30 and 37°C . Cells from 2 flasks at each temperature were trypsinized on alternate days for a total of 10 d, and average cell counts were calculated. In the same manner, the effect of differing concentrations of FBS (0, 5, 10 and 20%) and of different tissue culture media (RPMI 1640, medium 199 [M199], L-15 and minimum essential medium [MEM]) on cell growth was also assessed in duplicate 25 cm^2 flasks incubated at 25°C .

Cell-plating efficiency. The plating efficiency of BTMS and BTF cells was tested at passage 6 and 10 according to the method of Fryer et al. (1981). Cells in the exponential growth phase were harvested and diluted in L-15 medium containing 10% FBS. Following incubation at 25°C for 14 d, the cells were fixed, and stained. The cell colonies were counted and cell-plating efficiency was calculated using the method described by Middlebrooks et al. (1981).

Cryopreservation of cells. The stability of bluefin trevally cells in liquid nitrogen storage was examined. Briefly, cells growing logarithmically were harvested and diluted at a cell density of $2 \times 10^6 \text{ cells ml}^{-1}$ in L-15

medium containing 20% FBS and dimethyl sulfoxide (DMSO) at a final concentration of 10%. Aliquots (1.0 ml) of cell suspension were dispensed into 1.5 ml sterile plastic vials (NUNC), sealed, and processed for storage in liquid nitrogen. Cell viability was assessed at Day 180 by trypan blue exclusion (Wang et al. 2003).

Karyotyping of cells. Chromosome counts were conducted for BTF and BTMS at their 7th and 12th passages, respectively, according to the method described by Earley (1975). One hundred or more chromosome spreads were counted for each cell line using phase-contrast microscopy.

Ribosomal RNA analysis. Total DNA was extracted from 2.5×10^7 BTF cells at passage 18 according to the method described previously (Lu et al. 2000). Two sets of oligonucleotide primers were designed to amplify 16S bluefin trevally mitochondrial DNA: the first fragment (343 bp) using forward (16S-F1) 5'-CTCAAGC-CTCGCCTGTTTAC-3' and reverse (16S-R1) 5'-CCC AACCGAAGACATTAGGA-3' primers and the second fragment (312 bp) using forward (16S-F2) 5'-CAA GGGCGCAATAGAAAAAG-3' and reverse (16S-R2) 5'-GAGAAAGCCATGAGGCTGAAC-3' primers. The Invitrogen Platinum PCR SuperMix kit (Cat. No. 11306-016) was used for the PCR reaction. The final eluted volume of 50 μ l reaction mix includes: forward primer (20 μ M) 0.4 μ l, reverse primer (20 μ M) 0.4 μ l, and template 0.3 μ g. PCR cycling conditions were as follows: initial denature at 94°C for 5 min; followed by 40 cycles of 94°C for 1/2 min (denature), 55°C for 1/2 min (annealing) and 72°C for 1/2 min (extension); and final extension at 72°C for 7 min and then hold at 4°C.

The PCR products were subjected to 2% agarose gel electrophoresis and purified using a gel extraction kit (Qiagen). The purified PCR fragments were directly sequenced in both directions using an automated sequencer (373A, Applied Biosystems). The amplified sequences were identified by the Editseq and Seqman programs of the DNASTar package and then blasted in GenBank.

Viral susceptibility. Newly established bluefin trevally cell lines were tested for their susceptibility to 4 fish rhabdoviruses: snakehead rhabdovirus (SHRV), infectious hematopoietic necrosis virus (IHNV), spring viremia carp virus (SVCV), and viral hemorrhagic septicemia virus (VHSV); 1 fish herpesvirus, channel catfish virus (CCV) and 1 birnavirus, infectious pancreatic necrosis virus (IPNV) (Table 1), according to the methods described previously (Lu et al. 2003). Virus stocks were prepared and viral yields were determined in chinook salmon embryo

cells (CHSE-214) for IHNV, IPNV and VHSV, while SHRV and SVCV were prepared in epithelioma papulosum cyprini (EPC) cells and CCV was titered in brown bullhead (BB). BTF and BTMS cells (2.5×10^5) were incubated at 25°C until a subconfluent monolayer (80 to 90%) was achieved in 25 cm² tissue culture flasks and then inoculated with a 3.5 to 4.5×10^4 50% tissue culture infectious dose (TCID₅₀) of each virus stock (multiplicity of infection = ~0.2). Following virus adsorption for 1 h at the temperature optimal for each virus, unadsorbed virus was removed by washing cell monolayers twice with L-15 medium containing no FBS. Then 5 ml of L-15 medium containing 5% FBS was added to each flask and infected cultures were transferred to an incubation temperature optimal for viral infection (SHRV and CCV at 25°C, IHNV and VHSV at 15°C, IPNV and SVCV at 20°C). Infected cultures were monitored daily for virus-specific cytopathic effects (CPE). Cultures were frozen at -80°C when more than 80% of cells showed CPE or at Day 7 if no CPE developed. Viral susceptibility of trevally cells was evaluated by the appearance of viral-specific CPE and the production of infectious virus as determined by measuring TCID₅₀ (Reed & Muench 1938).

RESULTS

Cell morphology

Out of 6 selected tissues/organs from 1 bluefin trevally, we were able to establish 2 cell lines, BTMS and BTF, derived from muscle and fin tissues, respectively. These 2 cell lines were serially passed *in vitro* more than 37 times since their initiation in June 2002. Morphologically, fin-derived BTF appeared epithelial/fibroblast-like while BTMS derived from muscle was epithelial in appearance (Fig. 1).

Table 1. Summary of 6 viruses used in this study

Virus	Abbreviation	Family	Source
Channel catfish virus	CCV	<i>Herpesviridae</i>	Wolf & Darlington (1971)
Infectious hematopoietic necrosis virus	IHNV	<i>Rhabdoviridae</i>	Amend et al. (1969)
Infectious pancreatic necrosis virus	IPNV	<i>Birnaviridae</i>	Wolf et al. (1960)
Snakehead rhabdovirus	SHRV	<i>Rhabdoviridae</i>	Kasornchandra et al. (1991)
Spring viremia of carp virus	SVCV	<i>Rhabdoviridae</i>	Fijan et al. (1971)
Viral hemorrhagic septicemia virus	VHSV	<i>Rhabdoviridae</i>	Brunson et al. (1989)

Fig. 1. *Caranx melampygus*. Photomicrographs of bluefin trevally cells derived from (A,B) fins and (C,D) muscle tissue. (A) and (C) show primary cultures on Day 7 following seeding tissue explants; (B) and (D) show subcultured cells at passage 12. Magnification = 120 $\times$

Growth characteristics

Initial growth of cells derived from fins was observed on Day 2 following the explanting of minced tissue fragments, and a cell monolayer had formed by Day 10. In comparison, initial growth of muscle-derived cells was slower and no growing cells were observed until Day 4 of incubation. However, following 10 passages, BTMS cells exhibited growth kinetics similar to those of BTF. Both cell lines were then subcultured every 5 to 7 d at a 1:2–3 split ratio.

The newly established bluefin trevally cell lines grew well at a temperature range of 20 to 30°C with the optimal temperature falling between 25 and 30°C. As shown in Fig. 2, both BTMS and BTF cells grew very rapidly when incubated at 25 to 30°C, with cell doubling times (dt) of approximately 50 h. Although bluefin trevally cells were able to attach and spread in the cell culture flask at 20°C, the cells showed slower growth kinetics (dt > 8 d). No cell spreading and growth were observed when cells were incubated at either 15 or 37°C. Since bluefin

trevally is considered to be a tropical marine fish species, it is not surprising that these cell cultures propagate efficiently at 25 to 30°C.

Our experimental data also indicated that both BTMS and BTF cells grew well in L-15 supplemented with FBS, but not in L-15 alone (Fig. 2). BTMS cells grew more rapidly in L-15 containing 20% FBS (dt < 47 h) as compared to a slower growth with 10% FBS (dt = approximately 98 h), and an even slower growth rate in 5% FBS. Since the growth of BTMS and BTF were serum-dependent, this suggested that these cells were not yet transformed. Also, in the current passage the plating efficiencies of these cell lines were low (<25%), which further supported the untransformed status of the newly established omilu cell lines. Comparison of 4 different cell culture media commonly used for animal cells cultures showed that all these media supported the growth of BTMS cells equally well. However, the BTF cell line did not grow well in medium 199 as compared to the other 3 media. This may reflect the selective tendency of different cell types with respect to their nutrients.

Fig. 2. *Caranx melampygus*. *In vitro* growth curves ($\pm$ SE) of the 2 established bluefin trevally cell lines. (A,C) Bluefin trevally fins (BTF) and (B,D) bluefin trevally muscle (BTMS) cells were propagated at (A,B) different temperatures and (C,D) selected concentrations of fetal bovine serum (FBS)

Plating efficiency

Plating efficiency for the 2 bluefin cell lines ranged from 11 (BTMS) to 28.2% (BTF) tested at passages 6 and 10, respectively. These low and broad ranges of plating efficiency may suggest that the cells were in heterogeneous stages but not fully adapted to *in vitro* growth conditions during these early passages.

Cryopreservation

Assessment of the viability of BTMS and BTF cells stored in liquid nitrogen demonstrated that all cells were stable and were able to survive low temperature storage. Over 97.8% of the cell population remained viable for a period of 180 d storage at -196°C . All viable cells attached and grew well following seeding at 25°C . No apparent change in cellular morphology was observed following the storage.

Chromosomal analysis

Although bluefin trevally is the most abundant and ubiquitous carangid in Hawaii and throughout much of the tropical Pacific, little is known about the diploid chromosome number of this fish. Karyotyping of early

passages of BTMS and BTF indicated that these 2 bluefin-derived cell lines shared a similar pattern of chromosomal distribution (Fig. 3). Chromosomal counts from 100 or more randomly selected cells ranged from 36 to 98, but the majority of the counted cells had between 46 and 52 chromosomes (78 to 85% of all spreads) with a distinct peak of 48 for the 2 cell lines (Fig. 3). Our finding that these 2 cell lines had the same peak range of chromosomes during their early passage *in vitro* suggests that the diploid chromosome number of bluefin trevally may be 48 ($2n = 48$) and that these cell lines were diploid during the cell passages in the present study.

Sequence analysis of 16S ribosomal RNA (rRNA)

Heteroduplex analysis of mitochondrial 16S rRNA was also performed to verify the origin of these 2 cell lines (Miya et al. 2003, Wang et al. 2003). Amplification of the DNA extracted from BTF cells using the 2 pairs of PCR primers designed from the 16S mitochondrial DNA of bluefin trevally revealed the expected PCR products of 343 and 312 bp, respectively (Fig. 4). Comparative sequence analysis indicated that these newly identified sequences were nearly identical ($98.7 \pm 1.0\%$) to the respective mitochondrial DNAs of bluefin trevally (GenBank accession no. AP004445). In addition, these 2 sets of PCR primer pairs were also employed to amplify the DNA isolated from the BTF cells at passage 5 and 43, respectively, and the resulting sequences were identically matched with the respective sequences detected at the 18th passage (data not shown). These data indicated that the BTF and BTMS are truly derived from bluefin trevally *Caranx melampygus*.

Viral susceptibility

The sensitivity of the 2 fish cell lines to 6 viruses was determined. Both BTMS and BTF appeared to share a similar spectrum of virus susceptibility: sensitive to SVCV, IHNV, IPNV, SHR and VHSV, but refractory to CCV (Table 2). Viral infection resulted in extensive CPE in these 2 cell lines by Day 2 (Fig. 5), and SVCV infection induced visible CPE within 48 h post-infection (p.i.). It was of interest to note that although infec-

Fig. 3. *Caranx melampygus*. Frequency distribution of chromosomes in 100 cells from (A) bluefin trevally fins and (B) bluefin trevally muscle cell lines treated with colcemid. (C) Phase-contrast photomicrograph of bluefin trevally cellular chromosomes arrested in metaphase at the 11th cell subcultivation time. Magnification = 1300×

tion of BTMS and BTF cells with VHSV resulted in apparent CPE within 3 d, a very low yield of infectious virus was produced by these cells ($10^{3.3-3.6}$ TCID₅₀ ml⁻¹). In contrast, comparable virus yields to control cell lines were obtained in new bluefin trevally cell lines for SVCV ($10^{8.0-8.5}$ TCID₅₀ ml⁻¹) and IPNV ($10^{6.0-6.5}$ TCID₅₀ ml⁻¹). Based on viral-induced CPE and viral production, the BTMS cell line appeared to be more susceptible to viral infections than BTF. The wide range of viral susceptibility suggests that the 2 newly

established bluefin trevally cell lines can serve as sensitive biological indicators for future isolation and study of marine and freshwater fish viruses.

DISCUSSION

Animal tissue culture was initiated at the beginning of the 20th century as a simple technique to study the behavior of animal cells (Harrison 1907, Freshney 2000).

Fig. 4. *Caranx melampygus*. (A) PCR amplification of 343 and 312 bp sequences of the bluefin trevally genome using 2 pairs of oligonucleotide primers (F1/R1 and F2/R2) designed from the conserved portions of the 16S mitochondrial DNA. DNA (100 ng) isolated from bluefin trevally fin (BTF) cells was amplified and then subjected to 2.0% gel electrophoresis. Lanes 1 and 3: BTF-DNA; lanes 2 and 4: negative control; M (molecular marker): 100 bp DNA ladder (showing the range 100 to 800 bp). (B) Nucleotide sequences of the 343 and 312 bp fragments amplified by PCR using the respective primers. Underlined portions represent positions of the PCR primers

Table 2. Comparison of the 2 newly established bluefin trevally cell lines for the propagation of selected fish viruses. (For virus definitions, see Table 1.) CPE: cytopathic effect; TCID₅₀: 50% tissue culture infectious dose; BTMS: bluefin trevally muscle; BTF: bluefin trevally fins; BB: brown bullhead; CHSE: chinook salmon embryo cells; EPC: epithelioma papulosum cyprini; -: did not occur

Virus	Cell line	CPE occurrence (Day)	TCID ₅₀ (units ml ⁻¹ , log titer)	
			Day 0	Day 7
CCV	BTMS	-	3.5	0.0
	BTF	-		0.0
	BB	2		4.7
IHN	BTMS	3	3.7	6.0
	BTF	3		5.7
	CHSE-214	3		5.3
IPNV	BTMS	3	4.7	6.0
	BTF	3		6.5
	CHSE-214	1		6.5
SHRV	BTMS	3	4.5	5.7
	BTF	3		5.3
	EPC	2		7.3
SVCV	BTMS	4	4.5	8.5
	BTF	3		8.0
	EPC	3		8.7
VHSV	BTMS	3	4.5	3.3
	BTF	3		3.6
	CHSE-214	3		5.5

This technique has been well defined today and widely used in many diagnostic and research laboratories. A large number of cell lines have been established from a variety of marine species, including aquacultured fish (Wolf & Mann 1980, Fijan et al. 1983, Lannan et al. 1984, Chen & Kou 1988, Lu et al. 1990, Fryer & Lannan 1994, Wang et al. 2003, Zhao et al. 2003) marine mammals (Kadoi et al. 1992, Lu et al. 1998, 2001, Kumar et al. 2001), endangered marine species (Moore et al. 1997, Lu et al. 1999), and marine crustaceans (Tapay et al. 1995, Neuman et al. 2000). However, for *Caranx* species, there has only been one report of the establishment of an *in vitro* cell culture derived from omaka *Caranx mate* fish larvae (Lee & Loh 1975). There is no documentation of an *in vitro* cell line established from bluefin trevally. Furthermore, little is known about the chromosomal counts of this marine animal or the degree to which it is affected by viral infection. Since bluefin trevally is becoming an important aquacultural species in Hawaii and the Pacific, there

Fig. 5. *Caranx melampygus*. Microphotographs of viral-induced cytopathic effects in bluefin trevally. Bluefin trevally fin (BTF) cells infected with (A) IHN and (C) VHSV and bluefin trevally muscle (BTMS) cells infected with (B) IHN and (D) VHSV at Day 2 post-infection. Magnification = 110 $\times$. See Table 1 for definitions of virus abbreviations

is an urgent need to gain a biological understanding of this fish, and to develop a biological tool for the detection and diagnosis of infectious viruses which affect it. To that end, the aim of this study was to establish and characterize cell lines from bluefin trevally.

Although our initial attempt was to establish primary cell cultures from multiple tissues and organs including the liver, kidney, spleen, swim bladder, muscle, fin and heart, we were only able to establish 2 cell lines from the muscle (BTMS) and fin (BTF). Initially, cell growth was observed in the kidney, spleen, heart and swim bladder, but subculture was not successful. This was partially due to the limited amount of tissue specimen available for primary culture from the donor fish and also probably due to non-optimized *in vitro* cell culture conditions. Both the BTF and BTMS cells were serially subcultivated up to 37–41 passages *in vitro* and grew well at 25 to 30°C. Both cell lines can be easily maintained since they are stable either frozen in liquid N₂ storage or in cell culture flasks.

Among several major applications of established cell lines in virology is the use of permissive cell cultures to detect and diagnose viral infections, to isolate, propagate and assay infectious viruses, and to prepare and clone attenuated virus strains for antiviral vaccination. Cell lines established from a variety of marine species have been successfully used for the isolation, identification and detection of many kinds of marine viruses, including highly pathogenic viral isolates, which provides baseline information for the development of a long-term strategy for viral disease prevention and control (Wolf 1988, Hetrick & Hedrick 1993, Leong & Fryer 1993, Lu et al. 1995, Frerichs et al. 1996, Winton 1997, Watson et al. 1998, Alonso et al. 1999, Crane et al. 2000, Dannevig et al. 2000, Lopez-Doriga et al. 2001, Chen et al. 2004, Denis-Mize et al. 2004). Although there are currently no reports of natural viral infection in *Caranx* species, this does not necessarily mean that this species is free of viral infection. In fact, little viral surveillance of this fish has been done because of the lack of a cell culture system which can be used as a sensitive biological tool to monitor infection. A viral challenge test of the established BTMS and BTF cells indicated that these 2 new cell lines are sensitive to all of the viruses tested except for CCV, suggesting a relatively wide range of viral susceptibility. These findings may indicate that the BTMS and BTF cells could be potentially used to isolate and study pathogenic viruses affecting bluefin trevally and other fish species.

Cell cultures have been widely used in many other applications, including the determination of host genetics and chromosomal characteristics. The diploid chromosome number for bluefin trevally is not known. The present study on 2 established cell lines from this fish revealed that BTMS and BTF shared a similar pattern

of chromosomal distribution and had the same peak count of 48 (46 to 56 chromosomes). Since karyotyping analysis was performed during the early passages of these cell lines, this suggests that both cell lines may have a diploid number of chromosomes of $2n = 48$. In the future the modal chromosomal number of this species and the host origin of established bluefin trevally cell lines should be ascertained by employing G-banding or other karyotyping techniques.

In order to further identify the origin of the established cell lines, DNAs isolated from the cells during their early subculture (Passage 18) were amplified by PCR using 2 sets of oligonucleotide primer pairs designed from different portions of the conserved mitochondrial genome of bluefin trevally (accession number AP004445). Comparative analysis of the amplicon sequences showed that they are nearly identical (98.7%) to the respective portions of the reported bluefin trevally 16S mitochondrial DNA, indicating that BTF and BTMS are truly derived from bluefin trevally *Caranx melampygus*.

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