魚病研究 Fish Pathology, 33 (4), 447–448, 1998. 10

A Nodavirus Isolated from Grouper (*Epinephelus tauvina*) and Seabass (*Lates calcarifer*)

May Chew-Lim*[1], Seo Yen Chong*[1] and Mamoru Yoshimizu*[2]

*[1]*Primary Production Department, 5 Maxwell Road #03-00, National Development Building, Singapore 069110, Republic of Singapore*

*[2]*Faculty of Fisheries, Hokkaido University, Hakodate, 041-0821, Japan*

(Received April 13, 1998)

Key words: nodavirus, *Epinephelus tauvina*, *Lates calcarifer*, seabass cell line

At the request of Singapore netcage mariculture farmers of greasy grouper (*Epinephelus tauvina*) and seabass (*Lates calcarifer*) complaining of high mortalities in 2–4 cm juveniles, viral isolation from clinically affected fish was initiated. During 1986 to 1991 five viruses producing intracytoplasmic CPE were isolated from grouper and seabass in a seabass cell line (SB) which was immortalised from primary seabass larvae cell cultures[1]. Electronmicroscopy (EM) revealed small (approximately 30 nm) icosahedral virions. Three grouper and one seabass viral isolates were obtained from juvenile fish (2–4 cm in length) which had abdominal distension and manifestations of swimming abnormalities. Only one isolate was from market-sized 31–32 cm groupers. All these five isolates were completely neutralised in the serum neutralisation test using a rabbit anti-striped jack nervous necrosis virus (SJNNV) serum. Further confirmation was made by use of the oligonucleotide primer set designed for the amplification of T4 target region (426 bp) of SJNNV coat protein gene[2] in a reverse transcription-polymerase chain reaction (RT-PCR). The PCR method effectively established the identity between our isolates and SJNNV.

Because early attempts at isolation in BF-2 (ATCC CCL 91) were not successful, the SB was developed and by a process of passaging viral isolates can achieve titres of 10^{-7} to 10^{-8} $TCID_{50}$/m*l*. More recently an immortalised grouper cell line supported growth of one of the five isolates[3]. Since our first isolation of the virus from seabass larvae in 1986, other workers described viral nervous necrosis as a devastating disease in various fish species[4–8]. In 1992, Mori *et al.*[9] identified the SJNNV from larval striped jack (*Pseudocaranx dentex*) to be a new member of Nodaviridae. Chua and co-workers[10] presented a case of mass mortality in juvenile greasy grouper associated with a viral vacuolating encephalopathy and retinopathy.

Tissue suspensions of clinically affected fish were prepared for viral isolation as described by Chong *et al.*[11]. All the isolates were propagated in SB and stocked as ampoules of freeze-dried material of 0.2 m*l* virus in 20% fetal calf serum.

Cell free viruses were concentrated and semi-purified by ultracentrifugation at 200 000 × *g* for 3 h in the SW41Ti rotor (Beckman). Viral pellets were resuspended in phosphate buffered saline (PBS) and confluent control SB cells (CCSB) fixed in glutaraldehyde (3% in 0.1M PBS) were submitted for EM investigation. Particles of virus pellets were examined by negative staining technique using phosphotungstic acid stain. The CCSB were post-fixed with 1% osmium tetraoxide and ultrathin sections were positively stained with uranyl acetate and lead citrate.

Serum neutralisation was carried out with SB in microplate culture at 25°C using SJNNV antiserum. Antigens were titrated in 10 fold dilutions (10^{-1} to 10^{-10} $TCID_{50}$/m*l*) and reacted with an equal volume of fixed antibody (1:100 dilution). Normal rabbit serum was used as parallel control. Each aliquot of virus-serum mixture was inoculated into 5 wells of confluent day-1 SB microplate culture. On the 5th and 7th days post infection, the culture was checked for presence of CPE. Freeze-dried samples of the five isolates and two batches of CCSB were subjected to the RT-PCR. PCR amplification and assay of products were as described by Nishizawa *et al.*[2].

Table 1 summarises the investigation with background details of the five isolates. EM of the five isolates revealed virions sized 20–34 nm in diameter whilst the CCSB demonstrated the presence of a 57 nm-sized contaminant virus.

The results of the neutralisation test and PCR investigations confirmed that the local grouper and seabass isolates were comparable to SJNNV. The EM investigations indicated that the isolation of the nodavirus was possible in a CCSB which was persistently infected with an uncharacterised 57 nm virus. This is not inconsistent with a communication[12] describing isolation of a piscine neuropathy nodavirus from juvenile seabass (*Dicentrarchus labrax*) in a striped snakehead (*Ophicephalus striatus*) fish cell line which carries a C-type retrovirus[13].

Acknowledgements

The authors thank the Primary Production Department and National Science and Technology Board for support given to this work.

References

1) Chong, S. Y., G. H. Ngoh, M. K. Ng and K. T. Chu (1987): *Singapore Vet. Jour.*, **11**, 78–85. 2) Nishizawa, T., K. Mori, T. Nakai, I. Furusawa and K. Muroga (1994): *Dis. Aquat. Org.*, **18**, 103–107. 3) Chew-Lim, M., G. H. Ngoh, M. K. Ng, J. M. Lee, P. C. Chew, J. Li, Y. C. Chan and L. C. J. Howe (1994): *Singapore J. Prim. Indust.*, **22**, 113–116. 4) Glazebrook, J. S., M. P. Heasman and S. W. de Beer (1990): *J. Fish Dis.*, **13**, 245–249. 5) Renault, T., Ph. Haffner, F. Baudin Laurencin, G. Breuil and J. R. Bonami (1991): *Bull. Europ. Ass. Fish Pathol.*, **11**, 68–73. 6) Munday, B. L., J. S. Langdon, A. Hyatt and J. D. Humphrey (1992): *Aquaculture*, **103**, 197–211. 7) Breuil, G., J. R. Bonami, J. F. Pepin and Y. Pichot (1991):

Table 1. Investigation of five viruses isolated from grouper and seabass during 1986–1991

Year	Fish species	Size of fish (total length & weight)	Clinical signs recorded on case sheet	Specimens positive for virus isolation (in SB)	Neutralisation test with anti-SJNNV serum	PCR results
1991	Grouper	32 cm (500 g)	abdominal distension	brain*, liver, spleen	+	+
1991	Grouper	2 cm	spinning	mixed head & viscera*	+	+
1988	Grouper	3 cm	dark colour, lethargic	whole larva*	+	+
1988	Grouper	3-4 cm	abdominal distension	whole larva*	+	+
1986	Seabass	2 cm	dark colour, lethargic, red cyst-like pustules in the gill filaments	whole larva*	+	+
CCSB (118th passage & 334th passage)						–

* These isolates were subjected to neutralisation test and RT-PCR.

Aquaculture, **97**, 109–116. 8) Mori, K., T. Nakai, M. Nagahara, K. Muroga, T. Mekuchi and T. Kanno (1991): *Fish Pathol.*, **26**, 209–210. 9) Mori, K., T. Nakai, K. Muroga, M. Arimoto, K. Mushiake and I. Furusawa (1992): *Virology*, **187**, 368–371. 10) Chua, F. H. C., J. J. Loo and J. Y. Wee (1995): In "Diseases in Asian aquaculture II. (ed. by M. Shariff, J. R. Arthur and R. P. Subasinghe), Fish Health Section, Asian Fisheries Society, Manila, pp. 235–241. 11) Chong, S. Y., G. H. Ngoh and M. Chew-Lim (1990): *Singapore J. Prim. Indust.*, **18**, 54–57. 12) Frerichs, G. N., H. D. Rodger and Z. Peric (1996): *J. Gen. Virol.*, **77**, 2067–2071. 13) Frerichs, G. N., D. Morgan, D. Hart, C. Skerrow, R. J. Roberts and D. E. Onions (1991): *J. Gen. Virol.*, **72**, 2537–2539.