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

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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 cultures1). 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 gene2) 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 passages viral isolates can achieve titres of 10-7 to 10-8 TCID50/ml. More recently an immortalised grouper cell line supported growth of one of the five isolates3). Since our first isolation of SJNNV, this work. The authors thank the Primary Production Department and National Science and Technology Board for support given to this work.

Cell free viruses were concentrated and semi-purified by ultracentrifugation at 200 000 x 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 tetroxide 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 TCID50/ml) 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 communication3) 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 retrovirus4). The authors thank the Primary Production Department and National Science and Technology Board for support given to this work.

References

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

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

CCSB (118th passage & 334th passage) –

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