

Tissue culture isolation, electron microscopic characterization and PCR-detection of a betanodavirus isolated from diseased Asian sea bass.

ABSTRACT

A viral agent was successfully isolated from Asian sea bass juveniles (*Lates calcarifer*) exhibiting clinical signs of viral nervous necrosis (VNN) in southern Malaysia on February 2008. Eyes and brains of diseased fish were pooled, extracted and filtered. The filtrates were inoculated on SSN-1 cells and incubated at 25°C. Cytopathic effect (CPE) recognized as rounded cells continued to aggregate and the vacuolation. Electron micrographs of the infected SSN-1 cells revealed icosahedral nucleocapsid virions with 22-28 nm in diameter. Viral harvest was resistant to chloroform and iodine treatments, which indicated that it was naked and contained RNA genome. Identification using reverse transcription polymerase chain reaction (RT-PCR) and sequence analysis of PCR product was conducted and gave a single PCR product at 460 bp in agarose gel, followed by nested PCR with specific primers for PCR products at 220 bp. Partial nucleotide sequence of the nervous necrosis virus coat protein gene showed 94.0-96.1% homology to the nucleotide sequences of coat protein gene from nervous necrosis virus isolated from other countries in the Southeast Asia.

Keyword: *Lates calcarifer*; VNN; CPE; RT-PCR; Betanodavirus.