Genomic Similarity of Taura Syndrome Virus (TSV) between Taiwan and Western Hemisphere Isolates

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ABSTRACT—Mortalities of white shrimp Litopenaeus vannamei juveniles exceeding 80% have occurred in Taiwan since late 1998. It has been determined that Taura syndrome virus (TSV) is responsible for the epizootic outbreaks. In order to clarify the origin of these epizootics, a 3288 base pair fragment that encodes a TSV coat protein was amplified by RT-PCR. Analysis showed that the Taiwan isolate had 97% and 98% identity to the sequences in Genebank originated from isolates obtained from Mexico and Hawaii, respectively, both in cDNA and deduced amino acid sequences. These results suggest that TSV that infected the white shrimp in Taiwan in late 1998 and early 1999 is similar to the Western hemisphere isolates. We conclude that the Taiwan isolate originated in the Western hemisphere. Urgent and strict quarantine is advised to prevent Taura syndrome dissemination to other areas in Asia.

Key words: Taura syndrome virus, coat protein, Litopenaeus vannamei, RT-PCR, transmission, TSV

Since the outbreaks of Monodon baculovirus (MBV) and White spot syndrome virus (WSSV) caused catastrophic losses in cultured tiger shrimp (Penaeus monodon) (Lightner et al., 1987, Lo and Kou 1998), Taiwanese farmers switched to culture Pacific white shrimp (Litopenaeus vannamei) from 1994 and produced a volume of 8,500 metric tons in 1998. However, high mortalities of white shrimp juveniles exceeding 80% occurred within 3 days of disease onsets in late 1998 and early 1999. Similar disease outbreaks with the same magnitude of mortality happened in other localities in May and June 1999. As a result, white shrimp production dropped sharply to as little as 10% of the early 1998 production level. It has been determined that Taura syndrome virus (TSV), a member of the picornaviridae which was first recognized in Ecuador in 1992 (Brock et al., 1995, Lightner et al., 1995), was responsible for the epizootic outbreaks in cultured white shrimp in Taiwan (Tu et al., 1999, Yu and Song, 2000).

It is still unclear how TSV was introduced into Taiwan. A probable cause of transmission of TSV is the shipments of post-larvae from epizootic regions since 1994. Likewise, brooders imported from Central American countries could have transmitted the pathogen to larvae propagated locally since 1995 (Brock et al., 1997). Frozen shrimp products have been imported from Central America for local consumption since 1995 despite Brock’s comment (1997) that its importation should not be overlooked as a means of transmission of TSV (Lightner, 1995). Historically, white shrimp larvae were first introduced into Taiwan from Panama for culture trial in 1981 while brooders were introduced again from Panama in 1985. Taiwan Fisheries Research Institute successfully propagated and cultured the shrimp up to the third generation in a laboratory scale at those times (Liu et al., 1999). Whether there was a similar syndrome present but that did not cause significant losses hence did not attract attention at those times is not clear. Previously, when gross signs of “shell disease” were found in cultured penaeid shrimp in Taiwan, they were usually attributed to vibriosis. Because Taura syndrome shares gross and histopathologic features with bacterial shell disease (e.g. black spot), it cannot be differentiated solely by gross or microscopic criteria (Lightner et al., 1995). The possibility that TSV was pandemic cannot be excluded. We report here the genomic comparison of TSV coat protein of Taiwan, Mexico and Hawaii isolates in order to examine possible transmission origins of the virus.

Materials and Methods

In situ hybridization. Shrimp were collected during
naturally occurring TSV-like epizootics in 1999 and stored at -70°C. Samples were fix- ed in a neutral pH, RNA-friendly fixative for 48 hrs (Hasson et al., 1997). A paraffin block of each shrimp sample was prepared so that a portion of the gills and mid-lateral section from both the cephalothorax and the sixth abdominal segment were included. Following embedding, a series of consecutive sections, 4 μm thick, were mounted onto silane-coated slides (Silane-Prep, Dako) for in situ hybridization analysis. These tissue sections were evaluated using TSV-specific digoxigenin-labeled cDNA genomic probes, used in accordance with the manufacturer’s recommendations (DiagXotics, Inc., CT, USA). Parallel samples were collected for further experiments as the representative samples showing a positive gene probe signal (blue-black precipitate).

RNA template. For viral purification, local shrimp samples of gills, appendages and tissues adjacent to the exoskeleton were homogenized and then ultra-centri- fuged in a 15–30% (w/w) linear sucrose gradient followed by a 15–45% (w/w) CsCl gradient (Bonami et al., 1997). Extraction of viral genomic RNA was performed using TURIZOL Reagent (GIBCOBRL) according to the manufacturer’s instructions. RNA was stored in ethanol at -70°C until use.

RT-PCR of TSV RNA. The transcription of the TSV-RNA genome into cDNA was performed using Oligo(dT)20 as primer, using THERMOSCIPT™ RT- PCR SYSTEM (GIBCOBRL). Two oligonucleotide primers were used in the RT-PCR amplification proce- dure. Primers were designated 3272 (5’ CCGCC- GCCTTGGAGGTCAT 3’) and 9992 (5’ AAGTAGAC- AGCCGCGCTT 3’) to amplify a TSV cDNA fragment predicated to be about 3 kb. The 9992 primer was chos- en using the partial cDNA sequence information of TSV isolate originating from Mexico provided by Nunan et al. (1998) and 3272 primer using TSV (Hawaiian isolate) coat protein sequence provided by Robles-Sikisaka et al. (2001), respectively. These primers were synthesized at Mission Biotech Lt. Co. (Taiwan). Two μL of cDNA was used as template and PCR used 25 cycles of dena- turation at 94°C for 45 sec, annealing at 55°C for 30 sec and extension at 72°C for 2.5 min. The PCR conditions (final concentration in 50 μL total volume) were: primers (0.2 μM each), dNTPs (200 μM each), 2.5 U VioTaq DNA polymerase (Viogene), in 5 μL of 10x PCR buffer plus Mg (Viogene). The PCR products were electro- phoresed in 1.2% agarose gels (Amresco) and eluted by gel extraction system (Viogene) according to the manufacturer’s instructions. The purified PCR product was ligated, using T4 DNA ligase (Takara), into pUC-T vector (MDBio Inc.) and then used to transform Escheri- chia coli strain DH5α competent cells according to the method described by Inoue et al. (1990). Three clones were taken for sequencing in both directions. Plasmid primers T3 and T7 were used to obtain both end sequences. Internal primers were synthesized based on initial sequence data of both strands to complete sequencing of the clones. DNA sequencing was done using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, USA), and analyzed using an ABI Prism 377-96 DNA sequencer (Perkin-Elmer, CA, USA). Searches for similar nucleo- tide sequences were determined using Basic Local Alignment Search Tool (BLASTN, NCBI). The amino acid sequence was deduced and searches for similar protein sequences performed using BLAST X 2.1.2 version.

Results and Discussion

We used in situ hybridization to detect whether the shrimps were infected with TSV. Parallel samples of those showing a positive signal in the cuticular epithe- lium (Fig. 1) were collected and used for further viral purification. In this study, RT-PCR amplified a 3288 bp fragment (GenBank accession no. AF406789) that was reported to encode a TSV coat protein (Robles-Sikisaka et al., 2001). Nucleotide sequence analysis using the BLASTX 2.1.2 program showed that the coat protein of Taiwan TSV isolate shares limited identity to the related structures of other viruses including the capsid protein of acute bee paralysis virus, capsid protein precursors of Triatoma virus, Plautia stali intestine virus and himetobi P virus, capsid polyprotein of Drosophila C virus (DCV), and structural polyproteins of Rhopalosiphum padi virus, cricket paralysis virus (CrPV) and black queen cell virus. Intra-species analysis showed that Taiwan iso- late had 97% and 98% identity to the sequences in GeneBank originated from the isolates obtained from Mexico (AF277378) and Hawaii (AF277675) both in cDNA (data not show) and deduced amino acid sequences (Fig. 2). These results support the finding Fig. 1. In an in situ hybridization analysis using TSV-specific cDNA probes, the blue-black precipitate (arrow) appeared in the cuticular epithelium. Scale bar = 0.3 μm

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Fig. 2. Amino acid alignment of coat proteins of TSV isolated from Taiwan, Mexico and Hawaii. Taiwan isolate had 97% and 98% identity to the Mexico and Hawaii isolates, respectively, both in cDNA (data not show) and deduced amino acid sequences. The dots indicate identical amino acids. Numbers in parentheses show the accession numbers: Taiwan(AF406789), Mexico (AF277378) and Hawaii (AF277675).
that the TSV that caused the disease outbreaks in Taiwan in late 1998 and early 1999 is similar to the Western hemisphere isolates. Bonami et al. (1997) reported that Hawaiian and Ecuadorian TSV isolates are identical in their biophysical, biochemical and biological characteristics, and should be considered as the same virus. Recent data shows that most of the geographic and temporal isolates differ very little from the 1994 Hawaii and Ecuador reference isolates based on the full nucleotide sequences (D.V. Lightner, personal communication).

The representative and archived shrimp samples that showed shell disease symptoms had been preserved in an acidic Davidson’s fixative (acetic acid, formaldehyde, alcohol) for routine histopathological examination. Unfortunately, extraction of genomic RNA from those samples for re-examination purposes was not successful in this study, presumably due to the effect of the fixative. This agrees with the results reported by Hasson et al. (1997) that TSV genomic RNA was easily degraded due to either fixative-induced acid hydrolysis and/or acidophilic endogenous ribonuclease activity. The possibility that TSV was an indigenous species in Taiwan is remote. A highly TSV-susceptible species of shrimp from Asia, if given the opportunity to infect shrimp farming in the Americas, could potentially infect commercially important shrimp species. Hence, it is important to prevent dissemination of TS to other areas in the Asia.

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References


