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Transcriptional and translational expression profile of a white spot syndrome viral (WSSV) gene in different organs of infected shrimp

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Abstract

The occurrence of white spot syndrome virus (WSSV) infection causes high mortality and huge economic loss in shrimp aquaculture worldwide. The virus is known to infect only organs of ectodermal and mesodermal in origin. In this work, PCR, RT-PCR and Western blot analysis, for VP19, one of the four major structural proteins, were employed to study tissue distribution of WSSV in experimentally infected shrimp, *Penaeus indicus*. RT-PCR could detect the presence of virus at an earlier stage than PCR and Western blot analysis. In addition, differential expression of VP19 was observed in four different tissues (eyestalks, cephalothorax, gills and peeled tail muscle) and the results demonstrated a higher level of transcription and expression of VP19 in tail muscle, which indicated that infectivity was significantly higher in tail tissue than in the other three tissues, in both intramuscular and oral routes of infection.
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Keywords: Shrimp; *Penaeus indicus*; WSSV; Viral protein; RT-PCR; Western blot

1. Introduction

White spot syndrome virus (WSSV) is one of the most economically significant pathogens affecting hatchery-reared and wild shrimp world-wide (Lightner, 1996; Flegel, 1997). White spot disease caused by WSSV emerged for the first time in *Penaeus japonicus* in 1993 in Taiwan, and subsequently spread rapidly to shrimp farming areas in southeast Asia (Cai et al., 1995). Now its presence has been reported from Central and South America, Europe and Australia (Rosenberry, 2000). The disease is often fatal and characterized by circular white spots, ranging from 0.5 to 2 mm in diameter, most prominent in the cuticle of the cephalothorax. Sometimes, reddish-brown body coloration develops at late stages of infection. Mortality can reach 100% within 3–10 days from the first appearance of gross signs of disease (Flegel, 1997). WSSV infects a wide spectrum of hosts, including shrimp (penaeid and non-penaied), crabs and crayfish (Lo et al., 1996a; Chen et al., 1997; Flegel, 1997). The virus infects tissues of ectodermal and mesodermal

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origin (Wongteerasupaya et al., 1995). Although the complete genome sequence of WSSV is known (Yang et al., 2001; Van Hulten et al., 2001), the molecular mechanisms involved in its pathogenesis are poorly understood. Previous work has focused mainly on histopathology and development of diagnostic methods (Wongteerasupaya et al., 1995; Durand et al., 1997; Lightner et al., 1998; Wang et al., 1998; Chen et al., 2000).

A study of differential viral expression in different tissues of infected shrimp may give some insight into the mechanism of pathogenesis. Earlier studies on differences in tissue distribution and spread of virus have been done by PCR analysis, but the results can be interpreted qualitatively only. RT-PCR is a relative quantitation method where the transcript abundance across samples can be estimated more accurately. This work evaluates the difference in virus accumulation in four different target tissues of experimentally infected *Penaeus indicus* using RT-PCR and Western blot assay for VP19. VP19 is one of the four major structural proteins found in the envelope of WSSV (Van Hulten et al., 2000). The VP19 ORF182 encodes for a protein with molecular mass of 13.2 kDa, which differs substantially from its apparent mobility as 19 kDa protein in SDS-PAGE (Van Hulten et al., 2002). The results from infection by intramuscular injection and the oral route suggest that accumulation of WSSV is highest in tail muscle followed by gills, cephalothorax and eyestalks.

2. Materials and methods

2.1. Experimental animals

Shrimps, *P. indicus* (20–30 g body weight), were collected from the sea and maintained at room temperature (27–30 °C) with natural seawater at salinity 20–25 ppt in 1000-l aquaria equipped with an airlift biological filter. They were acclimatized for 2 days before starting the experiment and fed with commercial pellet feed (CP feed, Thailand).

2.2. Preparation of viral inoculum

WSSV infected *Penaeus monodon* with prominent white spots were collected from a shrimp farm located near Nellore, Andhra Pradesh, India. Hemolymph was drawn directly from the heart using a sterile syringe and pooled hemolymph was centrifuged at 3000×*g* for 10 min at 4 °C. The supernatant obtained was re-centrifuged at 8000×*g* for 20 min at 4 °C and the final supernatant was filtered through 0.45 μm filter. The filtrate was maintained as viral stock at −20 °C for infectivity studies.

2.3. Experimental challenge and sample collection

Total protein in the viral stock was determined by the Lowry method (Lowry et al., 1951) and the presence of WSSV was confirmed by PCR amplification using primers designed by Takahashi et al. (1996). Viral stock containing 200 μg of total protein was prepared in 50 μl of sterile NTE (0.2 M NaCl, 0.02 M Tris–HCl, 0.02 M EDTA, pH 7.4), and this was injected intramuscularly (i.m.) at the third abdominal segment into each individual in a batch of 10 shrimp in 2 replicates. One batch of 10 shrimp was fed with WSSV-infected shrimp for two consecutive meals and then with pellet feed for subsequent days. Another batch of 10 shrimp was maintained as the uninfected control group. The shrimp were then monitored closely for gross signs of disease. Various target organs were collected at different time intervals post-infection and stored at −80 °C for RNA isolation.

2.4. WSSV DNA isolation and PCR analysis

Template DNA for PCR tests was prepared from 100 mg tissue samples from experimental shrimp, following the method described by Lo et al. (1996b). Briefly, each tissue sample was homogenized with 1.2 ml of NTE and centrifuged at 3000×*g* at 4 °C, after which the supernatant fluid was placed in another centrifuge tube together with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulfate, 0.1 mg/ml Proteinase K). After incubation at 62 °C for 2 h, the digest was deproteinised by successive phenol/chloroform/iso-amyl alcohol extraction, recovered by ethanol precipitation and dried; the dried DNA pellet was resuspended in TE buffer.

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