

White spot syndrome virus biology essay

[Science](#), [Biology](#)



Kumari Swati, Sangeetha. S , Sudhakaran. R*
Aquaculture Biotechnology
Laboratory, School of Bio-Sciences and Technology, VIT University, Vellore -
632014, TamilNadu, Indiasweetysoni. swati@gmail. com; +91-86959-83346

Abstract:

Fresh water crab aquaculture accounts for very important part in the economics of the world. Economic losses due to disease in crab aquaculture have made it necessary to increase our knowledge of identify the specific gene and unravel the mechanisms of pathogenicity. Gene expression analysis of VP28 gene of white spot syndrome virus(WSSV) was carried out using RT-PCR. The VP28 gene from Indian WSSV isolate was sequenced and the sequence was deposited in gene bank(accession no. AY42228). RT-PCR was used to find out the VP28- specific transcript in DNase treated total RNA from WSSV infected fresh water crab. The WSSV cause 100% mortality at 72 h p. i. and transcript was detected at 12 h p. i. whereas in fresh water crab the transcript was detected at 2 days p. i. with 100% mortality at 84 h p. i.

INTRODUCTION:

Crab is very important part of aquaculture. According to the statistics of the Food and Agriculture Organization (FAO) of the United Nations, marine and brackish fresh water aqua culture production in the world expanded from less than 10, 000 metric tons in 1970 to more than 3, 000, 000 metric tons in 2011. Although the cultivated fresh water crab production has spectacular increase during these years, annual enormous losses was estimated to be approximately US \$1 billion per year. The diseases of crab are mostly caused by virus. Among the pathogenic viruses including white spot syndrome virus

(WSSV) is one of the most dangerous pathogen which can result in 90-100% mortality of crab . The nucleotide sequence analysis revealed that the WSSV genome encodes approximately 185 open reading frames(ORFs) of 50 amino acids or more. Now, this time research was focused mainly gene expressions. the WSSV contain five major and unknown minor polypeptides. There are five major proteins named as VP28, VP26, VP24, VP19 and VP15. The VP28 and VP19 proteins are present in the envelope while VP24, VP26, VP15 are located in nucleocapsid. The different host responded differently to WSSV the study was carriedout the expression of VP28 gene of WSSV infected fresh water crab. Zhang et al. (2002b) identified an ORF in C37 that encoded a 204 aa protein termed VP28, and expression of VP28 gene transcription revealed that the VP28 might be a late gene of WSSV, but the conserved motif (ATAAG) present in the late genes of insect baculoviruses could not be found in the DNA sequence of the VP28 gene. This suggests a difference between WSSV and baculovirus. In previous studies, major structural proteins of WSSV virions were separated by gel electrophoresis and analysed by mass spectrometry, and more than 30 polypeptides matching WSSV ORFs were identified with a molecular mass range of 7-660 kDa (Huang et al., 2002a; Tsai et al., 2004; Zhang et al., 2004). The infection can be transmitted vertically (trans-ovum), horizontally by consumption of infected tissue (e. g. cannibalism, predation, etc.), and by water-borne routes. Transmission of infection can occur from apparently healthy animals in the absence of disease. Dead and moribund animals can be a source of disease transmission.

METHODS AND MATERIALS:

Collection of experimental animal:

Fresh water crab (20 gms body) was collected from Palar river, Vellore Tamilnadu. The crab sample was maintained at room temperature (27 to 30) in fresh water. The animal were fed with commercial pellet feed. dissolved oxygen, salinity, pH and tempterure were measured in alternate days during the experimental period. Salinity was measured with a salinometer and dissolved oxygen was estimated by the Winkler method.

Experimental injection of virus to fresh water crab:

WSSV-infected crab with prominent white spots were collected from shrimp farms located Nellore, India. Hemolymph was drawn directly from the heart of infected crab using strile syringes. The pooled hemolymph was centrifuged at 3000Xg for 20 minutes at 4. The supernatant fluid was recentrifuged at 8000Xg for 30 minutes at 4and the final supernatant fluid was filtered through a 0. 4 The filtrate was then stored at -20 for infectivity studies.

Reverse transcriptase polymerase chain reaction (RT-PCR)

For the isolation of total RNA of different tissues samples (gills, head-soft tissue, heart, eyestalk, muscle and hemolymph) from crab were homogenized in 1ml of total isolation reagent TRIzol, incubate for 15 mint and transferred to 1. 5 ml centrifuge tubes. 200 of chloroform was added and centrifuge at 12000Xg for 15 mints at 4. The aqueous phase was removed and transferred to a new 1. 5 ml centrifuge tube 500f isopropanol was added and incubated on ice for 15 mint later it was centrifuged at 12000 rpm at 4. Now supernatant was discarded and the RNA was pellet was wash

with 1ml 75% ethanol. the pellet was air dried and resuspended in distilled water and quantified by spectrophotometer. RNA was treated with 200 U of RNase-free DNase I at 37 C for 30 min to remove any viral genomic DNA contamination and then reextracted with phenol-chloroform. The DNase treated total RNA was denatured by heating at 85C for 10 min in 10 DEPC-water containing 100-pmol oligo-dT primer . The first strand cDNA was synthesized by the addition of 3 5X M-MuLV buffer, 1 100 mM DTT, 1 10 mM dNTPs, 10 U rRNasin with total volume of 10 including 100 U M-MuLV reverse transcriptase . The reaction proceeded at 37C for 1 h. The cDNA reaction products were subjected to PCR with the primer set vp28-F (5'-ATGGATCTTTCTTTTAC-3') and vp28-R (5' - TTA CT CGGTCTCAGTGC - 3') for the VP28 gene. The b-actin transcript amplified by using actin-F (5' - GTGCCATCTACGAGGGATA - 3') and actin-R (5' - GTGTTGGCGTACAGGTCCTT - 3') primer set served as an internal control for RNA quality and amplification efficiency (Tsai et al., 2000; Zhang et al., 2002a). For VP28 and b-actin, 30 cycles were run and the PCR products were analyzed by electrophoresis in 1% agarose gels stained with ethidium bromide, and visualized by ultraviolet transillumination.

Results:

Confirmation of WSSV infection in experimentally infected crab

The penaeid shrimp virus, WSSV is experimentally infected with freshwater crab. After a week, infected crabs reached moribund stage and they were screened for WSSV. Different organs such eyestalk, heart, gills and head-soft tissue were taken for PCR analysis. Results revealed that the crabs were

infected with WSSV. 615 bppcr wetha0002. JPGFig. 1: PCR analysis of WSSV in eyestalk, heart, head-soft tissue and gills.

Reverse-transcriptase PCR:

Gene expression analysis of VP28 gene of WSSV was carried out using RT-PCR in fresh water crab. Various organs has been collected such as eyestalk, heart, head-soft tissue, gills, hemolymph and muscle has been taken for gene expression analysis. The results revealed that all the infected organs collected showed high expression of VP28 gene and also the internal control amplified well. 405 bp615 bpDiscussion: rtPCR wetha0002. JPGThe present study revealed that appearance of VP28 protein is related to infection in hostThe differential transcription of VP28 gene in different hosts has been observed and agrees with the results of the present study that WSSV caused 100% mortality in freshwater crab In fresh water crab, transcript of VP28 was clearly detected at 2 days p. i tissue and after 3 days p. i. The VP28 protein was slightly detected at 3 days p. i. in tissue, and disappeared after 3 days p. i. This protein was not detected during the experimental period of 15 days. The animals were lethargic and anorexic (initial clinical signs) at 3 and 4 days p. i. and after this period the animals recovered and survived without any mortality over the period of experiments. The observations indicate the response of freshwater crab to WSSV after injection as evidenced by the detection of the VP28 transcript and VP28 protein. The transcription was VP28 gene of WSSV in fresh water was studied using RT-PCR

Conclusion:

With reference to the study in penaeid shrimp, this work has been initiated. The results revealed that VP28 gene of WSSV is expressing high in all the tissues of infected freshwater *Paratelphusa hydrodomous*. Further studies can be carried out to analyze the gene-expression in protein level using western blot, dot blot and ELISA analysis.