PP-01

Cloning and Purification of Envelope Proteins (VP19, VP28) and Nucleocapsid Proteins (VP15, VP35) Genes of a Shrimp White Spot Syndrome Virus Isolates in Korea

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Introduction

White spot syndrome virus (WSSV) is the causative agent of a disease that has led to severe mortalities of cultured shrimps in Korea and many other countries. Since 1993, massive mortalities due to the viral infection have also occurred in the penaeid shrimps cultured in Korea. WSSV is a large, circular, double stranded (ds) DNA virus and an enveloped, ellipsoid virus with a rod-shaped nucleocapsid with flat ends. In order to identify the characteristics of this Korean isolate of WSSV, the genes for four virion proteins, VP15, VP19, VP28 and VP35 were cloned and their sequences were compared with the available pool of WSSV gene sequences in the GenBank/EMBL databases. From these comparisons, we confirm the occurrence of WSSV in Korea and deduce that, VP15, VP28 and VP35 genes are identically conserved among the Korean isolate and geographically different foreign isolates, but VP19 amino acid sequences of the Korean WSSV isolates changed valine of the foreign isolates into aspartate.

Materials and Methods

Moribund shrimps, which stayed at the edge of the pond, were collected. Internal organs, including lymphoid organ and stomach, of the shrimps were collected and homogenized. PCR was performed to amplify complete open reading frames (ORF) of structural virion proteins of WSSV, VP15, VP19, VP28, and VP35. The amplified PCR products were cloned into pBAD/Thio TOPO TA Cloning Kit (Invitrogen), and were sequenced at the GenoTech Corp (DaeJeon, Korea). We purificated these proteins, using ProBond Purification System (Invitrogen, USA).

Results and Discussion

The ORFs of four major virion proteins of WSSV, VP15, VP19, VP28, and VP35, were amplified by PCR and the products were analyzed by agarose gel electrophoresis. The sizes of the PCR products were almost the same as those expected from the database sequences. Blast analysis of these sequences revealed high similarity with those of WSSV virion protein genes in the GenBank/EMBL databases provided from Chinese, Indonesia, Japan and United States. The nucleotide sequences of VP15, VP28 of the Korean isolate were 100% identical to those from Chinese, Indonesia, Japan and United States. In VP35 was 100% identical to that from Taiwan. But in VP19, C and T in the foreign isolates were replaced by T and A in the Korean isolateat position 57 and 218 nt downstream of A (+) of the VP19 start codon, respectively. At position 218 nt downstream of the VP19 translational start codon, the deduced amino-acid, valine in the foreign isolates was replaced by aspartate in the Korean isolates. Others have demonstrated the utility of these proteins for screening of WSSV for antigen characterizationand for development of a simple and sensitive farmer-level ELISA. The performance of the test is going to be compared with PCR for screening shrimp from WSSV outbreaks.

References

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