

NOTE

Cloning and sequencing of envelope proteins (VP19, VP28) and nucleocapsid proteins (VP15, VP35) of a white spot syndrome virus isolate from Korean shrimp

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ABSTRACT: Since our first report in 1998, white spot syndrome virus (WSSV) has become widespread on the southern and western coasts of Korea. Almost all shrimp in ponds die within 3 to 4 d after the first dead shrimp are observed with gross lesions ranging from abnormal red body discoloration to white spots in the cuticle. From one isolate, we cloned and sequenced WSSV genomic DNA coding for VP19 and VP28 envelope proteins and VP15 and VP35 nucleocapsid proteins. Putative protein sequences were submitted to GenBank and assigned accession numbers AY316119 (VP19), AY324881 (VP28), AY374120 (VP15) and AY325896 (VP35). At the nucleotide level, VP19, VP28 and VP15 sequences were, respectively, 99, 100 and 100% identical to those of China, Indonesia, Japan and the United States and the VP35 sequence was 100% identical to that of a Taiwanese isolate. The deduced amino-acid sequences were 99 to 100% identical to those from other countries. In VP19, C and T in the foreign isolates were replaced by T and A in the Korean isolate at Positions 57 and 218 nt, respectively, downstream of A (+) of the VP19 start codon. The change at Position 218 nt resulted in valine in the foreign isolates being replaced by aspartate in the Korean isolate.

KEY WORDS: White spot syndrome virus · WSSV · Envelope proteins · VP19 · VP28 · Nucleocapsid proteins · VP15 · VP35

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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 shrimp in many countries (Flegel 1997, Park et al. 1998, Lo et al. 1999). Since 1993, it has caused massive mortalities in penaeid shrimp cultured in Korea (Park et al. 1998). WSSV is a large, double-stranded, circular dsDNA virus. It is enveloped and ellipsoid with a rod-shaped nucleocapsid with flat ends (Wang et al. 1995, Wongteerasupaya et al. 1995). Dif-

ferent sizes of the WSSV genome have been reported from China at 305107 bp (Yang et al. 2001), Thailand at 292967 bp (Van Hulst et al. 2001) and Taiwan at 307287 bp (GenBank, AF440570). These size differences are mostly due to several small insertions and 1 large (~12 kb) deletion (Chen et al. 2002a). In contrast, most of the 184 putative ORFs (open reading frames) identified on the WSSV genome are unassigned, as they lack homology to known genes in public databases (Van Hulst et al. 2001). Genetic analysis indicates that WSSV is a representative of a new viral

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family (*Nimaviridae*) and genus (*Whispovirus*) (Tsai et al. 2000, Van Hulten et al. 2000, Mayo 2002). The uniqueness of this virus means that a thorough study of its molecular biology is still urgently needed for a better understanding of its nature and replication strategy and the molecular mechanisms of its pathogenesis. Recently, transcription analysis of the major WSSV structural virion protein genes VP28, VP26, VP24, VP19, VP15 (Marks et al. 2003) and VP35 (Chen et al. 2002b) has been carried out.

In order to characterize a Korean isolate of WSSV, the genes for 4 virion proteins (VP15, VP19, VP28 and VP35) were cloned and their sequences compared with WSSV gene sequences available in the GenBank/EMBL databases. From these comparisons, we confirmed the occurrence of WSSV in Korea and showed that sequences for VP15, VP28 and VP35 were identical to those of other geographical isolates, while the putative amino acid sequence of Korean VP19 differed by 1 amino acid change from valine to aspartate.

MATERIALS AND METHODS

Virus samples. Juvenile *Penaeus orientalis* between 7 and 8 cm in length were collected from a shrimp farm at Taean in Chungcheong Province located on the western coast of Korea. Moribund shrimp that stayed at the edge of the pond were collected. Internal organs, including the lymphoid organ and the stomach of the shrimp, were collected and homogenized with a ceramic mortar in a 20-fold volume of sterile phosphate-buffered saline (PBS). The homogenate was centrifuged at 3000 rpm ($2300 \times g$) for 10 min, and the upper layer was collected and stored at -70°C in 1 ml aliquots.

Polymerase chain reaction (PCR) of WSSV VP15, VP19, VP28, and VP35 genes. PCR was performed to amplify the complete ORFs of structural viral proteins of WSSV, VP15, VP19, VP28, and VP35. Primers for PCR were designed from nucleotide sequences in the GenBank/EMBL databases of WSSV (AY249451 for VP15, AY160771 for VP19, AF272979 for VP28, and AF440570 for VP35). The PCR primers were as follows: VP15 upstream, 5'-ATA ATA CAA AAA TTA TAA ATT GGG AAG-3'; VP15 downstream, 5'-ACG CCT TGA CTT GCG GGC TCC-3'; VP19 upstream, 5'-GTC TTT ACG TTA CAT TGA CGT ACC-3'; VP19 downstream, 5'-CTG CCT CCT CTT GGG GTA-3'; VP28 upstream, 5'-CTC GTC ATG GAT CTT TCT TT-3'; VP28 downstream, 5'-CTC GGT CTC AGT GCC AGA GT-3'; VP35 upstream, 5'-TAC TAC TTG TTC TAC CCA CGA GTT-3'; VP35 downstream, 5'-CCA ACA AGG ATC ATC AAT CAA-3'. The template used was genomic DNA isolated from the infected tissues of *Penaeus ori-*

entalis in Korea. Briefly, the homogenate was mixed with SNET lysis buffer (20 mM Tris-HCl, pH 8.0; 5 mM EDTA, pH 8.0; 400 mM NaCl; 1 % (w/v) SDS; 1 mg ml⁻¹ Proteinase K) and incubated for 8 h at 55°C. DNA was extracted with phenol-chloroform, precipitated in isopropyl alcohol with sodium acetate, washed twice in 70 % alcohol, and resuspended in TE buffer (pH 8.0). The PCR mixture (100 µl) contained 2 µg of DNA, 100 pmol of each primer, 1 × PCR buffer, 2 mM MgCl₂, 200 µM dNTP, and 2.5 units of Taq polymerase (PCR Core Kit, Boehringer). The gene amplification reaction conditions were as follows: 1 cycle of 94°C for 7 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. The amplified PCR products were cloned using a pBAD/Thio TOPO TA Cloning Kit (Invitrogen) and were sequenced at GenoTech Corp (DaeJeon) using an ABI PRISM[®] 3700 DNA Analyzer (Applied Biosystems) according to the dye terminator procedure with forward and reverse primers designed from the sequencing results.

Computer-assisted analysis. Nucleotide and amino acid sequences were compiled and analyzed using the proteomics and sequence analysis tools, ExPASy Molecular Biology Server (Yonsei Proteome Research Center, Korea). The resultant amino acid sequences were analyzed for homologies to other proteins contained in the public database GenBank by BLASTp. These protein sequences have been submitted to the GenBank database and assigned the accession numbers AY316119 (VP19), AY324881 (VP28), AY374120 (VP15) and AY325896 (VP35).

RESULTS AND DISCUSSION

Researchers from several countries (Japan, Thailand, Taiwan) have isolated and partially characterized local isolates of WSSV and found that they appear to be variants of a single, if not identical, virus (Nadala et al. 1998). We obtained similar results with a purported isolate of the WSSV obtained from *Penaeus orientalis* from the western coast of Korea (Park et al. 1998, Lee et al. 2000). The infected shrimp exhibited abnormal behavior including a characteristic S-shape swimming pattern, lethargy, anorexia, opaque musculature and a laterally recumbent posture. This was accompanied by white spots in the cuticle of the cerebrothorax and red body coloration.

Using template DNA extracts from these shrimp for PCR, the amplicons for ORF VP15, VP19, VP28, and VP35 were as expected based on the database sequences of WSSV (i.e. about 185, 366, 615 and 684 bp, respectively). The PCR products were cloned into pBAD/Thio TOPO TA cloning vectors and sequenced. BLAST analysis of these sequences

ATGGCCACCACGACTAACACTCTTCCTTTTCGGCAGGACCGGAGCCCAGGCCGCTGGTTCCTTCTTAC	66
M A T T T N T L P F G R T G A Q A A G P S Y	22
ACCATGGAAGATCTTGAAGGCTTCATGTCTATGGCTCGCATGGGTCTCTTTTTGATCGTTGCATATC	132
T M E D L E G S M S M A R M G L F L I V A I	44
TCAATTGGTATCCTCGTCCTGGCCGTCATGAATGTATGGATGGGACCAAAGAAGACAGCGATTCT	198
S I G I L V L A V M N V W M G P K K D S D S	66
GACACTGATAAGGACACCGATGATGATGACGACACTGCCAACGATAACGATGATGAGGACAAATAT	264
D T D K D T D D D D T A N D N D D E D K Y	88
AAGAACACGACCAGGGATATGATGCTTCTGGCTGGGTCCGCTCTTCTGTTCCCTCGTTTGGGCCGCC	330
K N R T R D M M L L A G S A L L F L V S A A	110
ACCGTTTTTATGTCTTACCCCAAGAGGAGGCAGTAA	366
T V F M S Y P K R R Q	121

Fig. 1. Nucleotide sequence and deduced amino acid sequences of the structural protein genes of Korean isolate white spot syndrome virus VP19. The changed nucleotides (shaded) and amino acid (boxed) are indicated in comparison to those of China, Indonesia, Japan, and the United States

revealed high similarity to those of WSSV virion protein genes reported from China, Indonesia, Japan and the United States to the GenBank/EMBL databases. The nucleotide sequences of Korean VP15 and VP28 were 100% identical. VP35 was 100% identical to an isolate from Taiwan. In VP19, however, C and T in the GenBank isolates were replaced in the Korean isolate by T and A, respectively, at Positions 57 and 218 nt downstream of the VP19 A (+) start codon. To rule out the possibility that the nucleotide differences arose as an artifact during PCR amplification, 3 independently generated PCR products were sequenced using forward and reverse primers each with overlaps on both sides to diminish sequencing errors. The same base-pair replacement was present in all 3 PCR products. The changes resulted in 1 deduced amino acid change from valine in the GenBank isolates to aspartate in the Korean isolate at Position 218 nt downstream of the VP19 translational start codon (Fig. 1).

The results confirm previous observations that there is little genetic variation in geographic isolates of WSSV from China, India, Thailand and the United States (Lo et al. 1999). The amino acid sequence result for VP28 is also identical to that of a different Korean isolate previously reported (Moon et al. 2003). It is not known whether the single amino acid change in VP19 of the Korea WSSV isolate has any effect on virulence as has been reported for WSSV deletion variants (Lan et al. 2002). However, these and other findings from protein profiles (Wang et al. 2000) and restriction fragment length polymorphism (RFLP) (Nadala & Loh 1998) reveal that all geographical isolates of WSSV are not completely identical. This is also clear from the complete WSSV genome sequences at GenBank. Despite this, most of the differences in the records are

associated with deletions or with tandem repeat regions of highly variable length and of unknown significance and function (Wongteerasupaya et al. 2003) rather than coding regions for known functional proteins. Thus, our results add to the growing body of information indicating that WSSV outbreaks are the manifestation of a pandemic due to a single viral species.

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