Characteristic of a viral protein (VP-15) of white spot syndrome virus isolated from infected tiger shrimp

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Abstract

White spot syndrome virus (WSSV) causes mortality to tiger shrimp Penaeus monodon culture, which adversely affects the industry worldwide, included Indonesia. The structural proteins of VP-15 WSSV play very important roles in virus infection and morphogenesis process. The study aimed to isolate and characterize the VP-15 from infected WSSV tiger shrimp. The results showed that VP-15 was successfully isolated from the tiger shrimp in ORF DNA fragment size at 243 bp. The phylogenetic tree analysis was revealed three clusters corresponding to the time (year) of isolates collection. The VP-15 consisted of 80 amino acids, two start codons (ATG), one stop codon (TAA), and one Kozak context (AAAATGG). Hydrophilic amino acid was obtained to be a highest composition (44.2%) in VP-15, followed by neutral and hydrophobic amino acid group in value of 31.2% and 24.6%, respectively. The VP-15 was rich amino acid of lysine (21.3%), arginine (22.9%) and serine (24.6%). The successful to isolate VP-15 is a very important step in providing a material base to construct the RNAi technology for controlling the shrimp diseases in aquaculture.

Introduction

WSSV is one of the major virulent viruses infected the shrimp and causes the significant mortality in pond culture and hatchery. Recently, the smallest nucleocapsid protein discovered in WSSV of VP15 is thought to be involved in WSSV genomic packaging based on its DNA binding and condensing abilities. Moreover, the potential mechanism in improving fish diseases resistance is production of aquatic organism by genetic engineering through RNA interference (RNAi) technology. RNAi is a technology for inhibiting expression of virulence gene of pathogen, so the virus could not make infection to the fish/shrimp.

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Aim

To isolate and characterize a gene encoding viral protein VP-15 from infected tiger shrimp in providing the material base to construct the RNAi technology.

Materials and Methods

Tiger shrimp were collected from the brackish water pond culture. The nine selected samples from positive WSSV tiger shrimp consisted of 3 samples from diseases outbreak in 2012, 1 sample in 2013, and 5 samples in 2014.

DNA genome was extracted using CTAB. The DNA genome was used as a template for PCR technique using IQ-2000 kit to detect the positive infection.

VP-15 was isolated by PCR technique from the DNA genome of positive white spot disease. The DNA fragment was purified using purification kit and the gene was sequenced by using automatic ABI Prism.

Characterization of VP-15 was conducted by Genetyx program and the sequences were aligned with the gene bank using program of BLAST-N at NCBI.

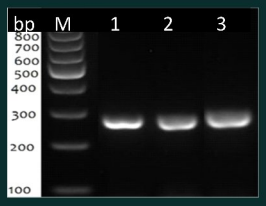

Figure 1. Gel electrophoresis of VP-15 WSSV (bp=base pair; M=marker DNA ladder; 1-3=representative samples of tiger shrimp.

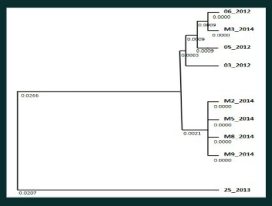

Figure 2. Phylogenetic tree of gene encoding VP-15 WSSV isolated from infected tiger shrimp collected in 2012, 2013, and 2014.

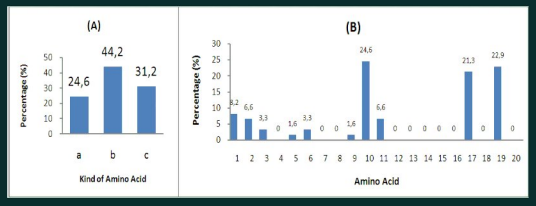

Figure 4. Amino acid composition of VP-15 WSSV isolated from tiger shrimp. (A) Amino acid group of hydrophopic (a), hydrophilic (b) and neutral (c). (B) Amino acid of glysine (1), alanine (2), valine (3), leucine (4), isoleucine (5), methionine (6), phenylalanine (7), tryptopan (8), proline (9), serine (10), threonine (11) asparagine (12), glutamine (13), cysteine (14), aspartic acid (15), glutamic acid (16), lysine (17), histidine (18), arginine (19), and tyrosine (20)

Results

VP-15 was isolated from the DNA genome of tiger shrimp positively infected WSSV. Electrophoresis result showed a single fragment of DNA at the position between 200-300 bp (Figure 1). The phylogenetic tree of VP-15 was revealed three clusters according to the time (year) of isolate collection (Figure 2). The gene VP-15 isolated in 2012, 2013, and 2014 showed the relatively high homology nucleotide. However, the isolate of 2013 showed the relatively different with other isolates. BLAST-N analysis also showed the high nucleotide identity (up to 99%) for the worldwide isolates of VP-15 gene deposited in GenBank. The size of ORF was 243 bp, calculated from first start codon (ATG) to stop codon (TAA). The ORF of VP-15 consisted of 2 start codons, 1 of stop codon, 80 of amino acids, 1 of Kozak context (AAAATGG). and the N-terminal sequence (Figure 3). The highest percentage of hydrophilic amino acid (44.2%) was obtained in VP-15, followed by neutral dan hydrophobic amino acid in value of 31.2% and 24.6%, respectively (Figure 4A). The VP-15 was rich in lysine (21.3%), arginine (22.9%) and serine (24.6%) (Figure 4B).

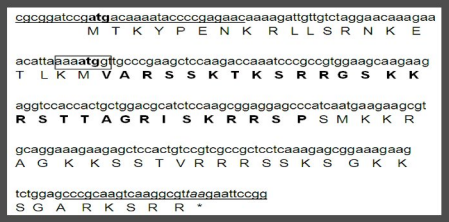

Figure 3. Nucleotide sequence and amino acid deduction of VP-15 (primers were underlined nucleotide, the Kozak context was boxed, start codon was in bold nucleotides, and stop codon was in italic nucleotide, the N-terminal sequence was in bold amino acids.

Conclusions

A gene encoding VP-15 was successfully isolated from infected tiger shrimp and had very high similarity (up to 99%) with the VP-15 deposited in GenBank. Three clusters were revealed in the present study corresponding to the time (year) of isolates collection. The VP-15 consisted of 80 amino acids, two start codons, one stop codon, and one Kozak context. The VP-15 was rich amino acid of lysine, arginine, and serine.