Characterization of VP28 and VP19-encoding Genes and Protein Sequence of White Spot Syndrome Virus (WSSV) Isolated from Indonesia

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Abstract

This study was aimed to compare VP19 and VP28 genes of WSSV isolated from Situbondo, Indonesia to other WSSV strains isolated from 12 different countries. The result indicated that VP19 gene from Situbondo appeared to be very uniqe. The nucleotide sequence of VP19 gene showed 100% identical to none of those 12 VP19 genes of WSSV. The nucleotide differences were observed initially at nucleotide position of 54, 108, 162, 201, and 218 nt. Meanwhile, VP28 gene of Situbondo appeared to be quite conserved. Compared to those 12 other WSSV strains, VP28 gene sequence of Situbondo showed 100% identical to VP28 gene sequence of WSSV isolated from China/Qindao, Japan, Korea01, South Carolina (USA) and also previous indonesian isolate (Indonesia97). However, the VP28 gene showed also some nucleotide differences compared to VP28 gene from China/Dalian, India, Iran, Korea Mexico, Thailand and Vietnam/Ho Chi Minh. The similarity was recorded at 99.4%, and the nucleotide differences were found at position 32, 255, 347, 396, 398, 430 and 458 nt. The variability in VP19 gene suggested that WSSV from Situbondo was quite different than the previous WSSV strain isolated from other region of Indonesia. However, based on VP28 gene, Situbondo isolate appeared to be 100% identical to previous Indonesia isolate. This result could be an important clue that VP28 gene is a conserved gene from which WSSV vaccine could be potentially developed.

Keywords: WSSV, VP28, VP19, homology.

Introduction

White spot syndrom (WSS) is the most virulent disease infected culture shrimp and lead to severe mortalities in many countries. Due to the disease, mortality of shrimp

could reach 100% in 3-7 days (Escobedo-Bonilla et al., 2008; Seok et al., 2004). Since firstly reported in a shrimp farm in Taiwan 1992, the disease has been quickly spreading around the world including Indonesia (Sunarto *et al*., 2004) and Japan in 1993 (Takahashi et al., 1994), China in 1995 (Huang et al., 2001), India in 1995 (Karunasagar et al., 1997), Thailand in 1995 (Wongteerasupaya et al., 1995), USA in 1996 (Lightner, 1996), Korea in 1998 (Park et al., 1998), Latin-Central and South America in 1999 (Rosenbery, 2000), Philipines in 2000 (Magbanua et al., 2000), Europe (France) and the Middle East (Iran) in 2002 (Rosenbery, 2002). Due to this disease, shrimp production has falled significantly and lead to great economic loses, at close to US$ 1 billion dollars across different countries (Mathew et al., 2005). The causative agent for this disease is white spot syndrome virus (WSSV). The virus has been isolated from a wide range of crustaceans including freshwater shrimp, crabs and lobsters (Chang et al., 1998; Hameed et al., 2001) posing a potential threat to shrimp culture.

WSSV is a rod-shaped virus containing double-stranded DNA with about 290-300 kbp genome (Lightner, 1996; Durand et al., 1996). Taxonomically, the virus belonged to family *Nimaviridae* and genus *Whispovirus*. The virion envelope contains two major proteins of VP28 and VP19 and the nucleocapsid consists of three major proteins of VP26, VP24 and VP15 (van Hulten et al., 2001). Two envelop proteins of WSS agent (VP28 and VP19) have gained a lot of interest for vaccine candidates, given their critical roles in infection processes.

Therefore, this study was aimed to characterize VP19 and VP28 gene of WSSV isolate from Situbondo, Indonesian. These 2 envelope proteins afterward were compared with gene and protein sequences of VP28 and VP19 amplified from WSSV strains isolated from other countires. This comparison results could be used as a main reason in vaccine design.

Materials and Methods

WSSV Isolation

Tiger shrimp (*Penaeus monodon*) collected from a shrimp farm at Situbondo, Indonesia, were homogenized with a ceramic mortar in a 20-fold volume of sterile phosphate-

buffered saline (PBS). Then, the homogenate was centrifuged at 3000 rpm (2300 × *g)* for 10 min, and the supernantant was collected and stored at -80°C in 1 ml aliquots. For VP19 and VP28 amplification, genomic DNA was isolated using standard procedures. Briefly, the homogenate was mixed with SNE 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).

Amplification of VP19 and VP28 encoding genes

PCR was performed to amplify the complete ORFs of structural viral proteins of WSSV, VP19, and VP28. Two sets of primers were used, VP19-F = 5'-CGG GAT CCA TGG CCA CCA CGA CTA A-3' and VP19-R = 5'-GCC TGC AGC CTG ATG TTG TGT TTC TAT A-3', and VP28-F= 5'-AAGGATCCCACACACTGTGACCAG-3', VP28-R= 5'-TAGCGGCCGCAAAAGCACGATTTATTTAC-3'). The amplification reaction was composed of 0,5 U *Ex Taq* DNA polymerase with their buffer (Takara Bio Inc., Otsu, Japan); 0,2 µM primer; 0,2 mM dNTP mix; 1 pg DNA template, and dH_2O. The amplification reaction was run under the following program: 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 30 s, 52°C for 25 s, and 72°C for 30 s; and 1 cycle of 72°C for 5 min. The amplified PCR products were cloned into pGEMT-Easy (Promega) 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 analyzed using Genious software version 5.3.2 to find sequence similarities. In addition, 13 sequences of each VP19 and VP28 genes isolated from different countries were downloaded from GeneBank, and used as comparisons. The accession numbers for VP19 gene which were used as the comparison

were: China95/Dalian AY249444 (Accesion number in GeneBank), China99/Qindao AY249445, India03 AY422227, Indonesia97 AY249448, Japan98 AY249447, US98/South Carolina AY249446, Korea01 AY316119, Korea03 GQ328028, Iran14 AB974691, Mexico05 AJ937860, Thailand01 AF369029, and Vietnam02/Ho Chi Minh AY160771. In addition, accession numbers for VP28 gene are: China95/Dalian AY249434, China99/Qindao AY249440, India05 AY422228, Indonesia97 AY249441, Japan98 AY249443, US98/South Carolina AY249442, Korea01 AY324881, Korea03 GQ328029, Iran11 KF723558, Mexico05 KF723558, Thailand07 EF194079, and Vietnam12 JX444994.

Results and Discussions

The result showed that VP19 and VP28 genes, which amplified using specific primers, were about 384 bp and 612 bp respectively (Figure 1). Both amplicon sizes of VP19 and VP28 appeared roughly to be the same size as VP19 and VP28 from VSSV isolated from other coutries including Korea (Seok et al., 2004), China, Japan, Thailand , Vietnam, Mexico, USA, and other countries.

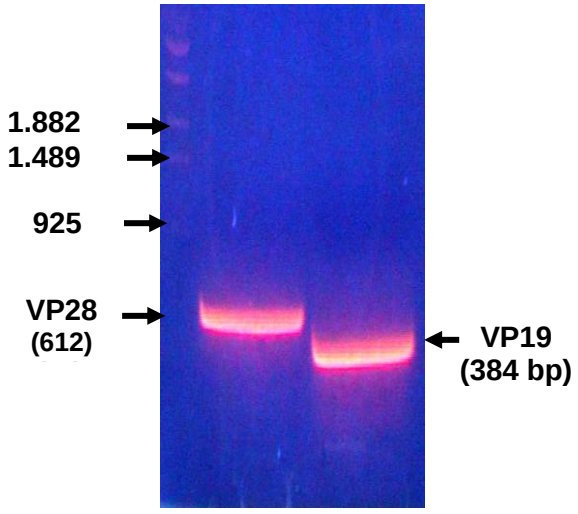

Figure 1. PCR products amplified by VP28 (left) and VP19 (right)-targeting primers.

Envelope protein VP19

Firstly, nucleotide sequence of VP19 gene isolated from Situbondo, East Java, Indonesia was compared to previous Indonesian isolate (Indonesia97, Access number: AY249448). The result showed Situbondo isolate was quite different, only 98.6% sequence similarities. The nucleotide differences were observed at position of 54 in which Thymine at Indonesia97 isolate was replaced by Guanine in Situbondo Isolate, at 108 and162 nt in which Guanine was replaced by Cytosine, at 201nt in which Cytosine was replaced by Thymine, and at poition 218 nt where Thymine in Indonesia97 isolate was replaced by Guanine in Situbondo isolate, Figure 2.

These nucleotide differences might indicate either the WSSV spreding Indonesia experienced some mutations or these isolates come from different regions/countires. In order to elucidate more specific answer, further studies including rRNA sequence homology analysis need to be done.

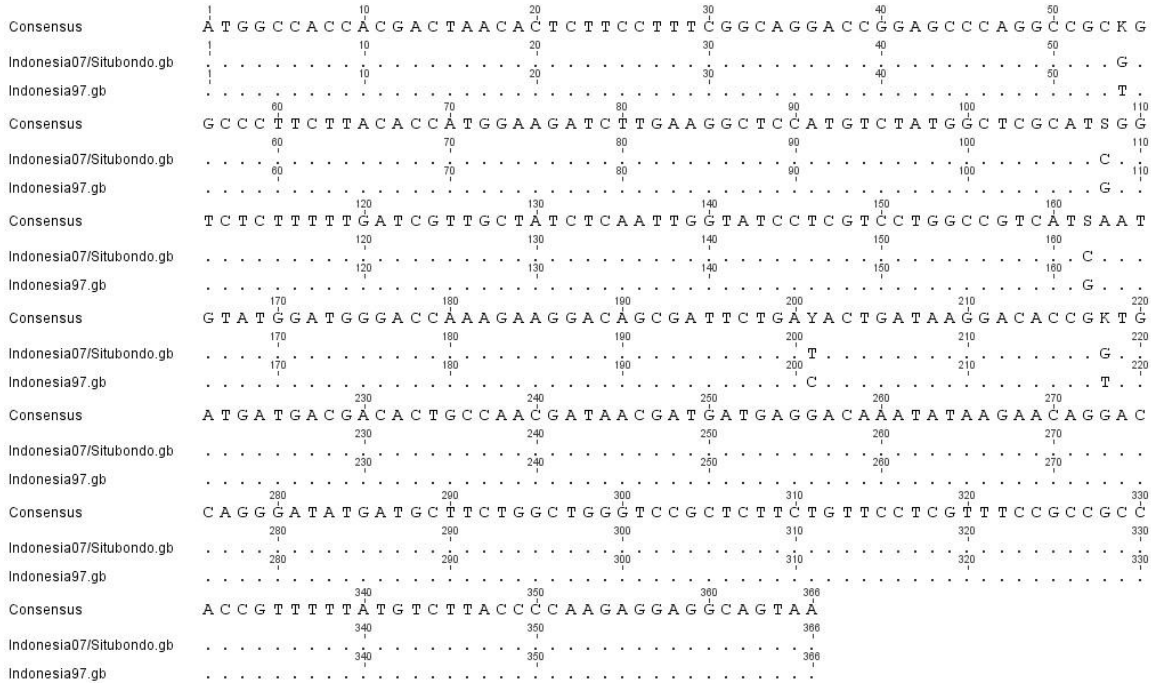

Figure 2. Comparison of VP19 gene sequence between Indonesia97 and Indonesia07, Situbondo isolate

Thereafter, the nucleotide sequence of VP19 from Situbondo isolate was also compared to 13 nucleotide sequences of VP19 isolated from other countries. The result showed that there was approximately 99.5% similarity. The differences were initially found at position 25 and 44 nt (Cytosine in Situbondo strain was replaced by Thymin in Indian isolate), at 54 nt (Guanine → Thymin from 12 other isolates), at 57 (Cytocine →Thymine in Korean01 and 03), at 108 and 162 (Cytosine → Guanine in 12 other isolates), at 201 (Thymine → Cytosine in 12 other isolates), and at 218 nt in which Guanine in Situbondo isolate was replaced by Thymine (China95/99, Indonesia97, Japan98, Thailand01 and US98/South Carolina), and by Adenine (India03, Korea01/03, Mexico05 and Vietnam02/Ho Chi Minh). Based on this result, nucleotide sequence of VP19 gene isolated from Situbondo appeared to be quite uniqe compared to other strains from different countries.

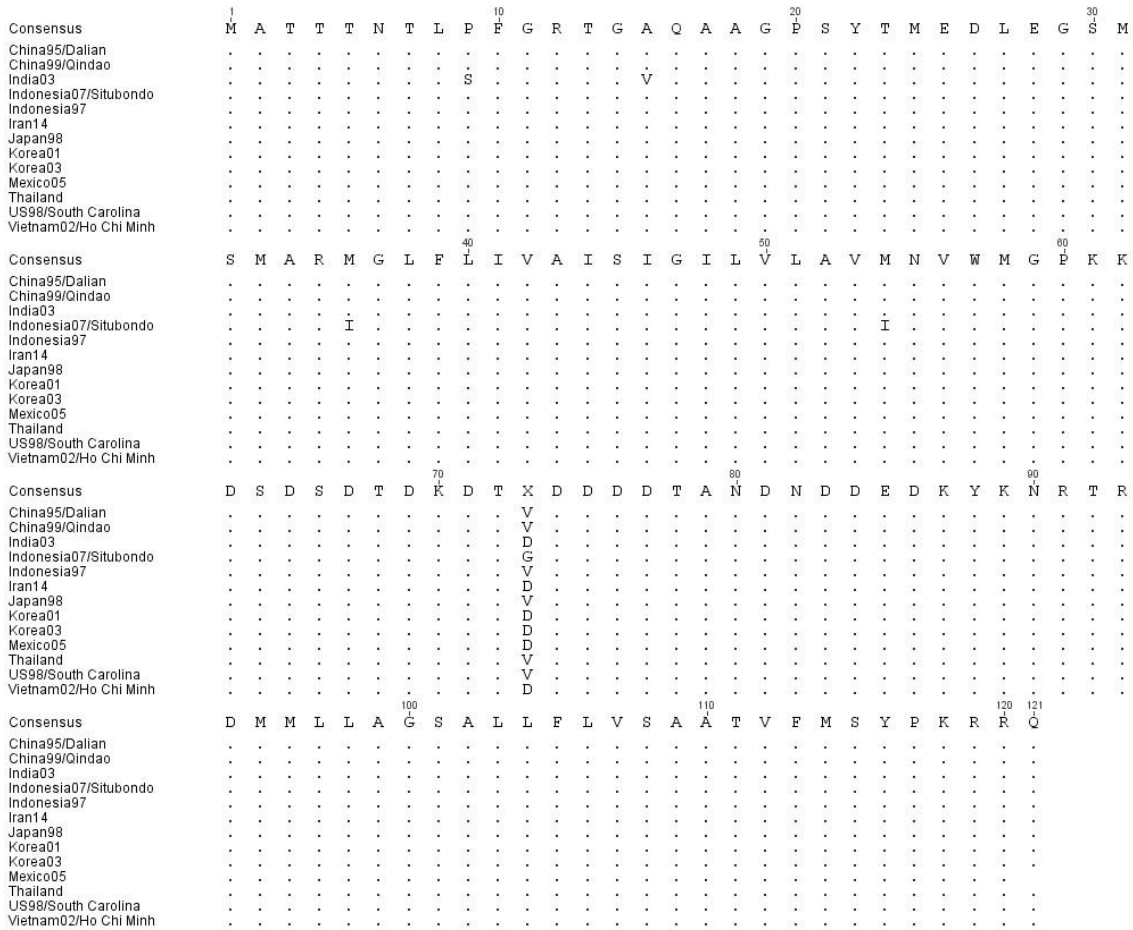

Figure 3. Comparison of VP19 nucleotide sequences isolated from Situbondo, Indonesia to 12 other VP19sequences from other countries.

The differences in nucleotide sequences of VP19 gene also caused some differences in the translation of the envelope protein, 99% similarity. Out of 121 translated amino acids (aa), at least five aa were observed to be different. The first difference was noticed to start at position 9, 15, 36, 54 and 73 of the letter (Figure 3). Started by Proline and Alanine at position 9 and 15 aa in situbondo isolate were replaced by Serine and Valine in India03, Isoleucine at 36 and 54 aa were replaced by Methionine in 12 othter isolates, Glycine was replaced by Valine (China95/99, Indonesia97, Thailand, Japan98 and US98) and by Aspartic acid (India03, Iran14, Korea01/03, Mexico05 and Vietnam02/Ho Chi Minh).

Envelope protein VP28

Based on nucleotide sequence of VP28-encoding gene, Situbondo isolate appeared to be 100 % identical to other strains Indonesia97, China99/Qindao, Japan98, Korea01 and US98/South Carolina. However, there were also some differences when it was compared to several strains isolated from China/Dalian, India, Iran, Korea Mexico, US/South Carolina, Thailand and Vietnam/Ho Chi Minh. The similarity was 99.4% (Fig. 4). Started at position 32 nt in which Adenin was substituted with Guanine (China/Dalian) and with Cytosine (Iran), at 255 Guanine was substituted by Adenine (Iran and Mexico), Cytosine was subtitued with Thymine at 347 in Thailand, and at position 396 and 398 nt in India strain, Tymine at position 430 nt was replaced by cytosine at Vietnam/Ho chi Minh, abd Cytosine at position 458 nt was replaced by Adenine in Iran and Mexico isolates.

The homology of nucleotide sequences in some countries was also followed by amino acid sequened of VP28 protein in 5 strains (Indonesia97, China/Qindao, japan, Korea01 and US/South Carolina). Therefore, these countries were excluded in further

comparison. The result showed that there was 98.9% similarity of VP28 amino acid sequences, Figure 5.

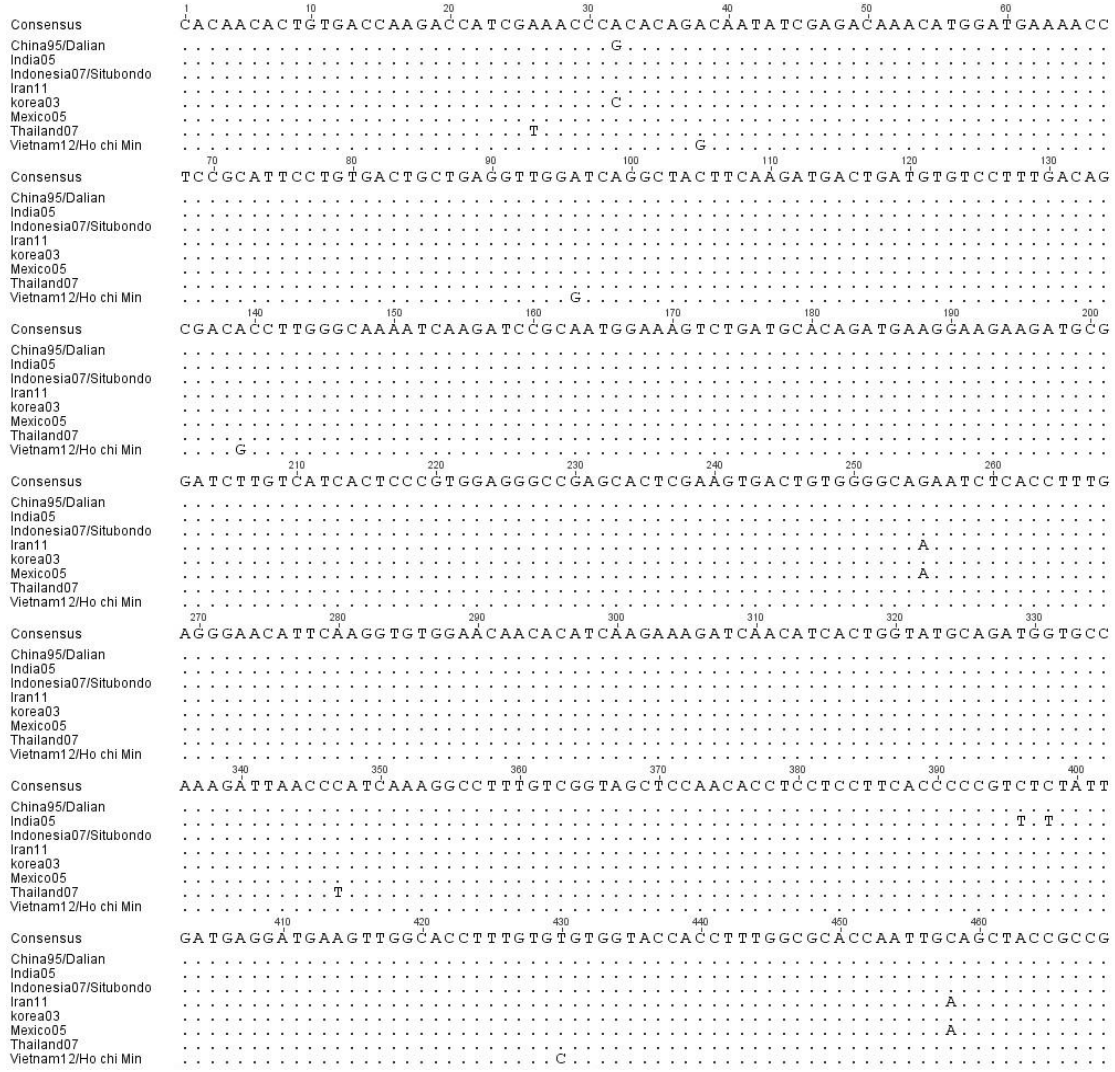

Figure 4. Nucleotide sequences of VP28 genes isolated from Indonesia other countries

The first difference was found at position 9 aa in which Glutamic acid was replaced by Valine (Thailand), Histidine at position 11 aa was substituted by Arginine (China/Dalian) and by Proline (Korea03), Threonine was replaced by Alanine in Vietnam/Ho Chi Minh, Proline at position 116 was substituted by Leucine in Thailand

isolate, Serine at 133 was replaced by phenylalanine (India) and lastly Alanine at 153 was replaced by Glutamic acid in Iran strain.

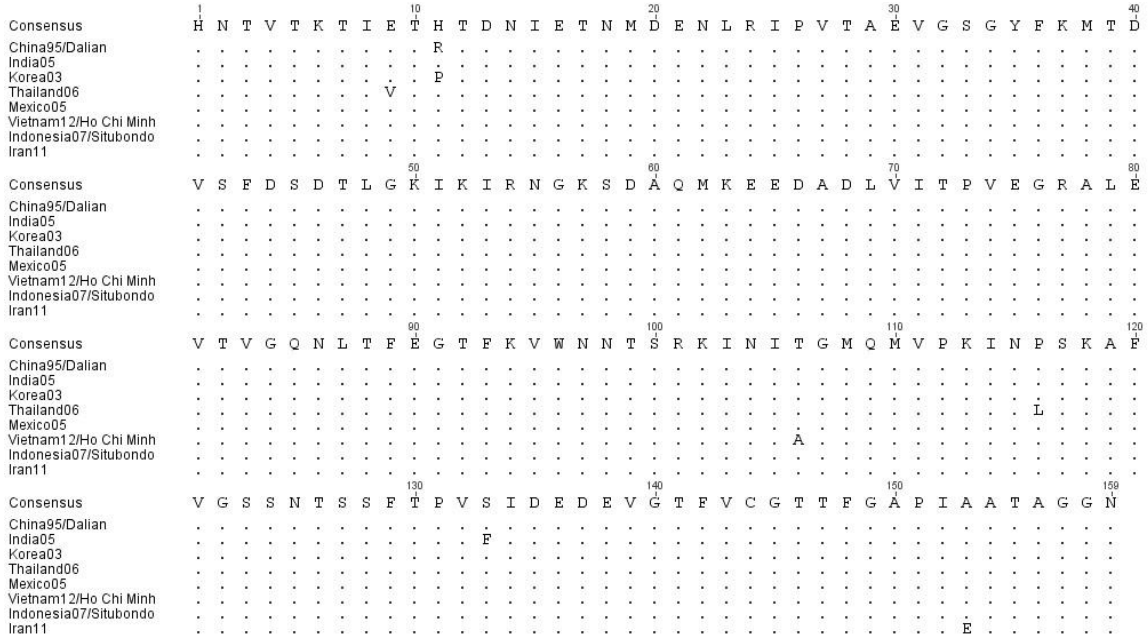

Figure 5. Sequence of an envelope protein VP28 isolated from Indonesia and VP28 protein sequences from other countries

The variability of VP19 and VP28 genes may also cause some differences in virulent activities of WSSV (Colin et al., 1993). In addition, the variability of these 2 envelope WSSV genes may also cause difficulties in developing a WSSV vaccine. Gilbert *et al*., (2011) suggested that the efficacy of a vaccine might be significantly influenced by a sequence of its amino acid sequences. However, further studies are needed to confirm the virulent activites and vaccine afficacy which is based on these 2 envelope proteins.

Given the sequence variability of VP19 in WSSV from sitobondo isolate and the fact that nucleotide sequences of VP28 gene appeared to be more conserved, this might indicate that the VP28 envelope protein could be a potential candidate from which WSSV vaccine should be developed.

Conclusion

Comparison of VP19 and VP28 genes indicated that WSSV from different countires were quite diverse. Based on nucleotide sequence of VP19, WSSV isolated from Situbondo, Indonesia appeared to be very uniqe, showed 100% identical to none of the 12 WSSV strains previously isolated from differerent countires. The nucleotide differences were observed at nucleotide position of 54, 108, 162, 201, and 218 nt. Meawhile, VP28 gene of Situbondo appeared to be quite conserved. Compared to 12 WSV strains, nucleotide sequence of VP28 gene showed 100% identical to at least 5 strains isolated from 4 different countries including China/Qindao, Japan, Korea01, and Mexico. However, the VP28 showed also some differences when it was compared to strains from China/Dalian, India, Iran, Korea Mexico, Thailand and Vietnam/Ho Chi Minh. The similarity was recorded at 99.4%, and the differences were found at position 32, 255, 347, 396, 398, 430 and 458 nt. These result suggested that Situbondo WSSV isolate was unique. However, based on VP28 gene, Situbondo isolate appeared to be 100% identical to previous Indonesia isolate. This result could be an important clue that VP28 gene is a conserved gene from which WSSV vaccine could be potentially developed.

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