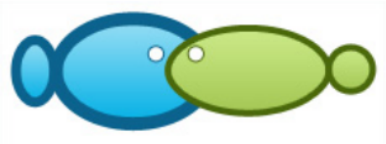
VP_19_Aliah.pdf

by

FILE	VP_19_ALIAH.PDF (179.49K)		
TIME SUBMITTED	05-JUN-2020 06:47PM (UTC+0700)	WORD COUNT	3155
SUBMISSION ID	1338317973	CHARACTER COUNT	16547



Isolation and characterization of an envelope protein (VP19) of a White Spot Syndrome Virus from diseased vannamei (*Litopenaeus vannamei*) in Indonesia

[1]Andi A. Hidayani, [1]Asmi Citra Malina A. R. Tassakka, [2]Andi Parenrengi

[1] Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia;
[2] Research Institute for Coastal Aquaculture, Maros, Indonesia.
Corresponding author: A. A. Hidayani, lhia_achsan@yahoo.com

Abstract. This study aimed to isolate and characterize White spot syndrome virus (WSSV) viral protein 19 (VP19) from Indonesian vannamei (*Litopenaeus vannamei*) isolates. Juvenile vannamei were collected from infected vannamei located at Pangkep, Barru and Pinrang regency of Indonesia. Total genomic DNA was isolated from the walking legs and telson of shrimp by DTAB-CTAB DNA extraction procedure. The length of DNA sequences of WSSV VP19 from Indonesian isolate was 387 bp. The homology of the Barru isolates with the other isolates from Pangkep and Pinrang was between 96-99%. Closer relationships were found between isolates from Barru and Pangkep. There could be a genetic flow between shrimp populations in Pangkep and Barru since these two areas are geographically close. BLAST-N (Basic Local Alignment Search Tool-Nucleotide) analysis showed that the highest similarity of WSSV VP19 was found between Indonesian and Mexican isolates. These findings reveal that all geographical isolates of WSSV VP19 are not completely identical.
Key Words: VP19, shrimp, virus, BLASTN, South Sulawesi.

Introduction. White spot syndrome virus (WSSV) is the cause of a viral disease which affects mainly cultured shrimp (Lightner 1996). It is a double-stranded rod-shaped DNA virus, and belongs to the Nimaviridae family (Wang et al 1995; Mayo 2002; Yi et al 2003). WSSV is the most pathogenic virus and causes high mortality among the penaeid shrimp (Sun et al 2015; Jariyapong et al 2015). The virus exists in an extremely wide range of potential hosts, including shrimps and other crustaceans such as crabs and crayfish (Wang et al 1998). WSSV was first detected in Asia, with occurrences in Taiwan (Chou et al 1995), Japan (Nakano et al 1994), and Korea (Park et al 1998). Since these first events, the disease has spread among most Asian countries, as well as in the Americas (Lightner 1996; O.I.E. 2003).

One of the known shrimp hosts of WSSV are *Litopenaeus vannamei*, which is one of the most commonly cultured species in Indonesia and is one of the most commercially important species comprising the wild shrimp fishery along the South Sulawesi coast of the Indonesia (Zainuddin et al 2014). Because WSSV has been catastrophic to the shrimp farming industry and may be a threat to the wild shrimp fisheries, 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. Tsai et al (2006) have identified the protein components of the WSSV virion by proteomic methods. VP26, VP36A, VP39A, and VP95 were all identified as tegument proteins distinct from the envelope proteins (VP19, VP28, VP31, VP36B, VP38A, VP51B, VP53A) and nucleocapsid proteins (VP664, VP51C, VP60B, VP15). More than 58 structural proteins had been identified, now, the envelope protein of WSSV has become the focus of numerous studies (Seok et al 2004; Tang & Hew 2007; Tsai et al 2004; van Hulten et al 2001; Wu et al 2005; Xu et al 2009; Liang et al 2011; Huang et al 2013). Viral envelope proteins play an important role in virus entry and assembly (Chazal & Gerlier 2003).

To our knowledge, there is no available information regarding an envelope protein (VP19) of a WSSV isolate from Indonesian *Litopenaeus vannamei*. Therefore, the objective of this work was to characterize WSSV VP19 from Indonesian *L. vannamei* isolate.

Material and Method

Sources of samples. Juvenile *L. vannamei* were collected from shrimp ponds located at Pangkep, Barru and Pinrang, South of Sulawesi, Indonesia from June–September 2014. Moribund shrimp in infected ponds were collected for PCR assays according to the methods described by Lightner (1996). Prior to DNA isolation, the walking legs and telson shrimp were preserved in 70% ethanol and subjected to PCR analysis in laboratory following the method of Corriveau et al (2010).

DNA isolation and PCR assay in shrimp. Total genomic DNA was isolated from the walking legs and telson of shrimp by DTAB-CTAB DNA extraction procedure with PCR kits (IQ2000, Taiwan) and subsequently precipitated with ethanol (Hoa et al 2011). The isolated DNA pallet was dissolved in DEPC ddH20 and quantified using NANODROP spectrophotometer (Hoa et al 2011). The nested PCR was carried out in a 0 mL thin wall PCR tube with 50 ng of genomic DNA, 10 pM of each primers, 50 µM of dNTPs, 1X PCR buffer, 1.5 mM $MgCl_2$, and 1.0 Ampli Taq Gold (Perkin Elmer, USA). Amplification conditions were followed 39 cycles at 94°C for 4 min, 55°C for 1 min, 72°C for 3 min and final extension at 72°C for 5 min in a PCR thermocycler. Positive and negative controls were included in all tests. PCR products were subjected to electrophoresis in 2% agarose gel and quantified as per the method of Hoa et al (2011).

PCR amplification and sequence analyses. PCR was performed to amplify the complete Open Reading Frames (ORFs) of structural viral protein of WSSV VP19. Primers for PCR were designed from nucleotide sequences in the GenBank/EMBL databases of WSSV (AY160771 for VP19). The PCR primers were as follows: VP19 upstream, 5'-GTC TTT ACG TTA CAT TGA CGT ACC-3'; VP19 downstream, 5'-CTG CCT CCT CTT GGG GTA-3'. The template used was genomic DNA isolated from the infected tissues of *L. vannamei*. Briefly, the PCR mixture consisted of 2 mL of each primer (5 pmol), 2 mL of 10x PCR buffer, 1.5 µL of 2 mM dNTP, 1 µL of 15 mM $MgCl_2$, 0.1 µL of *Taq* DNA polymerase (100 units) (Invitrogen, U.S.A.), 2 µL of the DNA template (1 µg mL^{-1}) and scaled up to 20 µL using distilled water. PCR amplification was carried out using the following conditions: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 sec and elongation at 72°C for 1 min; then a final elongation at 72°C for 5 min. PCR products (5 µL) were electrophoresed on a 0.8% agarose gel with 1% TBE electrophoresis buffer (pH 8.0) for 30 min, visualized using hand-held densitometer and photographed. The PCR products were sequenced using Big Dye Terminator ver 3.1 (Applied Biosystems, CA, USA) and comparative sequence analyses were performed by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI; http://blast.ncbi.nlm.nih.gov/). Multiple alignments of the sequences of VP19 were done using CLUSTAL W (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Phylogenetic tree was constructed using the Neighbor Joining Method with 1000 boostrap values of MEGA 4 (Tamura et al 2007).

Results and Discussion. Using one primer set to amplify a fragment of the VP19 genes, we were able to obtain sequences of the VP19 of an Indonesian isolate of WSSV. The sequence was 387 bp in length. Alignment of the sequences of the Barru isolate together with the isolates from Pangkep and from Pinrang is shown in Figure 1. The homology of the Barru isolates with the other isolates from Pangkep and Pinrang was between 96-99%.

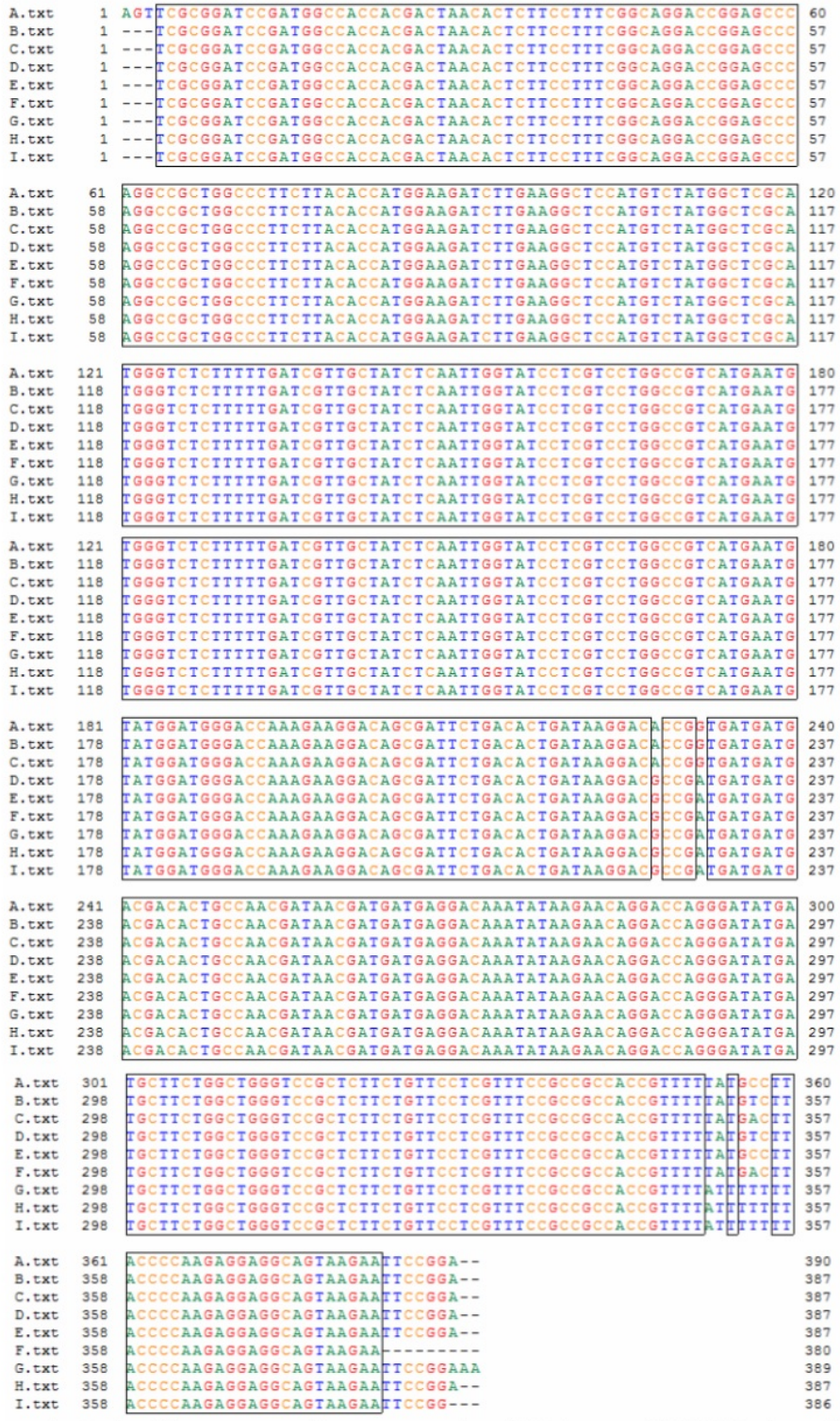


Figure 1. Nucleotide sequence alignment results of VP19 gene of WSSV isolates from Barru (A, B, C); Pangkep (D, E, F); and Pinrang (G, H, I).

In the phylogenetic tree the different places of isolates are all present in clades which are well bootstrap-supported. The computer-generated dendrogram revealed that all isolates were clustered into two distinct groups as shown in Figure 2. The Barru and Pangkep isolates clustered in one group as well as the Pinrang isolates. Closer relationships were found between Barru and Pangkep isolates than between Pinrang isolates. It can be hypothesized that there could be a genetic flow between shrimp populations in Pangkep and Barru since these two areas are geographically close.

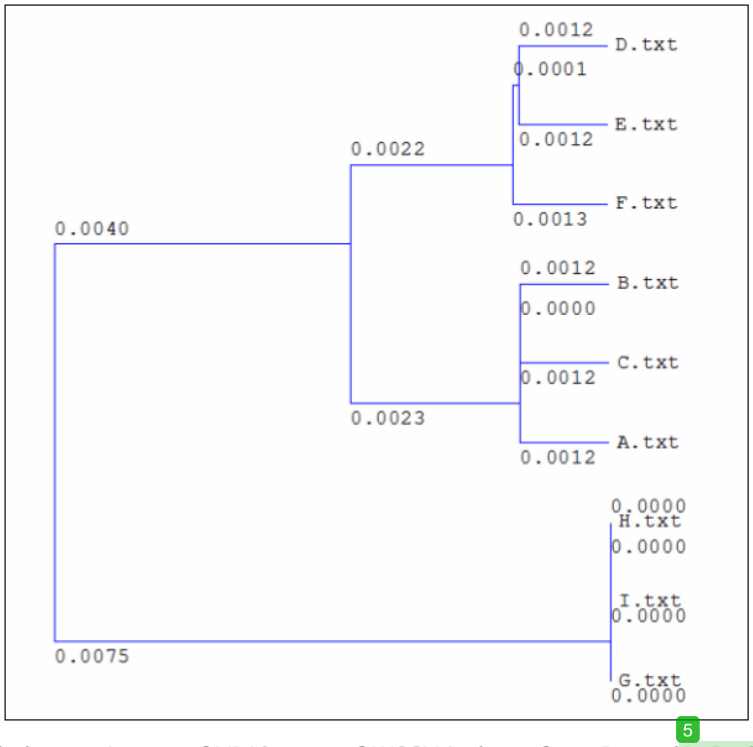


Figure 2. Phylogenetic tree of VP19 gene of WSSV isolates from Barru (A, B, C); Pangkep (D, E, F); and Pinrang (G, H, I). The Neighbor Joining method with 1000 bootstrap values was used for the analysis.

The amplified VP19 sequences were used as BLASTN queries against the NCBI database. The sequences of the PCR amplicons from Barru, Pangkep and Pinrang isolates were found to be 98-99% similar to the sequences of VP19 from other country isolates (Figure 3). The VP19 sequence amplified from Barru and Pangkep isolates showed 98% identity with VP19 from China (AY245790.1) and Xiang shan (DQ007314.1) isolates. These two isolates (Barru and Pangkep) also showed 99% identities with South Korea (JX515788.1), India (DQ681071.1), China (AY249445.1), China (AY249444.1), Netherland (AF369029.2), Taiwan (AF440570.1), India (GU734035.1), Cochin (EU012447.1), Vietnam (AY160771.1), China (AF332093.2), Korea (GQ328028.1), Korea (AY316119.1), China (AY220744.1), India (AY873786.1), Mexico (AY713372.1), India (AY422227.1), Singapore (AF402997.1), India (DQ902655.1), and Mexican Isolates (AJ937860.1). Similar patterns were observed amongst isolates of Pinrang that also showed 98% identities to isolates of China (AY245790.1), Xiang shan (DQ007314.1), South Korea (JX515788.1), India (DQ681071.1), China (AY220744.1), Netherland (AF369029.2), Taiwan (AF440570.1), India (GU734035.1), China (AF332093.2), Korea (AY316119.1), India (DQ902655.1) and Singapore (AF402997.1). Additionally, Pinrang isolates showed 99% identity to isolates of Cochin (EU012447.1), Vietnam (AY160771.1), Korea

(GQ328028.1), India (AY873786.1), Mexico (AY713372.1), India (AY422227.1), and Mexico (AJ937860.1) (Figure 3).

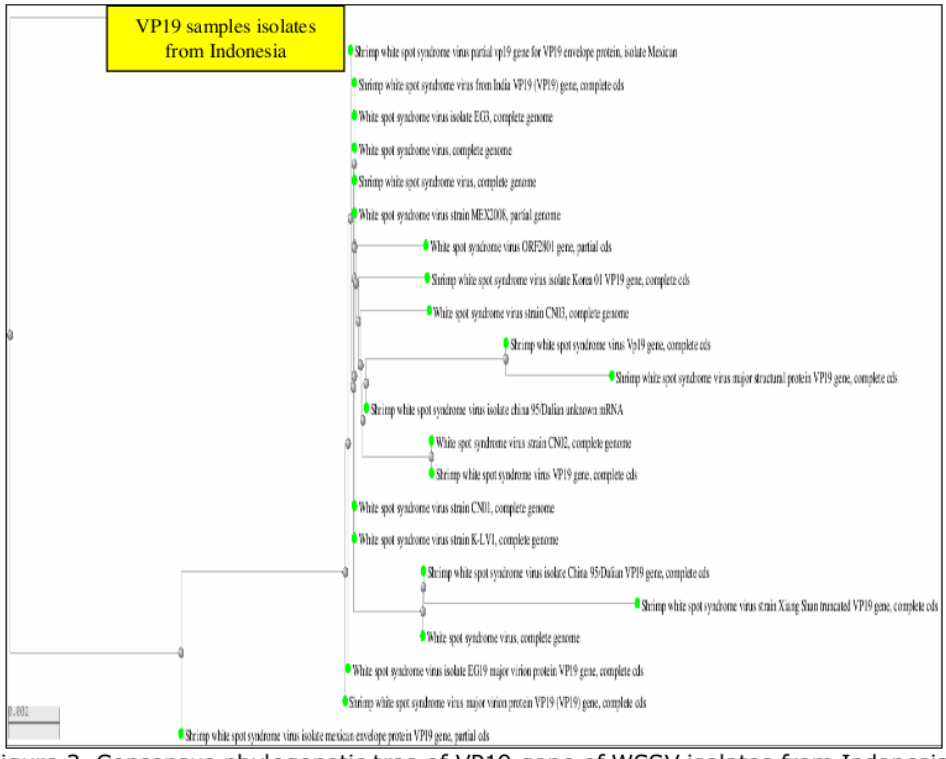


Figure 3. Consensus phylogenetic tree of VP19 gene of WSSV isolates from Indonesia.

In the present study, we have shown that BLASTn analysis of these sequences revealed high similarity to those of WSSV virion protein 19 genes reported from Mexico, China, South Korea, India, Netherland, Taiwan, and Vietnam. The highest similarity was found between isolates from Indonesia and Mexico. 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).

WSSV is an enveloped DNA virus. More than 30 proteins were classified as envelope proteins within the structural proteins (Huang et al 2013). Viral envelope proteins play a crucial role in viral infection. Seok et al (2004) demonstrated that at the nucleotide level, VP19, VP28 and VP15 sequences of Korean isolates were, respectively, 99, 100 and 100% identical to those of China, Indonesia, Japan and the United States. Molina-Garza et al (2008) revealed that VP19 may have application as a genetic marker. They found that VP19 of a WSSV isolate from Mexican *L. vannamei* was distinguishable from an American strain of WSSV, although homology was found with isolates from Taiwan and India. Furthermore, the major envelope proteins, VP19, VP26 and VP28 have been characterized from diseased *Penaeus vannamei* and moribund *Procambarus clarkii*. Homologous contrast showed that the sequences of VP19, VP26 and VP28 in the study possess over 97% DNA and 100% amino acid sequence similarity to WSSV in the NCBI database (Liang et al 2011).

Conclusions. Based on sequences of the genomic DNA of WSSV VP 19 and its phylogenetic analysis, there is likely a genetic flow between *L. vannamei* populations in Pangkep and Barru. BLASTN analysis showed that the highest similarity of WSSV VP19 was found between Indonesian and Mexican isolates. These findings reveal that all geographical isolates of WSSV VP19 are not completely identical.

Acknowledgements. This work supported by a grant from State Ministry of Research & Technology, Republic of Indonesia (National Innovation System Research Incentive).

References

Chazal N., Gerlier D., 2003 Virus entry, assembly, budding, and membrane rafts. Microbiology and Molecular Biology Reviews 67:226-237.

Chou H. Y., Huang C. Y., Wang C. H., Chiang H. C., Lo C. F., 1995 Pathogenicity of a baculovirus infection causing White Spot Syndrome in cultured penaeid shrimp in Taiwan. Diseases of Aquatic Organisms 23:165-173.

Corriveau M., Upstrom K., Klompen H., 2010 Effect of eight storage modes of DNA preservation. In: Trends in Acarology. Proceedings of the 12th International Congress. Sabelis M. W., Bruin J. (eds), Springer Science, Dordrecht, pp. 553-556.

Hoa T. T. T., Zwart M. P., Phuong N. T., Vlak J. M., de Jong M. C. M., 2011 Transmission of white spot virus in improved-extensive and semi-intensive shrimp production system - a molecular epidemiology study. Aquaculture 313:7-14.

Huang P. Y., Chen P. J., Huang H. T., Chen L. L., 2013 Identification and analysis of White Spot Syndrome Virus (WSSV) envelope protein VP41A. Journal of Marine Science and Technology 21:234-240.

Jariyapong P., Weerachatyanukul W., Direkbusarakom S., Hirono I., Wuthisuthimethavee S., Chotwiwatthanakun C., 2015 Enhancement of shrimp immunity against white spot syndrome virus by *Macrobrachium rosenbergii* nodavirus-like particle encapsulated VP28 double-stranded RNA. Aquaculture 446:325-332.

Liang T., Wu T., Du J., Ji H., Li Y., Gu W., Wang W., 2011 Characterization of a tailless white spot syndrome virus from diseased *Penaeus vannamei* and *Procambarus clarkii* in China. African Journal of Biotechnology 63:13936-13942.

Lightner D. V., 1996 A handbook of shrimp pathology and diagnostic procedures for diseases of cultured Penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA, 304 pp.

Lo C. F., Hsu H. C., Tsai M. F., Ho C. H., Peng S. E., Kou G. H., Lightner D. V., 1999 Specific genomic DNA fragment analysis of different geographical clinical samples of shrimp white spot syndrome virus. Diseases of Aquatic Organisms 35:175-185.

Mayo M. A., 2002 A summary of taxonomic changes recently approved by ICTV. Archives of Virology 147:1655-1663.

Molina-Garza Z. J., Galaviz-Silva L., Rosales-Encinas J. L., Alcocer-González J. M., 2008 Nucleotide sequence variations of the major structural proteins (VP15, VP19, VP26 and VP28) of white spot syndrome virus (WSSV), a pathogen of cultured *Litopenaeus vannamei* in Mexico. Journal of Fish Diseases 31:197-203.

Nakano H., Koube H., Umezawa S., Momoyama K., Hiraoka M., Inouye K., Oseko, N., 1994 Mass mortalities of cultured Kuruma shrimp, *Penaeus japonicus*, in Japan in 1993: epizootiological survey and infection trails. Fish Pathology 29:135-139.

Office International des epizooties (OIE), 2003 Spherical baculoviruses Chapter 4.1.5. In: Manual of diagnostic tests for aquatic animals. Paris, France, pp. 319-330.

Park J. H., Lee Y. S., Lee S., Lee Y., 1998 An infectious viral disease of penaeid shrimp newly found in Korea. Diseases of Aquatic Organisms 34:71–75.

Seok S. H., Park J. H., Cho S. A., Baek M. W., Lee H. Y., Kim D. J., Park J. H., 2004 Cloning and sequencing of envelope proteins (VP19, VP28) and nucleocapsid proteins (VP15, VP35) of a white spot syndrome virus isolate from Korean shrimp. Diseases of Aquatic Organisms 60:85-88.

Sun C. B., Wang G., Chan S. F., 2015 Effects of artificial infection of *Litopenaeus vannamei* by *Micrococcus lysodeikticus* and WSSV on the activity of immunity related enzymes. Fish and Shellfish Immunology 462:778-786.

Tamura K., Dudley J., Nei M., Kumar S., 2007 MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

Tang X., Hew C. L., 2007 Expression, purification and crystallization of two major envelope proteins from white spot syndrome virus. Acta Crystallographica Section F: Structural Biology and Crystallization Communications 63(7):624-626.
Tsai J. M., Wang H. C., Leu J. H., Hsiao H. H., Wang A. H., Kou G. H., Lo C. F., 2004 Genomic and proteomic analysis of thirty-nine structural proteins of shrimp white spot syndrome virus. Journal of Virology 78:11360-11370.
Tsai J. M., Wang H. C., Leu J. H., Wang A. H., Zhuang Y., Walker P. J., Kou G. H., Lo C. F., 2006 Identification of the nucleocapsid, tegument, and envelope proteins of the shrimp white spot syndrome virus virion. Journal of Virology 80:3021-3029.
Van Hulten M. C. W., Witteveldt J., Snippe M., Vlak J. M., 2001 White spot syndrome envelope protein VP28 is involved in the systemic infections of shrimp. Virology 285:228-233.
Wang C. H., Lo C. F., Leu J. H., Chou C. M., Yeh P. Y., Chou H. Y., Tuang M. C., Chang C. F., Su M. S., Kou G. H., 1995 Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus monodon.* Diseases of Aquatic Organisms 23:239-242.
Wang Y. C., Lo C. F., Chang P. S., Kou G. H., 1998 Experimental infection of white spot baculovirus in some cultured and wilds decapods in Taiwan. Aquaculture 164:221-231.
Wu W. L., Wang L., Zhang X. B., 2005 Identification of white spot syndrome virus (WSSV) envelope proteins involved in shrimp infection. Virology 332:578-583.
Xu H., Yan F., Deng X., Wang J., Zou T., Ma X, Zhang X., Qi Y., 2009 The interaction of white spot syndrome virus envelope protein VP28 with shrimp Hsc70 is specific and ATP-dependent. Fish and Shellfish Immunology 26:414-421.
Yi G., Qian J., Wang Z., Qi Y., 2003 A phage-displayed peptide can inhibit infection by white spot syndrome virus of shrimp. Journal of General Virology 84:2545-2553.
Zainuddin, Haryati, Aslamyah S., 2014 Effect of dietary carbohydrate levels and feeding frequencies on growth and carbohydrate digestibility by white shrimp *Litopenaeus vannamei* under laboratory conditions. Journal of Aquaculture Research and Development 5:274.
*** http://blast.ncbi.nlm.nih.gov/.
*** http://www.ebi.ac.uk/Tools/msa/clustalw2/.

Received: 23 December 2015. Accepted: 02 April 2016 Published online: 24 April 2016.
Authors:
Andi Aliah Hidayani, Faculty of Marine science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, Makassar 90245, Indonesia, e-mail: Lhia_achsan@yahoo.com
Asmi Citra Malina A. R. Tassakka, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, Makassar 90245, Indonesia, e-mail: citramalinaunhas@gmail.com
Andi Parenrengi, Research Institute for Coastal Aquaculture, Maros, Jl. Makmur Dg. Sittaka, 129 Maros, 90512, Indonesia, e-mail: litkanta@indosat.net.id

How to cite this article:
Hidayani A. A., Tassakka A. C. M. A. R., Parenrengi A., 2016 Isolation and characterization of an envelope protein (VP19) of a White Spot Syndrome Virus from diseased vannamei (*Litopenaeus vannamei*) in Indonesia. AACL Bioflux 9(2):389-395.

VP_19_Aliah.pdf

ORIGINALITY REPORT

%23	%10	%21	%14
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ajol.info Internet Source	%2
2	www.seer.furg.br Internet Source	%2
3	Soto, M.. "Epidemiological Parameters of White Spot Syndrome Virus Infections in Litopenaeus vannamei and L. setiferus", Journal of Invertebrate Pathology, 200107 Publication	%2
4	cancerres.aacrjournals.org Internet Source	%2
5	pt.scribd.com Internet Source	%2
6	webap.rsh.ncku.edu.tw Internet Source	%1
7	journals.plos.org Internet Source	%1
8	www.int-res.com Internet Source	%1

9	amsdottorato.unibo.it Internet Source	%1
10	sbr.sb-roscoff.fr Internet Source	%1
11	Basem M. Jaber, Rola Al-Silawi, Tariq Al-Najjar. "Isolation and molecular identification of Ascomycetes in sediments and waters of the Gulf of Aqaba, Red Sea", Natural Science, 2012 Publication	%1
12	Fujaya Yushinta, Ivo Asphama Andi, Aliah Hidayani Andi, Parenrengi Andi, Tenriulo Andi. "High genetic variation of Portunus pelagicus from Makassar Straits revealed by RAPD markers and mitochondrial 16S rRNA sequences", African Journal of Biotechnology, 2016 Publication	%1
13	anorexianervosabulimia.org Internet Source	%1
14	onlinelibrary.wiley.com Internet Source	%1
15	vir.sgmjournals.org Internet Source	%1
16	JW Do, SJ Cha, NS Lee, YC Kim, JW Kim, JD	

	Kim, JW Park. "Taura syndrome virus from Penaeus vannamei shrimp cultured in Korea", Diseases of Aquatic Organisms, 2006 Publication	%1
17	bmcbiotechnol.biomedcentral.com Internet Source	%1
18	Submitted to Ateneo de Manila University Student Paper	%1
19	Huang, P.-Y., J.-H. Leu, and L.-L. Chen. "A newly identified protein complex that mediates white spot syndrome virus infection via chitin-binding protein", Journal of General Virology, 2014. Publication	<%1
20	arizona.openrepository.com Internet Source	<%1
21	van Hulten, M.C.W.. "The White Spot Syndrome Virus DNA Genome Sequence", Virology, 20010720 Publication	<%1
22	Chae, H. S., G. N. Park, S. H. Kim, H. J. Jo, J. T. Kim, H. Y. Jeoung, D. J. An, N. H. Kim, B. W. Shin, Y. I. Kang, and K. S. Chang. "Rapid direct identification of Cryptococcus neoformans from pigeon droppings by nested PCR using CNLAC1 gene", Poultry Science, 2012. Publication	<%1

23	pubmedcentralcanada.ca Internet Source	<%1
24	www.scielo.br Internet Source	<%1
25	K. Sritunyalucksana, W. Wannapapho, C. F. Lo, T. W. Flegel. "PmRab7 Is a VP28-Binding Protein Involved in White Spot Syndrome Virus Infection in Shrimp", Journal of Virology, 2006 Publication	<%1
26	X. Tang, J. Wu, J. Sivaraman, C. L. Hew. "Crystal Structures of Major Envelope Proteins VP26 and VP28 from White Spot Syndrome Virus Shed Light on Their Evolutionary Relationship", Journal of Virology, 2007 Publication	<%1
27	M. Sarathi. "Silencing VP28 Gene of White Spot Syndrome Virus of Shrimp by Bacterially Expressed dsRNA", Marine Biotechnology, 03/2008 Publication	<%1
28	L. Fonseca, J. Cardoso, A. Lopes, M. Pestana, F. Abreu, N. Nunes, M. Mota, I. Abrantes. "The pinewood nematode, Bursaphelenchus xylophilus, in Madeira Island", Helminthologia, 2012 Publication	<%1

29	Sun, Yumiao, Fuhua Li, Zheng Sun, Xiaojun Zhang, Shihao Li, Chengsong Zhang, and Jianhai Xiang. "Transcriptome Analysis of the Initial Stage of Acute WSSV Infection Caused by Temperature Change", PLoS ONE, 2014. Publication	<%1
30	Submitted to Eiffel Corporation Student Paper	<%1
31	bmcgenomics.biomedcentral.com Internet Source	<%1
32	Submitted to Universiti Malaysia Sabah Student Paper	<%1
33	Liang Tingming, sect, Wu Ting, sect, Du Jie, Ji Hong, Li Yue, Gu Wei, Wang Wen. "Characterization of a tailless white spot syndrome virus from diseased Penaeus vannamei and Procambarus clarkii in China", African Journal of Biotechnology, 2011 Publication	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		