

Molecular Identification and Sequence Analysis of Virion Protein 28 (VP28) and Virion Protein 26 (VP26) from Saudi Arabia WSSV Isolates

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Abstract: White spot syndrome virus (WSSV) is a serious and lethal pathogen for the shrimp industry which causes great losses. In this study we applied molecular technique to identify the virulence related genes such as VP28 and VP26 from Saudi Arabia (SA) subsequently sequence based functionality were derived. The full-length of VP28 and VP26 obtained from SA isolates of WSSV and the sequences showed 99-100% homology with other WSSV known isolates. The identified gene sequences VP28 and VP26 contain an ORF of 615 bp and 612 bp encoding 204 and 203 amino acids respectively. SA WSSV isolates of VP26 amino acid sequences do not differ from known isolates in GenBank data base, whereas VP28 confirmed that a single amino acid differed, as a result of the substitution of G to D (Glycine to Aspartic acid). The phylogenetic analysis revealed that VP26 gene had a greater homology with other isolates of VP26; however, VP28 phylogenetic tree shown a subphylum among the isolates, in particular with Egypt and Thailand isolates. Putative conserved domain which codes for VP28 of WSSV superfamily and 3D form of structural relationships with the VP28 and VP26 amino acid sequences of WSSV SA isolates were generated. We anticipate that the present study will be helpful to reveal the genetic linkage between the isolates and to study the epidemiological relevance in harsh environmental conditions in SA.

Key words: White Spot Syndrome Virus • *Litopenaeus Vannamei* • *Fenneropenaeus Indicus* • Saudi Arabia WSSV Isolate And Virion Protein

INTRODUCTION

Amid aquaculture produced species, shrimp industry has grown as the most important and asset in seafood commodity over the past decades. Aquaculture in the Saudi Arabia (SA) began early 1980s and started culturing tilapia in freshwater bodies in inland areas. During late 1990s & 2010, *Fenneropenaeus indicus* and *Litopenaeus vannamei* were introduced and they have become major cultivated species in Red Sea coastal region of SA. Food and Agriculture Organization of the United Nations (FAO) reported that there was a phenomenal rise in aquaculture production of SA from 1950 to 2010, when total volume and value reached 26, 374 tone's valued US\$ 273 million [1]. Nevertheless, steep productions of shrimp industry

have a long-standing dilemma due to disease outbreaks, which have become an increasing challenge for the shrimp industry. Among the etiology of various diseases, viruses have given major impact on growth and survival of shrimp culture systems, in particular, white spot disease (WSD) which is caused by White spot syndrome virus (WSSV) and widely spread. [2, 3]. Occurrences of viral disease due to WSSV in *F.indicus* and *L.vannamei* have been reported 2010 and it continues to be a major problem for the shrimp aquaculture industry in SA [4, 5]. Recently, a new variant genotype of WSSV SA strain was found and it was suggested that it spread via wild broodstocks of *F.indicus* from Red Sea [6]. It was estimated that worldwide loss to the shrimp industry due to WSSV has been projected as about US\$ 10 billion since 1993 [7].

WSSV is a large enveloped virus containing double-stranded DNA genome and it has special characteristics such as the appendage extension at one end. It is classified in the genus *Whispovirus*, belonging to the family Nimaviridae [8]. WSSV genome length ranges from 292967 to 307107 bp based on the diverse genome sequences from different countries like China (KT995471/KT995472/KT995470/KY827813/AF332093/KX686117), Taiwan (AF440570), The Netherlands (AF369029), South Korea (JX515788), Ecuador (MH090824), Egypt (KR083866), Australia (MF768985), India (MG702567), Mexico (KU216744) and Brazil (MF784752). Sequence identity and similarities among these sequences showed a nucleotide identity of 99.32% with diminutive dissimilarity [9, 10].

Many studies have shown that WSSV virion envelope proteins and nucleocapsid proteins play a key role in the viral organization and infectivity with host cells. Among the structural proteins, VP19 and VP28 are located in virus envelope and VP15, VP24 and VP26 are found in nucleocapsid [11, 12]. Later, VP26 was found as a tegument protein and also it act as a molecular bridge between viral envelope and nucleocapsid [13, 14]. The role of VP28 in WSSV is attachment and entrance in the host cell and it is crucial to the systemic infection in shrimps [15]. N-terminal transmembrane region of VP28 and VP26 act as an anchor to connect or to fuse with the host cell for the viral infection [16]. VP28 and VP26 can interact with VP24 to facilitate the linkage between viral nucleocapsid and the envelope proteins to form a multiprotein complex [17, 18].

To date, the development of an effective vaccine strategy against WSSV was emphasized with VP28 and VP26 in many ways. Results with vaccinated shrimp with baculovirus expressing WSSV VP28 and with bacterial VP28 expression vector system, both given by oral route have shown higher survival rate on vaccinated animals [19, 20]. Induced gene-specific constructs of double-stranded RNA (dsRNA) approach with VP28 and VP26 was found to be successful protection for shrimp against WSSV; more importantly, the role of different WSSV proteins in infection has been studied by RNA interference [21-24].

Upon the functional significance of envelope and structural proteins, we focused on the identification and functional characterization of VP28 and VP26 genes from the SA strains of WSSV. Identification and understanding of phylogenetic relationships are important, especially for native strains and can pave the way to know the virus mechanisms for infection and anti-WSSV strategies.

MATERIALS AND METHODS

Sample Collection: WSSV infected animals (*F. indicus* and *L. vannamei*), were collected from Red Sea coast, Jazan, Saudi Arabia during epizootics in October 2014 and August 2015. At the time of sample collection, *F. indicus* exhibited visible macroscopic white spots in the carapace and red pigmentation of the body. The shrimp pleopod samples were dissected at the farm site, fixed in 95% ethanol, transported to Jeddah Fisheries Research Center, Ministry of Environment, Water and Agriculture and stored at -80°C for further analysis.

DNA Extraction: The ethanol fixed pleopod samples were dried and incised into small pieces and DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD) following the manufacturer instructions. The eluted DNA was assessed by spectrophotometer (Nanodrop 2000, Thermo Scientific) and its quality and quantity were determined.

Primer Designing, Amplification and Sequencing of VP28 and VP26 Genes: To amplify the full-length VP28 and VP26 genes from infected tissues, primers were designed based on database searches using gene or gene product names from National Centre for Biotechnology Information (NCBI). Available nucleotide sequences from the NCBI database (GenBank accession number) such as JX444994, DQ013883, AY249443 and AY249440 for VP28 and EU931452 for VP26. Sequences were submitted to a BLAST search and conserved regions were used for designing primers with DNA calculator, Sigma (<http://www.sigma-genosys.com/calc/DNACalc.asp>) and Primer Express software, Version 3.0.1 (Applied Biosystems, USA). The designed primers for respected genes and their product size were mentioned in Table 1.

The concentration of primer and annealing temperature were optimized. 1 µL (~100 ng) of DNA was used as template for PCR. The amplification of VP28 and VP26 gene was done with the DreamTaq Green Buffer (10x) (ThermoFisher Scientific, USA). The amplification was performed in 25 µL reaction mixture. The reaction mixture comprised the following reagents; 10X Reaction Buffer 2.5 µL, dNTPs (10mM each dNTP) 2.5 µL, Forward Primer (10 µM) 1.0 µL, Reverse Primer (10 µM) 1.0 µL, Nuclease-Free water 16.6 µL, Dream Taq DNA polymerase (5U/ µL) 0.4 µL and Template DNA (~100ng/ µL) 1.0 µL. Amplifications were carried out as initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 92°C for 30 s, annealing at 60°C for 1 min, extension at

Table 1: PCR primers for the amplification of VP28 and VP26 genes from Saudi Arabia WSSV isolates

Gene	Primer	Sequences 5' to 3'	Product Size
VP28	615F	ATG GAT CTT TCT TTC ACT CTT	615bp
	615R	TTA CTC GGT CTC AGT GCC AGA	
VP26	615F	ATG GAA TTT GGC AAC CTA AC	615bp
	615R	TTA CTT CTT CTT GAT TTC GTC	

72°C for 1 min and a final extension at 72°C for 5 min. The amplified products were electrophoresed through 1.5% agarose gel in TBE buffer at 100 V for 1 h. The gel was visualized under UV transillumination (GelDoc, BioRad). Amplified PCR products were recovered and purified using DNA Gel extraction kit (Norgen Biotekcorp, CA) according to the manufacturer's instructions. The purified VP28 and VP26 products were subcloned into pGEM-T Easy vector system (Promega, Madison, WI, USA) according to the manufacturer's instructions and sent to Apical Scientific Sdn Bhd, Selangor, Malaysia for sequencing. Four different clones were sequenced namely VP28Clone_Fi_2, VP28Clone_Lv_4, VP26Clone_Fi_2, VP26Clone_Lv_4.

VP28 and VP26 homology to other sequences in GenBank were identified using BLAST programme (<http://www.ncbi.nlm.nih.gov/BLAST>). Multiple sequence alignment of VP28 and VP26 were performed using ClustalW programme [25]. Neighbor-Joining phylogenetic tree of VP28 and VP26 amino acid sequences were run using neighbor-joining algorithm by Mega 7 programme [26]. Open reading frames (ORFs) and translated protein sequences of VP28 and VP26 gene were found in NCBI ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). Molecular weight and isoelectric point were predicted using ExPASy server [27]. Putative conserved domain (Motif) and superfamily were predicted with [28]. Identical amino acid sequences of VP28 and VP26 with toggle identity were found in Protein Data Bank (PDB). The 3D structure of VP28 and VP26 was predicted based on the homology modelling with SWISS-MODEL [16, 29, 30].

RESULTS

During epizootics of WSSV *F. indicus* and *L. vannamei* were collected from shrimp farms located in the Jazan, Red Sea coast of Saudi Arabia and the infection was confirmed [5]. PCR amplification of VP28 and VP26 genes (615bp) with SA isolates of WSSV infected *F. indicus* and *L. vannamei* and with the primers designed in this study was successful (Fig. 1).

The full-length sequences of SA WSSV isolate of VP28 and VP26 had an ORF of 615 bp and 612 bp encoding 204 and 203 amino acids respectively. (Fig. 2

and 3). The theoretical isoelectric point (pI) and molecular weight (MW) of VP28 were calculated to be 4.76, 22.01 kDa and 9.57 and 19.3 kDa for VP26 respectively. BLASTN and BLASTP for SA isolates of WSSV VP28 sequence showed 99-100% of similarity with other WSSV strains such as Australia MF768985, Bangladesh MF489076, China KY827813, India DQ013883, Ecuador MH090824, Egypt KR083866, South Korea JX515788 and Brazil MF784752. BLASTN and BLASTP for SA WSSV isolate of VP26 sequence resulted in 99% of similarity with other WSSV strains of isolates such as Ecuador MH090824, China KX686117, Brazil MF784752, Australia MF768985, Mexico KU216744, India KU556687, South Korea JX515788 and Egypt KR083866. Nucleotide and translated amino acid sequences of SA isolates of WSSV VP28 and VP26 were compared and the results showed that the sequences were highly conserved. The multiple sequence alignment of VP28 sequences of SA isolates showed to be 100% identical with strains from Australia, Bangladesh, China, India and Egypt, whereas with Brazil, Mexico, South Korea, USA, Indonesia, Japan and Thailand showed a single amino acid (D) sequence variation.

VP26 of SA did not have any dissimilarity in multiple sequence alignment with other known isolates of VP26 amino acid sequences (Fig. 4). Multiple sequence alignment has shown that VP26 from SA has a significant resemblance with known strains; however VP28 SA isolate presented a single different amino acid, as a result of substitution of G to D, positioned at 43rd amino acid, in comparison with the isolates of Brazil, Mexico, South Korea, USA, Indonesia, Japan and Thailand (Fig. 5).

To understand how the SA isolates of VP28 and VP26 genes were related with other known sequences of VP28 and VP26, a molecular phylogenetic analysis was performed. SA WSSV isolates were clustered together with the other known VP28 and VP26 sequences (Fig. 6 and 7). To understanding the phylogenetic relationship of SA isolates of WSSV VP28 and VP26, Neighbor-Joining method was performed with known amino acid sequences. The phylogenetic analysis revealed that VP26 had a greater homology with other isolates of VP26, however, the VP28 tree shown certain subphylum among the isolates, with Egypt and Thailand isolates. In VP28 of Egypt isolates formed a subgroup and Brazil, Mexico, South Korea, USA, Indonesia and Japan clustered together as an additional subphylum. However, the phylogenetic tree showed that Thailand isolate was distant from all other known isolates. In general, phylogenetic analysis of SA WSSV isolates of VP28 and VP26 showed an overall sequence similarity with other known WSSV isolates of the VP28 and VP26.

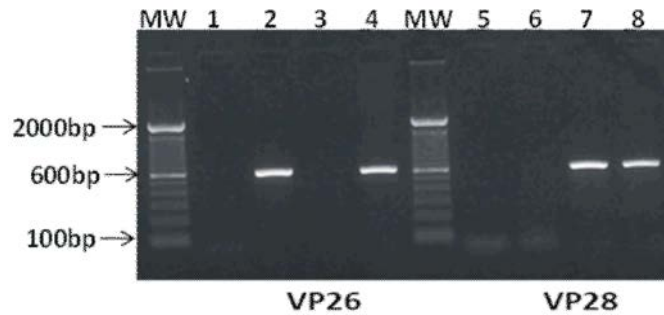


Fig. 1: Agarose gel showing PCR amplification of VP26 and VP28 products from WSSV isolates of Saudi Arabia, in *F. indicus* and *L. vannamei*. MW = Molecular weight maker (Thermo Fisher Scientific, USA, Cat No. 15628019). Lane 1 & 5 = Non template control (Nuclease free water). Lane 2 & 7 = Positive Control. Lane 3 & 6= Template Control (SPF-Specific Pathogen free DNA). Lane 4 & 8= WSSV infected *F. indicus* and *L. vannamei*.

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001 ATG GAT CTT TCT TTC ACT CTT TCG GTC GTG TCG GCC ATC CTC GCC
    MDLSFTLSVVSAILA
046 ATC ACT GCT GTG ATT GCT GTA TTT ATT GTG ATT TTT AGG TAT CAC
    ITAVIAVFIVIFRYH
091 AAC ACT GTG ACC AAG ACC ATC GAA ACC CAC ACA GGC AAT ATC GAG
    NTVTKTIEHTGNIE
136 ACA AAC ATG GAT GAA AAC CTC CGC ATT CCT GTG ACT GCT GAG GTT
    TNMDENLRIPVTAEV
181 GGA TCA GGC TAC TTC AAG ATG ACT GAT GTG TCC TTT GAC AGC GAC
    GSGYFKMTDVSFDS D
226 ACC TTG GGC AAA ATC AAG ATC CGC AAT GGA AAG TCT GAT GCA CAG
    TLGKIKIRNGKSDAQ
271 ATG AAG GAA GAA GAT GCG GAT CTT GTC ATC ACT CCC GTG GAG GGC
    MKEEDADLVITPVEG
316 CGA GCA CTC GAA GTG ACT GTG GGG CAG AAT CTC ACC TTT GAG GGA
    RALEVTVGQNLTFEG
361 ACA TTC AAG GTG TGG AAC AAC ACA TCA AGA AAG ATC AAC ATC ACT
    TFKVWNNTSRKINIT
406 GGT ATG CAG ATG GTG CCA AAG ATT AAC CCA TCA AAG GCC TTT GTC
    GMQMVPKINPSKAFV
451 GGT AGC TCC AAC ACC TCC TCC TTC ACC CCC GTC TCT ATT GAT GAG
    GSSNTSSFTPVSIDE
496 GAT GAA GTT GGC ACC TTT GTG TGT GGT ACC ACC TTT GGC GCA CCA
    DEVGTFVCGTTFGAP
541 ATT GCA GCT ACC GCC GGT GGA AAT CTT TTC GAC ATG TAC GTG CAC
    IAATAGGNLFDMYVH
586 GTC ACC TAC TCT GGC ACT GAG ACC GAG TAA
    VTYSGTETE*

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Fig. 2: Translated nucleotide and amino acid sequences of VP28 gene from Saudi Arabia isolate of WSSV. Conserved region of NNT sequence (motif) is indicated in red color. Total nucleotide length 615 and translated amino acid sequence length 204.

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001 ATGTTTATAATGATTGTATTCAACACACGTGTTGGAAGAAGCGTC
M V I M I V F N T R V G R S V
046 GTCGCTAATTATGATCAGATGATGCGAGTCCCAATTCAAAGAAGG
V A N Y D Q M M R V P I Q R R
091 GCAAAGGTAATGTCAATTCGTGGAGAGAGGTCCTACAATACTCCT
A K V M S I R G E R S Y N T P
136 CTTGGAAAGGTGGCCATGAAGAATGGTCTCTCCGATAAGGACATG
L G K V A M K N G L S D K D M
181 AAGGATGTTTCTGCTGATCTTGTCATCTTACCGTCACAGCCCCA
K D V S A D L V I S T V T A P
226 AGGACTGATCCCGCTGGCACTGGGGCCGAGAACTCTAACATGACT
R T D P A G T G A E N S N M T
271 TTGAAGATCTCAACAACACTGGCGTCGATCTCTTGATCAACGAC
L K I L N N T G V D L L I N D
316 ATTACTGTTTCGGCAACTGTTATTGCGAGAAACATTAAGGGAAAT
I T V R P T V I A G N I K G N
361 ACTATGTCGAACACTTACTTCTCGAGCAAGGACATTAATCTTCA
T M S N T Y F S S K D I K S S
406 TCTTCAAAAATTACCTCATTGACGTGTGCAGCAAATTTGAAGAC
S S K I T L I D V C S K F E D
451 GGCGCAGCCTTCGAAGCTACAATGAACATTGGATTACCTCCAAG
G A A F E A T M N I G F T S K
496 AATGTGATCGATATCAAGGACGAAATCAAGAAGAAGTAA
N V I D I K D E I K K K *

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Fig. 3: Translated nucleotide and amino acid sequences of VP26 gene from Saudi Arabia isolate of WSSV. Total nucleotide length 534 and translated amino acid sequence length 203

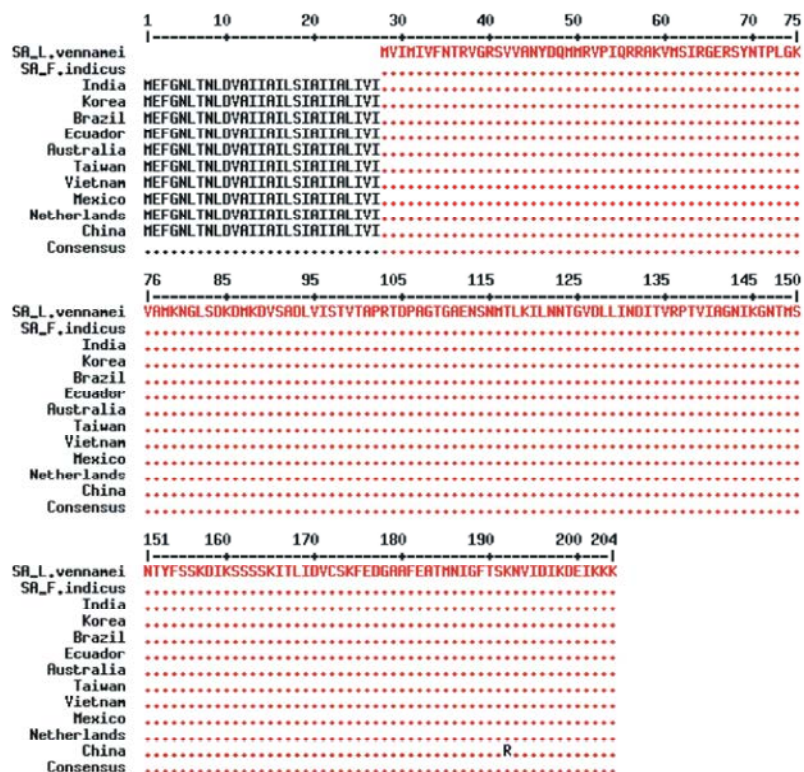


Fig. 4: Multiple sequence alignment of amino acid sequences of VP26 from WSSV infected *F. indicus* and *L. vannamei* in Saudi Arabia with other known isolates (India ABG75924, Korea AF380841, Brazil AUJ79481, Ecuador AWM67210, Australia ATU83474, Taiwan AF272980, Vietnam CAD83838, Mexico ACH61957, Netherland AF173992 and China AA069663). The predicted dissimilarity sequences are indicated single-letter code

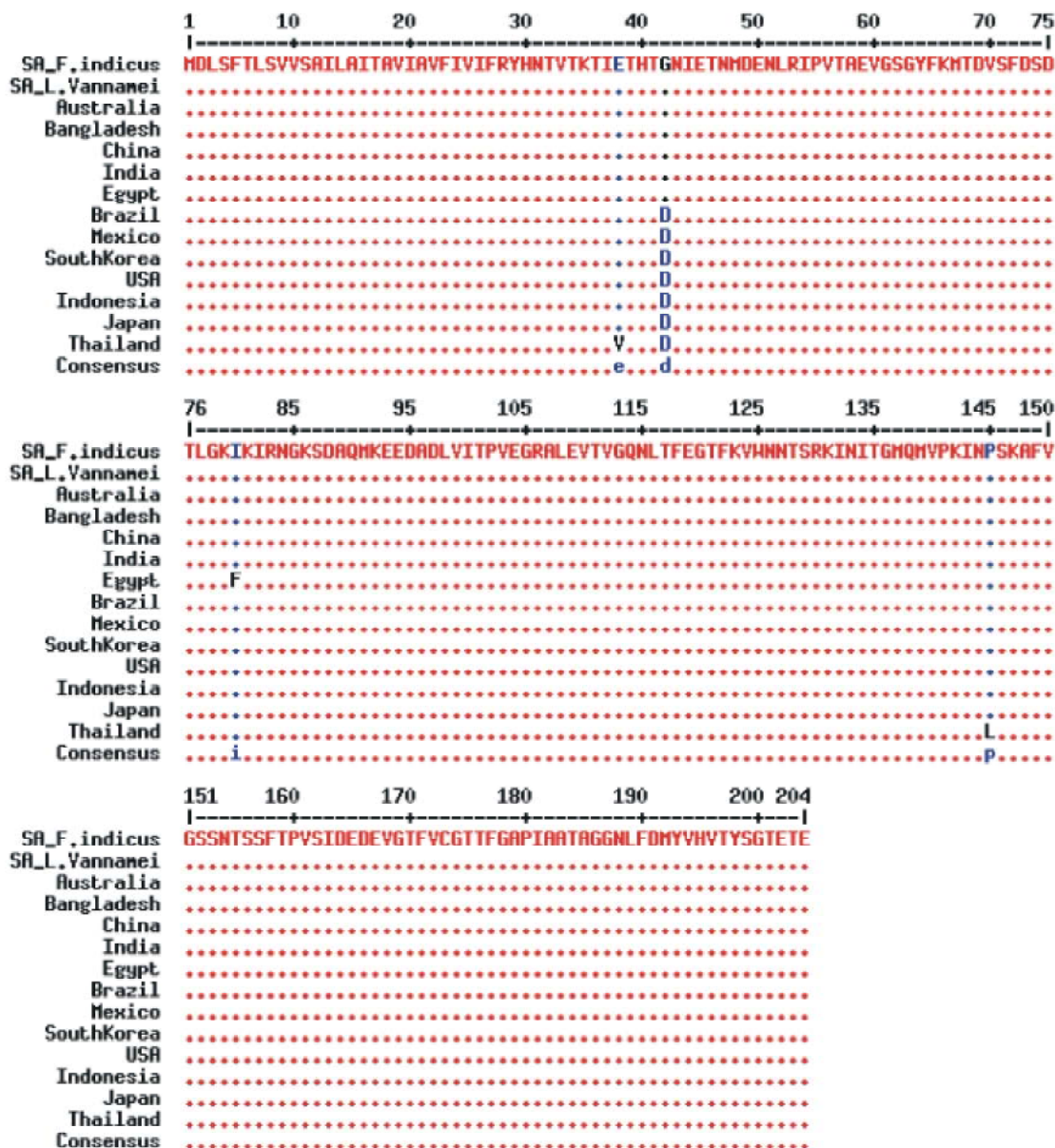


Fig. 5: Multiple sequence alignment of VP28 from WSSV infected *F.indicus* and *L.vannamei* in Saudi Arabia with other isolates (Australia ATU83471, Bangladesh ATE50874, China ALN66419, India AAY40276, Brazil AUR53624, Mexico ALK87217, South Korea AFX59798, USA AAP06670, Indonesia AAP06668, Thailand ABP01348, Japan AAP06670, Egypt ALB0064). The predicted dissimilarity sequences are indicated single-letter code.

VP28 sequence of SA isolates had conserved domain of sequence motif (NNT). Based on the toggle identity to known amino acid sequence of VP28 and VP26 with known crystal structure of VP28 (PDB ID; 2ED6) and VP28 (PDB ID; 2EDM) 3D structure was predicted (Fig. 8A & B and 9A & B). By threading analysis, it was found a single β barrel and α helix, which was protruded

from the β barrel sheet. The extended α helix, which is linked with two amino acid residues, can form the coil to the β barrel core of VP28 predicted structure. The predicted structure of VP26 consists of a nine β barrel sheet with typical antiparallel β strands. The N and C terminal ends together and it forms the β sheet which is extended from both ends.

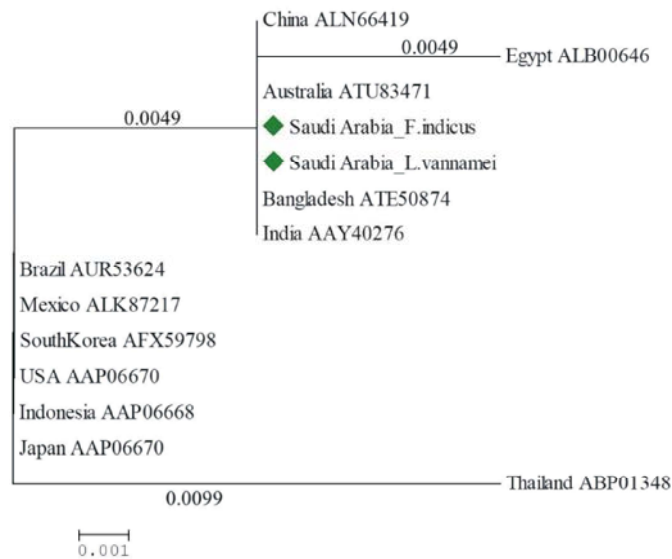


Fig. 6: Molecular phylogenetic analysis of VP28 gene from Saudi Arabia isolates of WSSV infected *F. indicus* and *L. vannamei* by Neighbor-Joining method. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.01972206 is shown. The unrooted phylogenetic tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 14 amino acid sequences. All positions containing gaps and missing data were eliminated. There was a total of 204 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

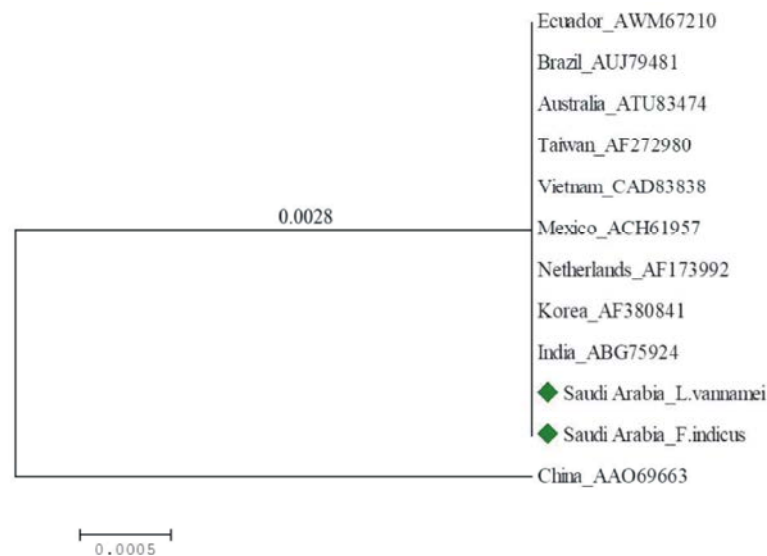


Fig. 7: Molecular phylogenetic analysis of VP26 gene from Saudi Arabia isolates of WSSV infected *F. indicus* and *L. vannamei* by Neighbor-Joining method. The optimal tree with the sum of branch length = 0.00566574 is shown. The unrooted phylogenetic tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There was a total of 177 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

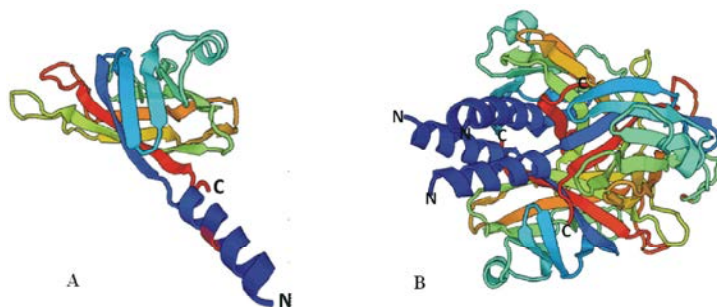


Fig. 8A & B: Predicted 3D structure (ribbon model) of VP28 based on the homology of 2ED6 (PDB ID). The model was predicted and modeled with SWISS-MODEL Homology Modelling. Fig. A: Monomer of VP28 and N and C termini are labeled. Fig. B: Trimer structure of VP28.

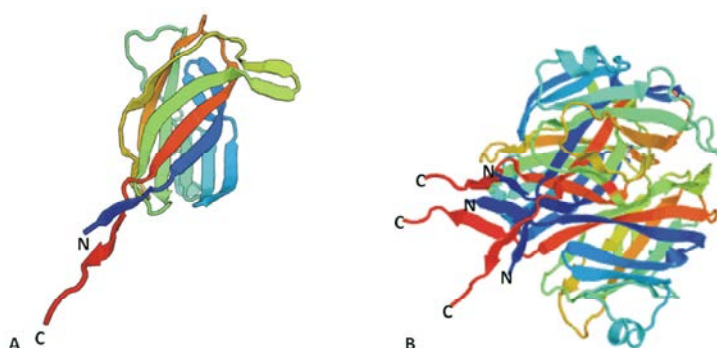


Fig. 9A & 9B: Predicted 3D structure (ribbon model) of VP26 based on the homology of 2EDM (PDB ID). The model was predicted and modeled with SWISS-MODEL Homology Modelling. Fig. A: Monomer of VP28 and N and C termini are labeled. Fig. B: Trimer structure of VP28.

DISCUSSION

Since 90s, WSSV is a serious pathogen for shrimp industry and it can infect the entire population followed by a mass mortality within a short span of time or days. WSSV infection was reported from Saudi Arabia during the period of 2010 in cultured *F. indicus* and 2014 in *L. vannamei* [5, 6]. The WSSV genome contains envelope and nucleocapsid proteins (tegument protein). Recently, a study revealed that VP28 protein is involved in systemic infection; more precisely, VP28 participates in the attachment and penetration into shrimp cells during entry of pathogen to host cell [15, 18]. VP26 is a key factor in morphogenesis and assembly of WSSV and for the development of virions by interacting between the envelope and the nucleocapsid of WSSV [14]. The present study aimed to identify and characterize the important envelope protein VP28 and tegument protein of VP26 from SA WSSV isolates.

SA WSSV isolates of full-length VP28 and VP26 was amplified and it showed significant similarities with other known WSSV isolates, which is consistent with other

reports. Multiple sequence alignment has shown that VP26 has a significant resemblance; however, SA isolate analysis of VP28 confirmed that a single amino acid was different. VP28 of the Mexican WSSV isolate was differed from two Indian isolates through non-conservative substitutions (S > F and D > G), whereas with Chinese isolate it was highly conserved [31]. Previous studies demonstrated that trivial genomic dissimilarities were found among the WSSV isolates and it's typically based on the different geographic locations [4, 9]. Recently, the complete genome sequence of an Indian isolate of WSSV was obtained and it was the smallest among the full available genome of WSSV isolates; as a result many genes and their annotations may have slight mutations [10].

The present study of phylogenetic relationship evidenced that among the WSSV isolates of VP28 amino acid sequences occurred minor substitutions and, as a result, Egypt and Thailand isolates of VP28 showed divergence by subsiding as subcluster in the phylogenetic tree. Similarly, the Bangladesh WSSV isolates of VP28 showed genetic divergence, which had a

variation at 500th nucleotide of VP28 coding gene [32]. Previous studies showed that a single amino acid substitution was found in South Korean isolates of WSSV VP28, which implies that a single strain of WSSV isolates has been circulating in this country and it may have a close genetic identity to other Asian isolates [33]. Molina-Garza *et al.* [31] demonstrated that VP28 fragment of WSSV isolates may undergo with non-conserved or conserved replacements of amino acid sequences. The present study resulted in di-phyletic clade of VP28 among the isolates, which could be attributed to point mutation or replacement of a single amino acid sequence and the virus might empower the virulence for the hosts. Phylogenetic analysis of VP26 sequences grouped WSSV isolates into single clusters, indicating a possible common origin of these isolates or may not undergo any mutations in amino acid sequences.

The predicted 3D structure of SA isolates of WSSV VP28 indicated composed extended α helix, core β barrel and VP26 composed of nine β barrel sheets with typical antiparallel β strands which are consistent with earlier report of crystal structure of VP26 and VP28 [16]. The families of structural proteins of among WSSV isolates are conserved and these domains are essential for entry into cells of the host. In this study, we found a putative conserved domain which codes for VP28 protein superfamily of WSSV.

CONCLUSION

Our data support the hypothesis that presently identified SA isolates of WSSV VP28 and VP26 genes highly conserved and might even have similar role such as attachment, penetration into host cells and development of virions. Single amino acid of VP28 was substituted, which might be a clear example for the development of virulence against the host. Phylogentic relationship of VP28 fell into subgroup which was evidence that the viral gene undergoes genetic variation for the endurance against the host. Structural similarities reveal that VP28 and VP26 functional implications. To understand the mechanism of VP28 and VP26, the present study is useful for developing the antigenic determinants as a remedial approach for the native strain. Further, this study will help in developing a strain specific diagnostic method and to understand the spread of disease.

GenBank Accession Numbers:

MH425505, MH425506, MH425507 and MH425508.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: This article does not contain any studies with animals performed by any of the authors.

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