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Cloning and evaluation of protein structure of *Vp28* gene involved in white spot syndrome virus

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Abstract

The White spot disease decrease performance of the shrimp. Despite the extensive measures taken to control it, this disease is still a major health problem in shrimp farms (Satheesh Kumar, Ananda Bharati *et al.* 2013). So use the best, most accurate and most cost-effective method for the detection and prevention of this disease is essential. The most important are the viral envelope protein Vp28 a key role in systemic infection and disease in white spot shrimp (Rosenberry 2003). Virus VP28 protein antigenicity has good properties. This study has tried to transfer genes into Microalgae pave the way for edible vaccines for shrimp. Samples were prepared from infected fields of Abadan and Razi Vaccine and Serum Research Institute and the Central Regional Branch (Arak) were transferred. DNA was extracted from the samples. After evaluation of bioinformation sequence corresponding to the gene bank, placing the position of primers were designed to shear Bam HI and Eco RI. The sequences of the PCR amplification of DNA samples infected with the vector pTZ57R / T and the vector pET28a were cloned and sequenced. Bioinformatic sequence structure was investigated. Translated into protein fragment sequencing revealed that the protein consists of 204 amino acid sequence of this gene have 13 beta strand, 2 alpha helical and 7 turn. Also the position of the protein within the cell, located outside the cell and the protein was detected in the plasma membrane.

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Introduction

Since 1992, a viral syndrome that usually white spot disease (White Spot Disease (WSD)) is called, Due to the casualties farms, shrimp overshadowed all the issues and causes heavy damage in the field (Sánchez-Paz 2010). Common cause white spots or White Spot Syndrome Virus in Iran viral genus Whispovirus in the family baculovirus name or part of a new family name was Whispoviridae (Van Hulten 2001). The disease causing white spots on the carapace of shrimps and severe mortality within 2 to 7 days to reach 70 to 100 percent is specified. Symptoms of the disease is easily visible in juveniles and adults. Infected shrimp too fast and low appetite lethargic and show signs of disease (Haq, Vignesh *et al*. 2012). Disease in Asian countries including China, Thailand, Malaysia, Singapore, Vietnam, Taiwan, India and Latin American countries like Guatemala, Nicaragua, Mexico and America have been reported. It Chuybdh Abadan in Iran in 1381, more than ten million dollars in damage to growers entered (Afsharnasab, Mortezaei *et al*. 2009).

Virion the symmetrical oval to rod-shaped zonate virus particles with a diameter between 120 and 150 nm and a length of about 270 to 290 nm. Virion-like tail at one end there. The virus has a genome of circular double-stranded DNA with a length of about 305 thousand pairs in which there are 181 ORF (Oidtmann B 2011). White spot syndrome virus, infects shrimp and other crustaceans. Virion proteins of the virus in at least five of the original. Three of them, namely, VP26, VP24 and VP15 is a rod-shaped nucleocapsid. The other two VP28 (KDa 30 ~) and VP19 are present in the membrane of the virus. None of the five major structural proteins of the virus are not glycosylated (Van Hulten 2001). This property is unusual among animal viruses is a membrane. Although the white spot virus-like morphology baculovirus insects, but these two aren't the sequences of amino acids linked together. Even more homology with genes of the virus are eukaryotic genes. The white spot virus sequences with different viruses. Virus VP28 protein is a major immunogen component of the vp28 gene (gene name is wsv421) of vp28 gene sequence is very similar to other species of white spot virus (Sangsuriya, Huang *et al*. 2014). The purpose of this study demonstrate understanding of the secondary structure and arrangement of these proteins is important when entering the host cell can be based on a better solution for dealing with this disease. The purpose of this study demonstrate understanding of the secondary structure and arrangement of these proteins during entry into the host cell, accordingly, it can be a better strategy for dealing with this disease.

Materials and methods

DNA extracted from infected shrimp

White spot virus-infected shrimp farms Abadan (Research Institute of Aquaculture south), Razi Vaccine and Serum Research Institute and the Central Regional Branch (Arak) were transferred. Sections carapas, pleopod and shrimp eyes the location is perfect for wssv viruses with scissors and forceps were isolated. Crucible DNA viruses were homogenized in PBS using a separate procedure proteinase K and phenol / chloroform extraction (Sambrook 1989). In this method, the precipitate obtained from shrimp tissues containing the virus suspension in PBS were centrifuged for 30 min 4500 rpm. Having discarded the supernatant, Lysis Buffer (sodium dodecyl sulfate 10%, proteinase K, EDTA ½ M, Tris chloride 1M, Nacl 5M) added and 3 h incubation and (every 15 min moved in) after cell lysate, DNA with phenol / chloroform extraction and µL 30 buffer TE with PH = 8 dissolved in 15 min at 35 ° C in Feb incubation and the DNA purified with the aid of optical absorption at a wavelength of 260/280 nm measured will.

Primer

Using vp28 gene sequences available in gene banks, forward and reverse primers were designed as follows.

5′-ATAGGATCCAGGATGGATCTTTCTTTCACT-3′ : Forward

5′-ATAGAATTCTTACTCGGTCTCAGTGCCA-3′ : Reverse

Primers containing sequences are for the detection of

enzymes EcoRI and BamHI.

PCR

Materials for PCR were used that are listed in Table 1. Schedule table (2) PCR was performed.

Coloning

The vp28 gene amplified PCR products on 1% agarose gel was used for confirmation. After seeing the band, the PCR products using Silica Bead DNA Gel Extraction Kit Company Fermentas (Vilnius, Lithuania) was purified. The vp28 gene purified enzyme Fermentas Inc. T4DNA Ligase vector PTZ57R/T cloned, Then DH5α bacteria susceptible to thermal shock approach was taken. To confirm the recombinant transfer vector, the bacterial colonies grown from colony PCR was performed.

Bioinfomation

Sequences cloned, sequenced and the sequences were analyzed bioinformatic. Sequences were translated by expasy site. 204 amino acid using the protein database structure were investigated. Center for Biological Sequence analysis of the protein sequence database of the Technical University of Denmark, took the place of its position in the cell was determined.

Results and discussion

Chromosomal DNA concentrations obtained from the WSSV virus 1500 ng/µl respectively. This DNA was used as template for amplification of vp28 gene. To confirm gene amplification, the PCR product was checked on a 1% agarose gel. The size of amplified gene fragment of approximately 615 bp was indicative comparison (Fig. 1).

Table 1. The amount of material required for the PCR reaction.

Material	Amount
10X PCR Buffer	2.5 µl
MgCl2 (50mM)	1 µl
dNTPs (10mM)	1 µl
Forward Primer	1 µl
Reveres Primer	1 µl
Taq DNA polymerase	0.5 µl
Tempelet	3 µl
D.D.W	15 µl
Final volume	25 µl

Table 2. Thermal cycler devices for a given application in a PCR reaction.

cycle	Temperature(°c)	Time(minute)	Number of cycle
1	95	3	1
2	95	1	34
	57	1	
	72	1	
3	72	10	1
4	4	hold	-

The colonies obtained from the recombinant transfer vector PTZ57R / T into competent cell DH5α bacteria susceptible to 5 colonies were selected randomly. Two colonies from five colonies containing recombinant vector PTZ57R / T (Fig. 2).

Vp28 gene and protein sequence analysis from Network, The following results were obtained; According to Figure 3, the structure of this protein is 204 amino acids in length that is, 13 beta strand, the two helices seen. Importantly, in this structure, there are 7 turn that plays an important role in the three-

dimensional structure of proteins and post-translational modifications of proteins are also rotated.

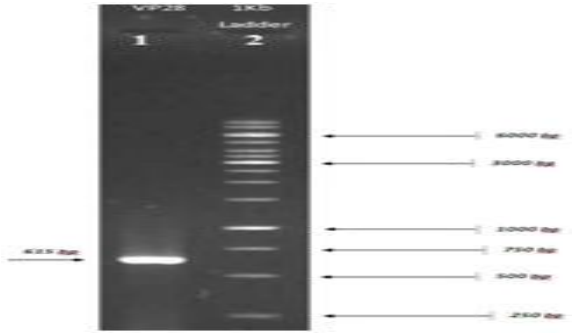

Fig. 1. Gene size on 1% agarose gel, Well 1:Vp28 gene Well 2: DNA ladder.

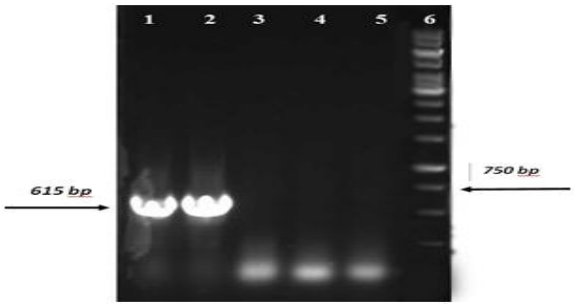

Fig. 2. PCR from colonies transformed with the vector PTZ57R / T vp28 gene on 1% agarose gel, 1&2; Positive colonies (containing the vp28 gene), 3&4&5; Negative colonies (no gene vp28), 6; Ladder 1Kb.

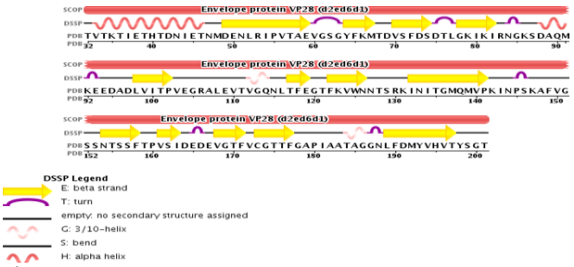

Fig. 3. VP28 protein secondary structure that is represents different parts of the protein.

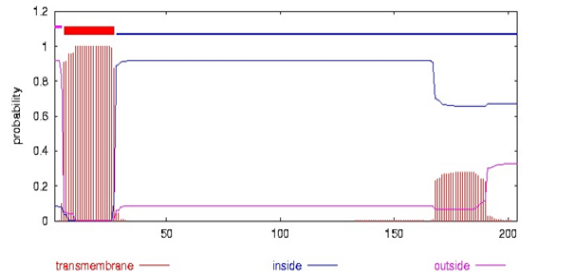

Fig. 4. No arrangement VP28 protein amino acids in different parts of the cell.

Vp28 gene and protein sequence analysis from Network, The following results were obtained; According to Figure 4. In this analysis, how the placement, number and amount of amino acids in proteins within the cell, outside the cell and plasma membrane shows. According to Figure 4 from amino acid 1 to 4 are placed in the extracellular space. Amino acids are numbered from 5 to 27 in the plasma membrane into the cell. And amino acids 28 to 204 are located inside the cell.

References

Afsharnasab M, Mortezaei R, Yegane V, Kazemi B. 2009. Gross sign, histopathology and polymerase chain reaction observations of white spot syndrome virus in shrimp specific pathogen free Litopeneaus vannamei in Iran. Asian Journal of Animal and Veterinary Advances **4(6),** 297-305. http://dx.doi.org/10.3923/ajava.2009.297.305

Haq M, Vignesh R, Srinivasan M. 2012 Deep insight into white spot syndrome virus vaccines: A review. Asian Pacific Journal of Tropical Disease, **2(1)**, 73-77, http://dx.doi.org/10.1111/are.12045

Oidtmann B. 2011. "White spot syndrome virus (WSSV) concentrations in crustacean tissues: a review of data relevant to assess the risk associated with commodity trade." Transbound Emerg **58,** 82-469. http://dx.doi.org/10.1007/s11033-013-2805-y

Rosenberry B. 2003. "World Shrimp Farming 2003." San Diego, California, Shrimp News International. http://dx.doi.org/10.1111/j.17497345.2005.tb00328.x

Sambrook J. 1989. "Fritsch. EF and Maniatis. T.(1989) Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory, Cold Spring Harbor, NY **267,** 9289-9293.

Sánchez-Paz A. 2010. "White spot syndrome virus: an overview on an emergent concern." Veterinary

research **41,**43.
http://dx.doi.org/10.1051/vetres/2010015

Sangsuriya P, Huang JY, Chu Y F, Phiwsaiya K, Leekitcharoenphon P, Meemetta W, Lo C. (2014). Construction and application of a protein interaction map for white spot syndrome virus (WSSV). Molecular & Cellular Proteomics, **13(1)**, 269-282.
http://dx.doi.org/10.1074/mcp.M113.029199

Satheesh Kumar S, Ananda Bharati R, Rajan J, Alavandi S, Poornima M, Balasubramanian C, Ponniah A, 2013. "Viability of White spot syndrome virus (WSSV) in sediment during sun-drying (drainable pond) and under non-drainable pond conditions indicated by infectquivity to shrimp." Aaculture.
http://dx.doi.org/10.1016/j.aquaculture.2013.04.001

Van Hulten 2001. "The white spot syndrome virus DNA genome sequence." Virology **286,** 7-22.
http://dx.doi.org/10.1006/viro.2001.1002