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Time-Mortality Relationship Between Dna Vaccination of Recombinant Viral Proteins (Vp19 and Vp28) Against WSSV in Marine Ornamental Squat Shrimp *Thor amboinensis*

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Abstract: Viruses are by far the most abundant 'life forms' in the oceans and are the reservoir of most of the genetic diversity in the sea, with their numbers reaching about 10 billion per liter. The present study revealed that, the protection from WSSV infection in shrimp by the vaccination with recombinant viral proteins could be accomplished by the prevention of entry of WSSV due to the shrimp immune system activated by recombinant protein vaccines the experimental studies were performed using the marine ornamental shrimp species as an alternate host for WSSV. In this present study, marine ornamental shrimp species, Squat shrimp *THOR AMBOINENSIS* were orally fed with DNA vaccination of recombinant viral proteins (VP19 and VP28), over expressing VP19 and VP28 and found to be tolerant to WSSV following challenge by oral delivery vehicle. The experimental 1 and 2 shrimp selected were divided into three groups and each group was further divided into another experimental setup of ornamental shrimp species for VP19 and VP28 recombinant protein vaccination trials. After the vaccination, the shrimps were challenged by the injection of specific WSSV dilution, except for the negative control that was mock infected.

Key word: DNA vaccination · Ornamental shrimp · WSSV

INTRODUCTION

Viruses are by far the most abundant 'life forms' in the oceans and are the reservoir of most of the genetic diversity in the sea. Viruses are the simplest forms of life yet they play a crucial role in regulating planetary processes. WSSV is a large DNA virus with five major proteins with expected sizes of 28 kDa (VP28), 26 kDa (VP26), 24 kDa (VP24), 19 kDa (VP19) and 15 kDa (VP15). VP28 and VP19 are associated with the virion envelope and the others are associated with the nucleocapsid [1]. The present study revealed that, the protection from WSSV infection in shrimp by the vaccination with recombinant viral proteins could be accomplished by the prevention of entry of WSSV due to the shrimp immune system activated by recombinant protein vaccines

MATERIALS AND METHODS

Sample Collection and Extraction of DNA: Healthy marine ornamental Squat shrimp sub-adults (2–5 g body weight) were collected from a Gulf of Mannar islands located near in Mandapam, Tamil Nadu, India. Healthy shrimp were maintained in 200 - 500-l fiberglass tanks with an airlift biological filter at room temperature (27–30 ?C), with salinity between 25 and 30 ppt. Natural seawater was used in the experiments. Then the DNA was extracted using standard protocol.

PCR Condition: The primers are constructed (WSSV 530 F/530 R) in Marine virology laboratory, CAS in Marine Biology, Parangipettai and had published earlier [2]. The primers designed using Primer expression software (Version 2.0) and registered in NCBI in under accession number JX198549.The WSSV 419 protein gene encoding hypothetical protein (about ~530bp) was polymerized using P1 and P2 primer set sequences assembled from conserved region of WSSV-419 gene. The amplification series proceeded was about 94°C for 5min in the first cycle then the process continues as 94°C for 30sec, 55°C for 30sec, 68°C for 60sec and 68°C for 7min. The resultant polymerized DNA (6-10µl) was electrophoreses with 2% agarose gels mixed with ethidium bromide (1µgml^{-1}).

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Production of Recombinant Proteins VP19 and VP28: The recombinant plasmids pHCE VP19 and pHCE VP28 were obtained from a previous study [3]. Both VP19 and VP28 from the E. coli BL21 (DE3) transformed with pHCE VP19 and pHCE VP28 were produced by culturing at 37ºC for 8h in LB medium containing 100 ìg/ml ampicillin respectively. For the harvest of recombinant protein, cultured bacterial cells were centrifuged at 6,000 ×g for 10 min at 4ºC. Bacterialellets were thoroughly resuspended in 1ml of PBS and then centrifuged at 9,800 ×g for 4 min at 4ºC. The harvested cell was disrupted by sonication in PBS buffer. The expression of VP19 and VP28 proteins was further confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [4].

Recombinant Viral Proteins, rVP19 and rVP28 vaccination by Oral Feeding: Commercial pellets weighing approximately 36g were mixed with 18ml (200ìg/ml of protein) of recombinant proteins VP19 and VP28. The feed pellets were mixed with recombinant proteins and coated with 2.8il of Freund's Complete Adjuvant (FCA; Sigma, St. Louis, MO, USA) per gram of commercial pellet and incubated on ice to allow the absorption of suspension with FCA to prevent dispersion of the recombinant protein suspension in the water. In the vaccination experiments, group of 30 shrimps were vaccinated by feeding feed pellets at 5% of body weight for 7 days, as indicated in Table 4.3. During the vaccination of the test groups (experimental 1 (VP19) and experimental 2 (VP28), the commercial pellets without protein vaccine were fed to the positive and negative control groups. The experimental 1 and 2 shrimp selected were divided into three groups and each group was further divided into another experimental setup of ornamental shrimp species for VP19 and VP28 recombinant protein vaccination trials. In each group, the first subgroup was fed with the commercial pellets with protein vaccine coated with the expressing VP19 and VP28, the second subgroup with the commercial pellets without protein vaccine were fed to the positive and the third subgroup was fed with the PBS-coated pellets to the negative control groups respectively. After the vaccination, the shrimps were challenged by the injection of specific WSSV dilution, by in vivo titration, except for the negative control that was mock infected and the time mortality was recorded up to 100%.

RESULT AND DISCUSSIONS

The PCR result shown The band size was identified in ~530bp without any overlapping bands that makes clear visualization of bands in the gel.The recombinant proteins vaccinations of squat shrimp *THOR AMBOINENSIS* were carried out using rVP19 and rVP28 as protein vaccines by oral delivery. rVP28, rVP19, FCA control, host control and positive control groups were challenged by the injection of 2×10^2 WSSV dilutions. The negative control was mock challenged using PBS. During the challenge, shrimps were fed with C.P feed pellets. The positive and FCA control groups showed 100% cumulative mortalities at 37 days after the challenge. The host control group showed 100% mortality at 12 days later than that of positive and FCA control groups owing to the adjuvant effect of bacterial

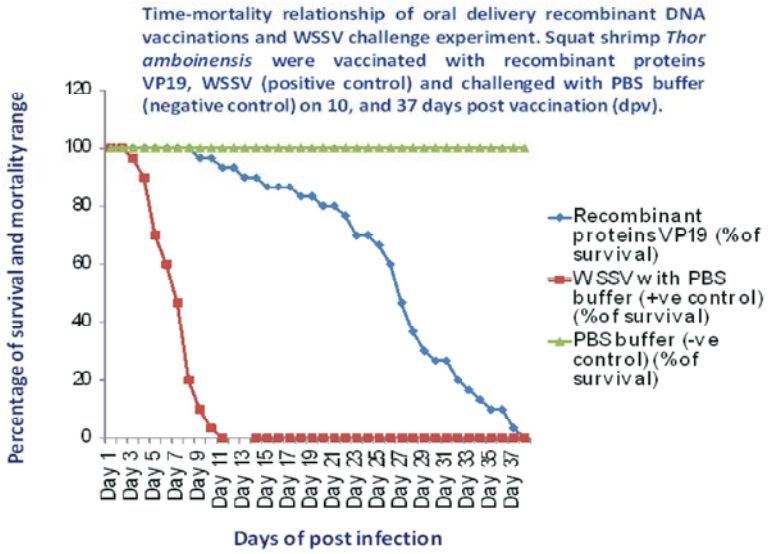

Fig. 1: Time-mortality relationship of oral delivery recombinant protein vaccination.

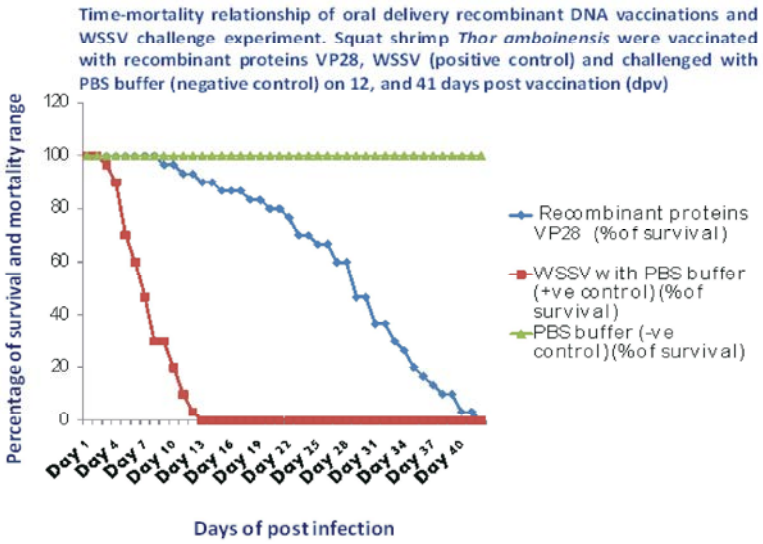

Fig. 2: Time-mortality relationship of oral delivery recombinant protein vaccinations.

host cells. The cumulative mortalities at 37 and 41 days after the challenge with WSSV were determined and groups vaccinated with rVP19 and rVP28 showed cumulative moralities of 96.66% at 10 day, comparing WSSV with PBS buffer control group (VP19) and 93.33% at 12 day for VP28 respectively. The cumulative mortalities for vaccinated groups were significantly lower as compared with control groups. rVP28 showed a better protective efficacy against WSSV in shrimps than rVP19 vaccinated experiment.

The vaccination by recombinant viral proteins (VP19 and VP28) significantly increased the survival ratio of ornamental squat shrimp *THOR AMBOINENSIS* compared with that of control groups showed in the fig 1and 2. Recently, plasmid DNA vaccines using VP28 envelope protein and other envelope proteins as an antigen were injected in black tiger shrimp, resulting in resistance that was effective for at least one month post vaccination [5].

CONCULSION

The obtained results could explain the decrease of cumulative mortalities by the vaccination with recombinant viral proteins by oral methods.

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