Fish & Shellfish Immunology (2008) **24**, 467–478

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/fsi

Immunological responses of *Penaeus monodon* to DNA vaccine and its efficacy to protect shrimp against white spot syndrome virus (WSSV)

S. Rajesh Kumar, V.P. Ishaq Ahamed, M. Sarathi, A. Nazeer Basha, A.S. Sahul Hameed*

Aquaculture Biotechnology Division, Department of Zoology, C. Abdul Hakeem College, Melvisharam-632 509, Vellore District, Tamil Nadu, India

Received 21 September 2007; revised 23 December 2007; accepted 3 January 2008
Available online 11 January 2008

KEYWORDS
White spot syndrome virus;
DNA vaccine;
Penaeus monodon;
VP28;
Superoxide dismutase;
Prophenoloxidase;
Superoxide anion;
SISK cell line

Abstract White spot disease is an important viral disease caused by white spot syndrome virus (WSSV) and is responsible for huge economic losses in the shrimp culture industry worldwide. The VP28 gene encoding the most dominant envelope protein of WSSV was used to construct a DNA vaccine. The VP28 gene was cloned in the eukaryotic expression vector pcDNA3.1 and the construct was named as pVP28. The protective efficiency of pVP28 against WSSV was evaluated in *Penaeus monodon* by intramuscular challenge. *In vitro* expression of VP28 gene was confirmed in sea bass kidney cell line (SISK) by fluorescence microscopy before administering to shrimp. The distribution of injected pVP28 in different tissues of shrimp was studied and the results revealed the presence of pVP28 in gill, head soft tissue, abdominal muscle, hemolymph, pleopods, hepatopancreas and gut. RT-PCR and fluorescence microscopy analyses showed the expression of pVP28 in all these tissues examined. The results of vaccination trials showed a significantly higher survival rate in shrimp vaccinated with pVP28 (56.6–90%) when compared to control groups (100% mortality). The immunological parameters analyzed in the vaccinated and control groups revealed that the vaccinated shrimp showed significantly high level of prophenoloxidase and superoxide dismutase (SOD) when compared to the control groups. The high levels of prophenoloxidase and superoxide dismutase (SOD) might be responsible for developing resistance against WSSV in DNA vaccinated shrimp.

Introduction

White spot syndrome virus (WSSV) is an important viral pathogen and is responsible for huge economic losses in shrimp culture worldwide [1,2]. White spot syndrome was

* Corresponding author. Tel./fax: +91 4172 269487.
E-mail address: cah_sahul@hotmail.com (A.S. Sahul Hameed).

1050-4648/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2008.01.004

first recognized in 1992–1993 in North East Asia [3,4] and has spread throughout most shrimp culture areas of the Indo-Pacific. Cumulative mortalities in infected populations may reach 100% within 2–10 days of the onset of clinical signs [4,5]. It affects penaeid shrimp such as Chinese shrimp (*Fenneropenaeus chinensis*), Kuruma shrimp (*Marsupenaeus japonicus*), black tiger shrimp (*Penaeus monodon*) and other crustaceans, such as salt, brackish and fresh water crayfishes, crabs and lobsters [6–8]. Any practical method to eradicate or inactivate WSSV in the culture systems would have enormous practical benefits for the shrimp farmers and hatchery operators. Conventional control strategies such as improvement of environmental conditions, stocking of specific pathogen-free shrimp post-larvae and augmentation of disease resistance by oral immunostimulants, are currently employed to control WSSV infection [9,10]. However, due to the extreme virulence of this virus and its wide host range, control and prevention are difficult [1,11,12]. To control the infection of WSSV in the culture system, the development of vaccines against WSSV would be desirable. Since shrimp lack a true adaptive immune response system and rely solely on innate immune responses, the possibility of vaccinating shrimp is not feasible. However, some studies have reported the protection of shrimp using either inactivated pathogens or recombinant proteins against bacterial [13–15] or viral pathogens [16–21] suggesting that protective responses can be inducible [22,23].

There are several recombinant proteins effectively used to vaccinate shrimp against WSSV. Injections with r-VP26 or r-VP28 protein induced resistance against WSSV [11,20,24]. The protection conferred by these recombinant protein vaccines has been found to be short lived. Hence, long-term antigen expression through genetic immunization could be a useful strategy to adopt against viral diseases.

Current vaccine research is oriented towards the replacement of conventional recombinant protein vaccines with new more effective and safer approaches, such as DNA vaccines. Immunization with antigen-encoding plasmid DNA can elicit very strong and long-lasting humoral and cellular immune responses in vertebrates. This approach also offers economic, environmental and safety advantages, which are particularly attractive for the aquaculture industry [25,26]. Several reports have demonstrated the effectiveness of DNA vaccination in fish against viral infections, including infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHS) [27,28]. The expression of the exogenous reporter gene, β galactosidase, in shrimp tissues following intramuscular injection of CMV promoter based plasmid DNA construct has been demonstrated earlier [29]. This observation encouraged us to explore the possibility of vaccinating shrimp with plasmid DNA encoding viral genes.

The aim of the present study was to construct a DNA vaccine using VP28 gene of WSSV in pcDNA3.1 and to evaluate its protective efficiency in black tiger shrimp (*P. monodon*), against WSSV by experimental infection. The present study also measures the changes in the level of important immunological parameters such as prophenoloxidase, superoxide dismutase and superoxide anion in the hemolymph of VP28 DNA vaccinated and control shrimp. The study also correlated the changes in the level of immunological parameters with the survival percentage and protective efficacy of DNA vaccination (pVP28) against WSSV in shrimp.

Materials and methods

Collection of experimental animals

Shrimp, *P. monodon* (7–10 g body weight), were collected from commercial grow-out ponds and were maintained in 1000-l fibre glass tanks with air-lift biological filters at room temperature (27–30 °C) with salinity between 20 and 25 ppt. Natural seawater was used in all the experiments. It was pumped from the Bay of Bengal, Chennai. The seawater was first chlorinated by treating it with sodium hypochlorite at the concentration of 25 ppm and then dechlorinated by vigorous aeration, before being passed through a sand filter and used for the experiments. The animals were fed with artificial pellet feed (CP feed, Thailand). Temperature and pH were recorded; salinity was measured with a salinometer (Aquafauna, Japan) and dissolved oxygen was estimated by the Winkler method. The animals were kept in these tanks for 7 days for acclimatization prior to the experiments. From the experimental animals, five from each group were randomly selected and screened for WSSV by polymerase chain reaction (PCR) using the primers designed by Takahashi et al. [30]. Only animals shown to be healthy were used for the experiments.

Preparation of viral inoculum

WSSV infected *P. monodon* with prominent white spots were collected from shrimp farms. WSSV infection in shrimp was confirmed by PCR analysis of DNA isolated from gills as described elsewhere [31]. The hemolymph was drawn directly from the heart of infected shrimp using sterile syringes followed by centrifugation (3000 × g for 20 min at 4 °C). The supernatant fluid was then recentrifuged (8000 × g for 30 min at 4 °C) and the final supernatant fluid was filtered through a 0.2 μm filter (Millipore Corporation, Bedford, MA, USA). The filtrate was then stored at −80 °C for infectivity studies.

Extraction of WSSV-DNA

WSSV-DNA was extracted from the viral suspension following the method described by Lo et al. [32]. Briefly, the viral suspension was mixed with an appropriate amount of digestion buffer (100 mM NaCl, 10 mM Tris–HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulphate and 0.1 mg ml^{-1} proteinase K). After incubation at 65 °C for 2 h, the digests were deproteinized by successive phenol/chloroform/isoamyl alcohol extraction and DNA was recovered by ethanol precipitation and dried. The dried DNA pellets were resuspended in TE buffer and used as a template for PCR amplification of the VP28 gene of WSSV.

Construction and preparation of DNA vaccine

The gene encoding for VP28 was amplified from WSSV genomic DNA by the polymerase chain reaction (PCR) with