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Aquaculture 253 (2006) 98–104

Aquaculture

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Neutralization of white spot syndrome virus of shrimp by antiserum raised against recombinant VP28

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Received 20 February 2005; received in revised form 15 July 2005; accepted 25 July 2005

Abstract

An attempt was made to neutralize white spot syndrome virus of shrimp using the antiserum against recombinant VP28 protein of WSSV. The dilutions of virus stock ranged from 1×10^1 to 1×10^8 were used to determine the dilution resulting in 90–100% mortality in *Penaeus monodon* by intramuscular injection of WSSV. A constant amount of WSSV (at 1×010^4) was incubated with various antiserum concentrations and injected into shrimp. No shrimp died in the negative control injected only with NTE buffer. The shrimp in the positive control, which were injected with WSSV only showed 100% mortality at 10-day post infection (d p.i.). The WSSV preincubated with preimmune serum caused 100% mortality of shrimp at 10 d p.i. None of the shrimp injected with the preimmune serum only died. When the WSSV was preincubated with two or one time diluted anti r-VP28, shrimp mortality was 100% and 75%, respectively, at 50 d p.i. apparently the anti r-VP28 at these dilutions is not able to neutralize the WSSV. When the WSSV was preincubated with undiluted anti r-VP28, none of the shrimp died, indicating that WSSV can be neutralized by the anti r-VP28 in a dose-dependent manner.

Keywords: *Penaeus monodon*; WSSV; Recombinant VP28; Antiserum; Neutralization assay

1. Introduction

White spot disease is an important viral disease caused by white spot syndrome virus (WSSV) and is responsible for huge economic loss in the shrimp culture industry worldwide. Recent studies on individual genes and analysis of the complete genome sequence have resulted in transfer of this virus to a new virus family *Nimoviridae* and genus *Whispovirus* (Tsai et al., 2000; van Hulten et al., 2001a; Liu et al., 2001; Mayo, 2002). The virions contain one nucleocapsid with a typical striated appearance and five major and at least 13 minor proteins (Huang et al., 2002; van Hulten et al., 2001a,b, 2002). The *VP28* gene encoding an envelope protein (VP28) of WSSV was amplified from WSSV-infected shrimp from Malaysia and recombinant VP28 protein (r-28) was

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doi:10.1016/j.aquaculture.2005.07.032

expressed in *Escherichia coli* and used as an antigen for production MAbs, and three murine MAbs selected were able to recognize viral VP28 protein as well as r-28 on Western blot (Liu et al., 2002). WSSV protein of 27.5 kDa has been expressed as a histidine tag fusion protein in *E. coli* and antiserum from a rabbit immunized with the recombinant protein recognized the 27.5 kDa viral envelope protein of WSSV isolated from different geographical regions (You et al., 2002). The genes encoding VP28 and VP22 were expressed in *E. coli* and antisera raised against these proteins, and temporal analysis showed that these two genes might be late genes (Zhang et al., 2002a,b). Yoganandhan et al. (2004) produced polyclonal antibodies against recombinant VP28 protein of WSSV in rabbit.

Injections with recombinant proteins of rVP26 or rVP28 induced a higher resistance, with relative percent survival values of 60% and 95%, respectively, in the shrimp on the 30th day post-vaccination and this study indicates the possibility of vaccination of shrimp with recombinant proteins against WSSV (Namikoshi et al., 2003). Witteveldt et al. (2004a) used two WSSV envelope recombinant proteins of VP19 and VP28 to evaluate their potential to vaccinate shrimp against WSSV and the results show that protection can be generated in shrimp against WSSV using its structural proteins as a subunit vaccine. In another experiment, shrimp were fed food pellets coated with inactivated bacteria overexpressing two WSSV envelope proteins, VP19 and VP28, and results showed a significantly lower cumulative mortality in vaccinated shrimp compared to control groups (Witteveldt et al., 2004b). These experiments open the way to new strategies to control viral diseases in shrimp and other crustaceans. The present study was carried out to neutralize the WSSV using the antiserum against recombinant VP28 protein of WSSV.

2. Materials and methods

2.1. Preparation of viral inoculum

WSSV-infected shrimp, *Penaeus monodon*, with prominent white spots were collected from shrimp farms located near Nellore, India. Hemolymph was drawn directly from the heart of infected shrimp using sterile syringes. The pooled hemolymph was centrifuged at $3000 \times g$ for 20 min at 4 °C. The supernatant fluid was recentrifuged at $8000 \times g$ for 30 min at 4 °C and the final supernatant fluid was filtered through a 0.4 μm filter. The filtrate was then stored at − 20 °C for infectivity studies. The total protein in hemolymph was determined by the method of Lowry et al. (1951). The presence of WSSV in the hemolymph was checked by PCR using published primers (Yoganandhan et al., 2003).

2.2. Collection of experimental animals

Shrimp, *Penaeus indicus* or *P. monodon* (10–15 g body weight), were collected from grow-out ponds or the sea and were maintained in 1000-l fibreglass 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, near Chennai and allowed to sediment to remove the sand and other suspended particles. The seawater was first chlorinated by treating the seawater 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 pelleted 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 this tank for 5 days for acclimatization before the experiments. From the experimental animals, a small portion of pleodpods were cut and used for screening for the WSSV by polymerase chain reaction (PCR) using the primer designed by Yoganandhan et al. (2003). Only animals shown to be healthy were used for the experiments.

2.3. WSSV propagation and purification

Fifty shrimp *P. indicus* or *P. monodon* (15–20 g body weight) collected from stocking tanks were placed into five tanks and injected intramuscularly with hemolymph from WSSV-infected shrimp (300 μg of total protein per animal). They were examined at intervals of 8 h and moribund shrimp were removed for hemolymph collection. Pooled hemolymph was