Journal of Invertebrate Pathology 121 (2014) 24–27

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Short Communication

First report on vertical transmission of a plasmid DNA in freshwater prawn, *Macrobrachium rosenbergii*

Labrechai Mog Chowdhury [a], P. Gireesh-Babu [a], A. Pavan-Kumar [a], P.P. Suresh Babu [b], Aparna Chaudhari [a,*]

[a] Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Versova, Mumbai 400 061, India
[b] Central Institute of Fisheries Education, Kakinada Centre, Beach Road, Kakinada 533007, India

ARTICLE INFO

Article history:
Received 28 February 2014
Accepted 2 June 2014
Available online 13 June 2014

Keywords:
Plasmid DNA
Antisense RNA
Vertical transmission
Macrobrachium rosenbergii

ABSTRACT

Outbreak of WSSV disease is one of the major stumbling blocks in shrimp aquaculture. DNA vaccines have shown potential for mass scale vaccination owing to their stability, cost effectiveness and easy maintenance. Development of economically feasible delivery strategies remains to be a major challenge. This study demonstrates vertical transmission of a plasmid DNA in a decapod *Macrobrachium rosenbergii* for the first time. Females at three different maturation stages (immature, matured and berried) and mature males were injected with a plasmid DNA and allowed to spawn with untreated counterparts. Using specific primers the plasmid DNA could be amplified from the offspring of all groups except that of berried females. For this confirmation genomic DNA was isolated from 3 pools of 10 post larvae in each group. This presents an ideal strategy to protect young ones at zero stress.

1. Introduction

More than 90% of the global aquaculture production comes from Asia, where it contributes significantly to livelihoods and national economies. Out of the total world aquaculture production of about 59.9 million tonnes, crustaceans contribute 9.6% by volume and 18% by value (FAO, 2012). Of the cultured decapod crustaceans, the marine black tiger shrimp *Penaeus monodon* and freshwater giant prawn *Macrobrachium rosenbergii* led the market share until 2010. Crippling losses due to white spot syndrome virus (WSSV) in the former and *M. rosenbergii* nodavirus (MrNV) in the latter have caused the aquaculturists in India and S.E. Asian countries to adopt alternative decapod species like *Litopenaeus vannamei* (FAO, 2011). However, there are reports of WSSV affecting the production of *L. vannamei* too in India (Balakrishnan et al., 2011). Effective and economically viable vaccination strategies and therapeutic approaches can recover and revive the culture of these important species. Since decapods lack an adaptive immune response like the one present in vertebrates, the standard vaccination strategies are not applicable. Nevertheless, the RNA interference pathway was recently shown to be active in shrimp, fuelling considerable effort towards developing RNAi based vaccines (Krishnan et al., 2009; Bartholomay et al., 2012). We have reported a DNA vaccine for WSSV capable of expressing long hairpin RNA (lhRNA) against vp28 envelop protein *in vivo* and providing 75% protection on challenge (Krishnan et al., 2009). Recently, Ahanger et al. (2014) reported successful use of antisense constructs. However, intramuscular injection of individual shrimp is not a feasible option in India where extensive farming is practiced. In this study, we have used a plasmid DNA to test the possibility of vertical transmission from the injected brooder to the offspring in the decapod *M. rosenbergii* at different maturation stages. Five maturation stages are recognized in *M. rosenbergii* females viz., immature, maturing, matured, berried and spent. In immature and maturing females, ovaries appear like flabby off white or slightly orange mass of tissue and are not visible through carapace. In mature females, ovaries are well developed, orange in color and visible through carapace. Berried females carry fertilized eggs in the brood chamber between the swimming legs for incubation and subsequent hatching after 3–4 weeks. As the eggs hatch, the free-swimming larvae called zoeae are dispersed and undergo a number of microscopically distinct larval stages before metamorphosizing into post-larvae (PL). Three maturation stages of females (immature, matured and berried) were selected for the experiment.

* Corresponding author. Address: Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Versova, Andheri West, Mumbai 400 061, India.
E-mail address: achaudhari67@gmail.com (A. Chaudhari).

http://dx.doi.org/10.1016/j.jip.2014.06.001
0022-2011/

2. Material and methods

2.1. Experimental animals

A total of 40 male and female mature brooders of *M. rosenbergii* were procured from Balabadhrapuram freshwater fish farm, CIFE. The brooders were reared in FRP (Fibre-reinforced plastic) tanks ($2 \times 0.5 \times 0.5$ m) with aeration by providing commercial pelleted prawn feed (CP, India) for 2 days. Prior to use, the experimental animals were checked for the presence of any external abnormalities, disease symptoms, erratic swimming and lethargy. The animals were screened for the presence of WSSV and MrNV by PCR and only healthy animals were used for the experiment.

2.2. Antisense construct

The antisense construct against WSSV carried a 330 bp fragment of the vp28 gene of the virus in reverse orientation downstream to the Cytomegalovirus (CMV) promoter in pcDNA3.1 vector. Briefly, fragment to be cloned was PCR amplified using primer set Vp28-F: AAATCTAGATGTGACCAAGACCATCGAAA and Vp28-R: AAAGATATCTGCACCATCTGCATACCAGTG containing linkers for *Xho*I and *Eco*RV restriction enzymes to facilitate directional cloning. Genomic DNA purified from WSSV-infected shrimp was used as template. The correct size of the amplified fragment was confirmed using agarose gel electrophoresis, purified by Gel Extraction kit (Qiagen, Netherlands), and cloned into pcDNA3.1 vector following Sambrook et al. (2001). The positive clones were identified by colony PCR. The orientation of the cloned fragment was confirmed by sequencing and the clone was named pCMV-VP28AS.

2.3. Experimental design

Four different treatments were designed to study the vertical transmission of DNA vaccine (Table 1). First three treatment groups include intramuscular injection of the plasmid DNA into females at three different maturation stages viz., immature, matured and berried. In the fourth treatment group, mature males were injected with plasmid DNA to test the transfer of plasmid DNA through milt. The pCMV-VP28AS construct was injected intramuscularly (i.m.) using an insulin syringe. The concentration of the pDNA was maintained at 1 μg/gm of body weight and injection volume was 100 μl. Control group received 100 μl of STE buffer (10 mM Tris Cl, 100 mM NaCl and 1 mM EDTA, pH: 8.0). The injected brooders were maintained in FRP tanks ($2 \times 0.5 \times 0.5$ m). Two mating pairs with 1:2 or 1:3 (female:male) ratio were kept in one FRP tank (Table 1). The tanks were covered with net to prevent crawling. Feeding was done twice a day with commercial prawn feed for 3 weeks until the females were berried. In 3rd week, the berried females with dark grey colored eggs were shifted to spawning tank with pre-chlorinated water with 7 ppt salinity.

2.4. Breeding setup

An initial dip treatment of formalin (500 ppm) for 5 min in aerated water was given to disinfect the brooders. The brooders were then transferred to FRP tanks of 300L capacity filled half with formalin treated (30 ppm) bleached seawater (7 ppt). In each tank, two to three brooders were maintained. They were fed with commercial prawn feed four times a day. Tank cleaning was done daily to remove the left over feed and 30% water exchange was done daily with fresh 7 ppt water until hatching took place. Immediately after spawning, the proto-zoea were collected and transferred to 13 ppt saline water in separate FRP tanks of 250L capacity filled half with formalin treated sea water. The temperature of the water was maintained between 28 and 30 °C.

2.5. Feeding zoea

The zoea were fed twice a day with artemia nauplii until 6th stage at the rate of ~5 artemia nauplli per zoea. From 6th stage onwards artemia flakes were also included in the diet. For 7th, 8th and 9th stages egg custard was included once in the daily diet. Conversion from one stage to the next took 2 days on average. The post larvae (PL) were obtained in 19–20 days.

2.6. Tissue distribution

To study the distribution of plasmid construct in different parts of the body after injection, both male and female brooders were dissected after mating and heart, hepatopancreas, muscle, gill, pleopod, and intestine tissues were collected. Ovaries were also collected from females. Genomic DNA was isolated and presence of construct was confirmed by PCR amplification of the CMV promoter using specific primers (CMVpF: AAAGCTAGCGAATCTGCTTAGGGTTAGG and CMVpR: AAATCTAGAAA TTTCGATAAGCCAGTAAGC) that amplified a 700 bp fragment carrying the CMV promoter from the plasmid DNA. The PCR reaction mix was prepared following Sambrook et al. (2001). The PCR cycling conditions included an initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and an extension at 72 °C for 45 s and final extension at 72 °C for 5 min. The correct size of the amplified fragment was confirmed using agarose gel electrophoresis and imaging system (BioRad XR$^+$ Gel Documentation System).

2.7. Vertical transmission

Genomic DNA was isolated from 3 pools of 10 post larvae (15 days old) in each group using Qiagen DNeasy Blood and Tissue kit following manufacturer's instructions. During sampling two washes with distilled water and one with sterile STE buffer were performed before setting up lysis for DNA isolation to prevent cross contamination with any extraneous material like feces, etc. Vertical transmission of the injected pCMV-VP28AS plasmid was confirmed by PCR amplification of CMV promoter as described in

Table 1
Experimental design for confirming vertical transmission.

Group	Treatment	Mating design	
		Male	Female
T1	Intramuscular injection to immature female	2	5 (injected)
T2	Intramuscular injection to matured female	2	5 (injected)
T3	Intramuscular injection to berried female	2	5 (injected)
T4	Intramuscular injection to matured male	3 (injected)	7
C	Un-injected control	2	5