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Viral resistance in shrimp that express an antisense Taura syndrome virus coat protein gene

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Abstract

Taura syndrome virus (TSV) is a major cause of mortality and morbidity in shrimp, and has a profound economic impact on commercial U.S. shrimp farming. This paper describes the stable expression of an antisense Taura syndrome virus-coat protein (TSV-CP) gene construct in shrimp zygotes, via transfection using jetPEI reagent, over a period of at least 236 days. The transgenic shrimp showed no statistically significant difference from normal control shrimp in terms of weight gain or their appearance, morphology, swimming and eating activities. When challenged with live TSV, the transgenic shrimp exhibited increased resistance to the TSV infection (83% survival rate) as compared to control animals (44% survival rate). This work demonstrates that transgenic shrimp, which stably express an antisense transcript from the TSV-CP gene, are partially resistant to TSV infection. These data may have an important implication for commercial shrimp farming.

Keywords: Virus resistance; Transgenic shrimp; Taura syndrome virus; Antisense RNA; TSV coat-protein; Transfection

1. Introduction

In the last decade, world shrimp aquaculture has suffered significant losses due to serious virus outbreaks (Chamberlain, 1994; MacMillan, 1996; Brock, 1997; Lightner and Redman, 1998). Over 22 shrimp viruses have been described, several of which are highly pathogenic—including white spot baculovirus, yellow head virus and Taura syndrome virus (TSV). Collectively, these agents pose a serious problem to global shrimp production (You et al., 2004). However, there are currently no effective chemicals or drugs to treat viral diseases in shrimp. Strategies generally used in combating shrimp diseases include immuno-stimulation, vaccination, quarantining, and environmental management (Xiang, 2001). These strategies are non-specific in combating infectious diseases and cannot boost the shrimp's ability to cope with future infection even with the same pathogen. However, vaccine-based approaches are likely to prove difficult in shrimp since these organisms lack the ability to produce antibodies (Reichhart et al., 1992; Bachere et al., 1995; Glinski and Jarosz, 1997). Thus, controlling viral diseases clearly represents a great challenge in the shrimp aquaculture industry. The serious impact of viral disease on cultured shrimp, which is coupled with a decline in natural fisheries of healthy shrimp (Pullin et al., 1998), has led to a critical demand for advanced biotechnological applications.

In the present study, we have used genetic transfer technology to produce a TSV-resistant shrimp strain, through the expression of an antisense RNA that corresponds to a 493-bp fragment of the TSV coat protein (CP) gene. The expression vector, consisting of the chimeric shrimp beta-actin promoter (Sun et al., 2002) from *Penaeus vannamei* and 493 bp partial sequence of the target gene, TSV-CP, was constructed by the method described previously (Sun, 1997). Briefly, the pSV-Galactosidase vector (Promega) was used as the base vector; through PCR, *Nco*I and *Hin*dIII restriction enzyme sites were created at the 5′ end and 3′ end, respectively, of the beta-actin promoter, actP2. The SV40 promoter and enhancer of the pSV-Galactosidase vector were excised through restriction enzyme digestion with *Nco*I and *Hin*dIII, and the βactP2 was inserted into the vector to construct the expression vector, pβactP2-β-Gal. In addition, *Hin*dIII and *Sal*I restriction enzyme sites were added to the 493-bp TSV-CP target gene.

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Again through PCR, the *lac* Z gene of the pβactP2-Gal vector was replaced with the TSV-CP (AS) target gene in antisense orientation, thus producing the expression vector, pβactP2-TSV-CP (AS). The orientation of the strand of TSV-CP gene was confirmed by DNA sequencing in both directions using an automated DNA sequencer (model 373A, Applied Biosystems Inc., Foster City, CA, USA). General procedures of ligation, cloning, and plasmid DNA purification followed the methods of Sambrook et al. (1989).

An established jetPEI-based transfection protocol in this laboratory was employed for gene delivery into shrimp eggs (Calderon and Sun, 2003). Briefly, 800–1000 fertilized shrimp eggs approximately 5 min post-spawning were transferred into a Petri dish (35 mm × 10 mm) containing 1 μg of the expression vector, pβactP2-TSV-CP (AS), and 1.2 μL of the transfecting reagent, jetPEI (7.8 mM) in 2.0 mL of sterile seawater. Following a 50-min incubation at room temperature, the reaction mixture of approximately 2.5 mL was transferred into a 1-L beaker filled with sterile aerated seawater at 28 °C for the hatching process. After recording the hatching rate at 24–36 h after treatment, the transfected shrimp were transferred to 55-gallon tanks for further development and growth. The control shrimp were treated in the same manner except that no plasmid DNA was introduced into the shrimp eggs. The putative transgenic shrimp and control animals were raised in aerated seawater at 28 °C and fed with commercial pelleted shrimp feed twice a day, and screened for the transgene expression by reverse transcription-polymerase chain reaction (RT-PCR) or by genomic PCR assay at various developmental stages (130, 190, and 236 days). For TSV-CP screening, two pleoplods (swimmerets) from experimental shrimp were excised and homogenized with a mortar and pestle pre-chilled in liquid nitrogen. RNA or genomic DNA was isolated using the Purescript RNA or DNA isolation kits (Gentra). For RT-PCR reaction, total RNA (0.2–1.0 μg) isolated from jetPEI/DNA transfected shrimp and normal control animals was used as a template, with TSV-CP gene-specific primer pair A (primer 1: 5′-CTTAATTAATGCCTGCTAACCC-3′ and primer 2: 5′-ATTGATGTCTGCTTAGCATTCA-3′). In order to confirm the presence of the target gene in the transgenic shrimp, a second round PCR reaction was performed using 1 μL of the RT-PCR product as a template and the TSV-CP nested gene-specific primer pair (primer 3: 5′-TGATACAACAACCAGTGGAGGAC-3′ and primer 4: 5′-TGTCATCAGGTAGGGAAATTTC-3′). These two sets of oligonucleotide PCR primers were designed basing on the published genomic sequence of the TSV-CP (GenBank Accession No. AF277378). The RT-PCR procedure was as described by Sun (1995) using the GeneAmp PCR Kit (PE Biosystems). For genomic PCR assay, 1 μg of genomic DNA isolated from the pleopods, was used as template. Either gene-specific oligonucleotide pairs of primer 1 and primer 2 or primer 3 and primer 4, that are complementary to the TSV target genes were used as primers in the PCR reactions. Genomic DNA amplification was performed by following the instruction suggested by the vendor (Perkin Elmer). In brief, reaction mixtures were initially denatured at 94 °C for 5 min, then programmed for 1 min at 95 °C and 1.5 min at 55 °C for 35 cycles, followed by 7 min at 67 °C for 1 cycle as a final extension in a DNA Thermal Cycler (Perkin-Elmer 9600). PCR products were subjected to 1.0% agarose gel electrophoresis and the expected DNA band of either 493 bp (when primer 1 and primer 2 were used) or 302 bp (when primer 3 and primer 4 were used) was revealed following ethidium bromide staining, indicating the presence of the TSV-CP target gene in the shrimp.

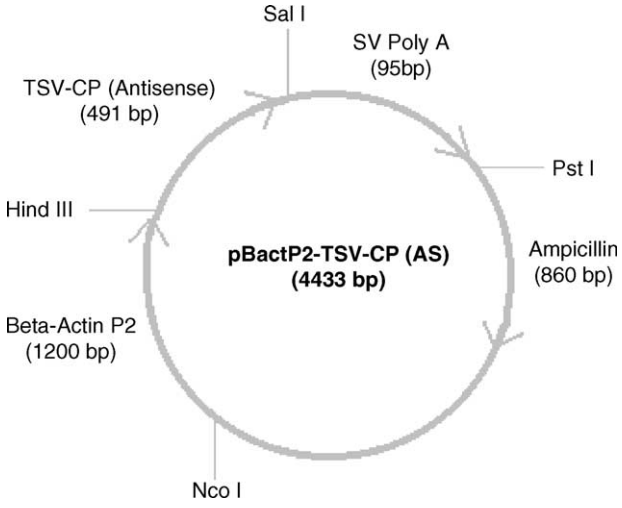

Fig. 1. Schematic structure map of the expression vector pβactP2-TSV-CP (AS).

As expected, the fertilized eggs of *Litopenaeus vannamei*, transfected with the expression vector, pβactP2-TSV-CP (AS) (Fig. 1) were found to be positive for the presence of the transgene. Under the described laboratory conditions, shrimp zygotes that were transfected with plasmid DNA via jetPEI showed a 50–60% hatchability, 35–50% post-hatching survival rate, and a 64% gene transfer efficiency as determined by the RT-PCR method (Table 1). This rate of gene transfer efficiency was substantially greater than that obtained using other gene transfer techniques including microinjection and electroporation (Sun et al., 2005). As such, this finding is consistent with the recent reports of successful use of transfection reagents for transferring desirable genes into mammalian

Table 1
A summary of gene transfer into shrimp zygotes

Experimental[a] shrimp	Hatchability (%)	Post-hatching survival (%)	Transfection[b] efficiency (%)
Test group	50–60	35–50	64 (64/100)
Control group	50–60	40–60	0 (0/25)

N/A: not applicable.

[a] Delivery of plasmid DNA into fertilized shrimp eggs (test group) by jetPEI-based transfection as described in the materials and methods; control animals were untreated.

[b] Transfection efficiency was determined by PCR screening of genomic DNA isolated from the swimming legs of individual juvenile and adult shrimp for the presence/absence of the target gene, TSV-CP.