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GENOTYPING OF WHITE SPOT SYNDROME VIRUS ON WILD AND FARM CRUSTACEANS FROM SONORA, MEXICO

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Abstract - White spot syndrome is a viral disease affecting wild and farm crustaceans that serve as reservoirs. Previous reports have demonstrated high genomic variation in WSS viruses (WSSV) isolated from distinct geographical regions. In this study, we collected wild shrimps (*Litopenaeus stylirostris*), crabs (*Callinectes arcuatus*) and farmed shrimp (*L. vannamei*) in Sonora, Mexico, between 2008 and 2010. DNA was extracted, and the variable regions and transposase genes were subjected to PCR and sequencing. Compared to strains of WSSV from other sites, Mexican samples exhibited a distinct number of repeat units (RUs) in ORF94, ORF75 and ORF125, which ranged between 1-11, 3-15, and 8-11 RUs respectively, and a unique single nucleotide polymorphism (SNP) at position 48 of ORF94. A total of six Mexican genotypes were found in organism from shrimp farm and natural environment.

Key words: White spot syndrome virus, VNTR, SNP, ORF94, ORF75, ORF125.

INTRODUCTION

Wild and farmed shrimp are the most commonly harvested marine species in Mexico, with an average yearly production of 187,000 MT in 2009 (CONAPESCA, 2009). However, white spot syndrome virus (WSSV) continues to be the most pathogenic among the penaeid shrimp viruses and often leads to mass shrimp mortalities and severe economic losses to shrimp farmers (Molina et al., 2008).

WSSV has a rod-shaped nucleocapsid with a thin polar extension at one end and a circular double-stranded (ds) DNA genome of approximately 300 kb and 184 open reading frames (ORFs), of which only 11 are homologous with those listed in public databases (Van Hulten et al., 2001).

The variable number of tandem repeats (VNTRs) associated with three minisatellites, ORFs 94, 75, and 125, have been suggested as potential molecular markers for epidemiological studies; these VNTRs are DNA elements consisting of repeating units and are highly polymorphic (Wongteerasupaya et al., 2003). VNTR analyses have been used to demonstrate high levels of genetic variation within or between populations. Additionally, distinct RU numbers have been linked to the disease severity (Dieu et al., 2004). In this study, we genotyped Mexican WSSV isolates using a putative transposase sequence and ORFs 94, 75,

and 125 as molecular markers. We compared these results to those obtained from American and Asian WSSV isolates.

MATERIALS AND METHODS

A total of 56 samples, including cultured shrimp (*Litopenaeus vannamei*), wild shrimp (*Litopenaeus stylirostris*), and wild crabs (*Callinectes arcuatus*), were collected from six different localities, including 11 culture ponds where WSSV outbreaks were in progress, and from four natural ecosystems, such as creeks and canals, between 2008 and 2010. DNA from 25-50 mg of pleopods or gills were extracted using PureLink Genomic DNA Kits (Invitrogen, Carslbad, CA, USA) according to the manufacturer's instructions. For PCR, 10 ng of DNA template and 30 pmol of each primer were added to a 50-µL reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.25 mM each of dATP, dTTP, dCTP, and dGTP, and 2.5 U of Taq DNA polymerase (Bioline, MA, USA). WSSV-positive were identified by PCR (Nunan et al., 1998). Three minisatellite regions were then analyzed: ORF94, ORF125, ORF75 (Dieu et al., 2004; Pradeep et al., 2008), and a transposase gene (Dieu et al., 2004).

PCR products were purified with Wizard SV Gel and PCR Clean-Up System and sequenced using an automatic DNA sequencer (ABI 3130, Applied Biosystems). Sequence data were analyzed to determine the number of variable tandem repeats using the Tandem Repeats Finder (TRF) software (Benson et al., 1999).

RESULTS AND DISCUSSION

WSSV was present in only 14 of the 56 samples. The length of amplicons for all the genes and RUs are summarized in the Table 1. Genotypes of samples from Atanasia and Tobari harbored three RUs, but had the same nucleotide at position 48 of the ORF94 repeats. The above results are consistent with reports characterizing samples from Thailand, for which two WSSV samples consisting of 8 RUs in ORF94 in two geographical regions demonstrated the same pattern of repetition with no difference in SNP at position 48 (Wongteerasupaya et al., 2003). These results suggest that the identical pattern of repetition (7 RUs in ORF94) of WSSV samples analyzed from the shrimp farms of Santa Barbara, Siari and Riito is due to the geographical proximity of these viruses. Samples analyzed in Brazil were identified as having 4-19 RUs in ORF94, 7-11 in ORF125, or 6-15 RUs in ORF75. In contrast, genotyping of samples from Santa Catarina, Brazil, had the same pattern of RUs over three growing cycles, but exhibited variation in the SNP at position 48 in ORF94. This study also identified an absence of the gene encoding the transposase (Muller et al., 2010), which is similar to our findings.

During the sampling that took place in 2010, the WSSV genotype obtained from wild crab (*C. arcuatus*) had 11 RUs in ORF94, 3 RUs in ORF75 and 8 RUs in ORF125. This pattern is different to the genotype of WSSV from cultured shrimp (*L. vannamei*) from the same locale, which had 7 RUs in ORF94 and 10 RUs in ORF125. WSSV can adapt depending on the type of infectious organism, which is supported by the presence of differences in the VNTR genotypes derived from wild organisms versus farmed shrimp. These variations may be due to adaptation within the host and viral mechanisms of virulence (Pradeep et al., 2008) and thus, we can infer that this is due to different genotypes or strains of WSSV, but further studies are needed to test this hypothesis. The genotype of WSSV shrimp samples taken during 2008 in the region of Tastiota was unique among all WSSV genotypes analyzed between 2008 and 2010. This WSSV sample had only 1 RU of 54 bp in ORF94.

In conclusion, we report the presence of six different WSSV genotypes based on the analysis of SNPs and ORF94, ORF125, and ORF75 of samples from six different geographical regions within Sonora, Mexico. Our studies indicate that differences among WSSV genotypes are influenced by environmental conditions, the type of host in which the virus replicates, and perhaps repeated introductions of the virus through imported crustaceans. Our results also support the hypothesis that variations in VNTR can be used as molecular markers of geographical origin.

Table 1. Crustacean samples originating from Sonora, Mexico and summary of repeats units (RUs) in ORF94, ORF75 and ORF125.

Region	Month/Year	Host	No. of samples/WSSV positives	ORF94		ORF75		ORF125	
				Amplicon size (bp)	No. RUs	Amplicon size (bp)	No. RUs	Amplicon size (bp)	No. RUs
S. Barbara	June/2010	*L. vannamei*	5/2	543	7	ND	--	763	10
Atanasia	June/2010	*L. vannamei*	9/1	324	3	565	4	ND	--
Siari	June/2010	*L. vannamei*	5/2	543	7	567	4	767	10
Riito	June/2010	*L. vannamei*	9/1	545	7	ND	--	763	10
Tobari	June/2010	*L. vannamei*	5/1	330	4	ND	--	ND	--
Riito	January/2010	*C. arcuatus*[1]	2/1	762	11	444	3	684	9
Tobari	March/2010	*C. arcuatus*[1]	2/1	275	3	450	3	ND	--
Riito	March/2010	*L. stylirostris*[1]	2/1	ND	--	ND	--	ND	--
Riito	March/2010	*C. arcuatus*[1]	2/1	ND	--	450	3	ND	--
Riito	November/2008	*L. vannamei*	9/2	489	6	1169	15	869	11
Tastiota	November/2008	*L. vannamei*	6/1	217	1	ND	--	ND	--
			56/14						

[1)]Wild samples
ND: Not detected

ORF94 is especially useful for this purpose because the number of repeating units is highly variable and may be used in epidemiological studies to follow the movement of the virus in other geographic regions.

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