PCR based DNA marker analysis for WSSV resistant populations of *Penaeus monodon* Fabricius in Sri Lanka

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Abstract

White Spot Syndrome Virus (WSSV) is the causative agent of White Spot Disease (WSD) and it creates major problems in shrimp aquaculture industry throughout the world. The WSD was first reported in Sri Lanka in 1996 and still appears as sporadic outbreaks causing extensive production losses in farmed *Peneaus monodon*. Selective breeding using DNA markers would be a cost effective strategy as a long term solution for this problem. Therefore, this research was conducted aiming at developing a suitable DNA marker for the identification of WSSV resistant cultures. DNA samples of disease infected and healthy *P. monodon,* giant black tiger shrimp originated in Sri Lanka were amplified using previously reported markers in the Asian region, namely, 457bp SCAR marker, KM 19/20 marker and RS0622 marker that were linked with WSSV resistance. Successful amplification was detected in shrimp samples with all DNA markers except RS0622 marker. However, any genetic diversity among the shrimp samples or any association between marker genotype and the infection phenotype were not observed in this study. This implies that although 457bp SCAR marker, KM 19/20 marker and RS0622 marker are useful as a tool in the investigation of genetic diversity or resistant genotypes to WSSVamong the *P. monodon* cultures in some countries, no such relationship could be explained with the cultures originated in Sri Lanka, probably due to the different genetic background. Therefore, further studies on DNA markers are necessary for the detection of resistant populations of giant black tiger shrimps in Sri Lanka.

Keywords: *Penaeus monodon,*WSSV,457bp SCAR marker, KM 19/20, RS0622

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Introduction

White spot syndrome virus (WSSV), the causative agent of WSD infects nauplii to adult shrimps and can be transmitted both vertically and horizontally. The severity of infection is very high in *Penaeus monodon* thus it can result in mortality up to 100% within 3 to 7 days causing huge economical losses (Chang *et al.*, 1996).

Formerly, the existence of WSSV resistance or tolerant*P. monodon*has been reported based on the clinical signs by WSSV challenge experiment (Maheswarudu, 2007). Thereafter, DNA markers, 457bp SCAR marker, KM 19/20, RS0622 that can

differentiate disease resistant and susceptible populations were observed (Dutta *et al.*, 2013, Mukherjee and Mandal, 2009, Dong et al., 2008).

This research was aimed at identifying a suitable DNA marker for the detection of WSSV resistant/tolerant populations in giant black tiger shrimp, *P. monodon* Fabricius for generating specific pathogen resistant (SPR) brood stock for the development of disease-free shrimp aquaculture industry in Sri Lanka. As the first step of the study, here we describe the feasibility of using previously reported makers in *P. monodon* cultures of Sri Lanka.

Materials and Methods

Seventy one tissue samples of *P. monodon* brood shrimps (pleopods) and juveniles (pleopods or gills) were obtained from shrimp hatcheries and grow-outs situated in the North Western Province and brood shrimp collectors from Ambakandawilla, Hendala and Negombo during the period from February to July 2014.

The genomic DNA extraction and WSSV screening (Nested PCR) was done using reagents supplied in IQ2000TM WSSV detection kit (Farming IntelliGene technology crop, Taiwan) following the manufactures guidelines.

PCR amplification was done using selected markers (Table 1) and electrophoresis was done for amplified PCR products to identify WSSV resistant populations.

Table 1: DNA sequences of the primers used in the study

Name of the primer	Annealing Temp. (°C)	Fragment Size (bp)	F/R	Sequence (5'- 3')	Accession No*
457bp SCAR primers	57	457	NM-F	ATCCTCTGGAGTGG AAAGCA	KC461829
			NM-R	CACCTGGGCTCACC TTACT	
KM 19/20	50	317 and 71	F	GATCATCATCATCG GCTG	AF077565
			R	TATGTACGTTAGTC CAAG	
RS0622	55	494 and 482	F	TCAGTCCGTAGTTC ATACTTGG	AY132778
			R	CACATGCCTTTGTG TGAAAACG	

*Accession no. obtained from NCBI genebank

Results

Identification of WSSV positive and negative samples

Using IQ2000^{TM} WSSV Detection and Prevention System, infected samples were differentiated into four different levels of infection; very light infection, light infection, moderate infection and severe infection. They were considered as WSSV positive samples. The negative results of this test indicate that the specimen is either not infected or the infection level is lower than the detection limit (10-20 copies/reaction) of the test kit.

PCR amplification with the 457bp SCAR marker

According to the results of this study, most of the WSSV positive and negative samples generated the relevant band of 457 bp after PCR reaction (Table 2, Figure 1). These values did not show any significant difference between WSSV positive and negative samples that were used in this study.

PCR amplification with the KM 19/20 microsatellite allele marker

In this study only one band with 317bp was observed in 25 samples tested (Table 2, Figure 1) regardless of the WSSV infection. The expected 71bp band was not observed in any animal.

PCR amplification with the RS0622 microsatellite allele marker

Expected amplified bands with the sizes of 494 bp or 482 bp were not observed in any sample of *P. monodon*. But it gave another DNA band of around 90bp for *P. monodon* cultures (Figure1).

Primer name	Allele (bp)	WSSV positive samples			WSSV negative samples		
		Number of samples	Number of observations	Frequency of allele	No.of samples	Number of observations	Frequency of allele
457bp SCAR primers	457	38	32	0.842	33	32	0.970
	Null allele		6	0.158		1	0.030
KM 19/20	317	13	13	1.000	12	12	1.000
	71		0	0.000		0	0.000

RS0622	494	9	0	0.000	6	0	0.000
	482		0	0.000		0	0.000
	<100		9	1.000		6	1.000

Table 2. DNA markers for P. monodon and their frequencies

**Frequency of allele (number of observation at the given allele/total number of observations).*

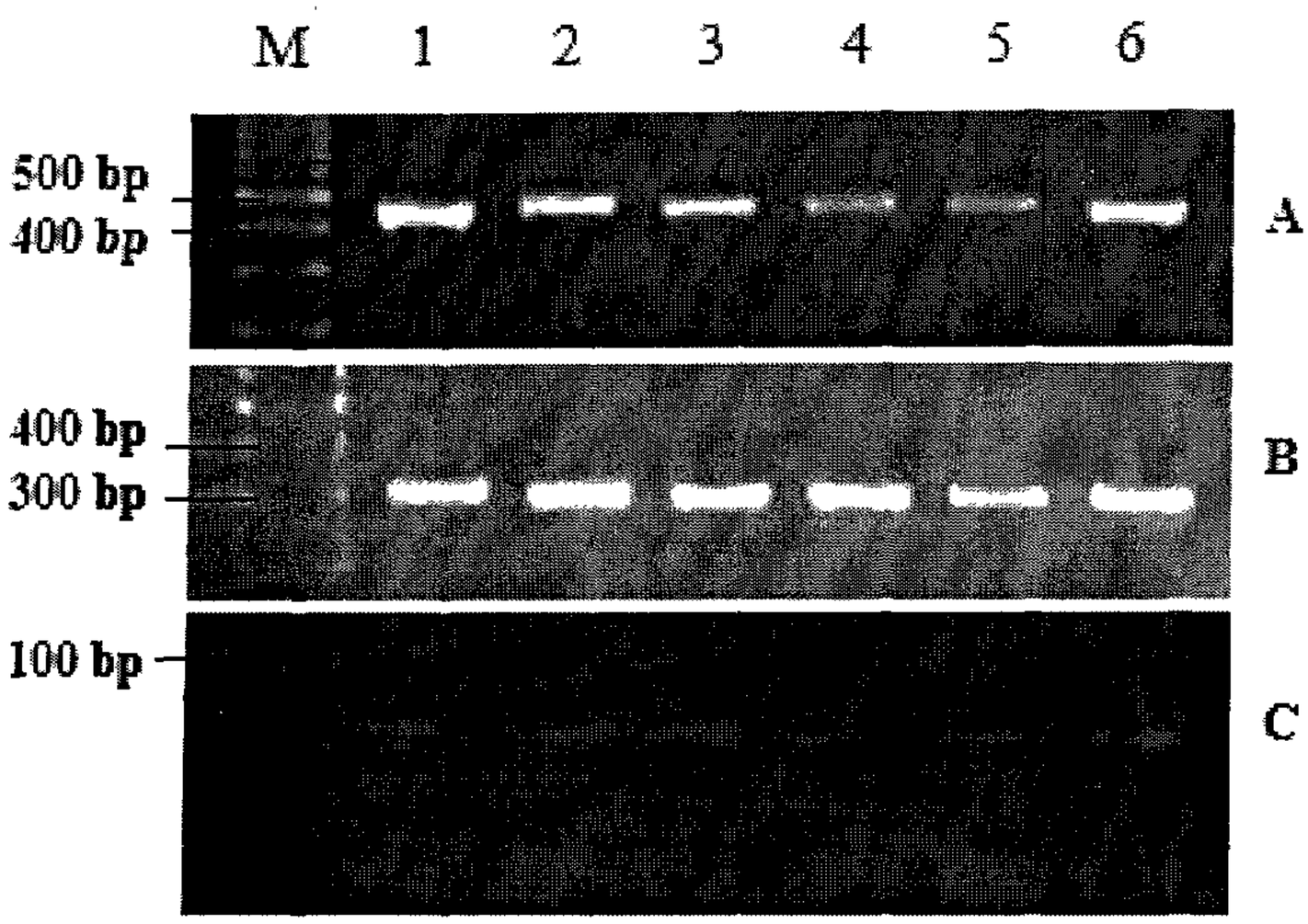

Figure 1. Agarose gel electrophoresis of PCR products generated by A) 457 bp SCAR marker B) KM 19/20 C) RS0622

Lane M: 100 bp ladder DNA marker,
Lane 1, 2: Sample of severe WSSV infection,
Lane 3, 4: Sample of very light WSSV infection, Lane 5, 6: WSSV negative sample.

Discussion

Infected viral concentration has to reach 10~100 fold of the detection limit for the white spot syndrome to occur. Therefore, samples that did not show any amplification were considered as WSSV negative samples. A negative control and quantified positive standard were run with the samples to ensure accurate and reliable results.

The phenotypic variation in survival to WSSV, explained by the presence or absence of the 457bp DNA band by the SCAR marker which has been developed from the sequence of 502 bp DNA fragment (Dutta *et al.*, 2013). As this is a dominant marker, absence of the band is considered as null allele and null allele frequency was very low in the population.

According to Mukherjee and Mandal, (2009) disease resistant populations should show only one microsatellite DNA band of 317 bp, while disease susceptible populations show an additional unique microsatellite DNA band of 71 bp by KM 19/20marker. However, the results of this study indicate that there is no genetic variation with regard to the KM 19/20 locus of the genome of *P. monodon* strains originated around Sri Lanka and that this has no association with disease resistance.

An allele at microsatellite locus RS0622 was reported (Dong et al., 2008) as associated with resistance to WSSV in *F. chinensis*. This marker had amplified a 494 bp or 482 bp DNA band and showed polymorphism in their population.But though we could not observe either of these two bands in *P. monodon*, it gave another DNA band around 90bp. This may probably be due to non-availability of the relevant sequence in the *P. monodon* or availability of different sized microsatellites that amplifies only about 90bp allele. Unsatisfactory optimization of PCR might also be a causal factor for this situation. Hence, the marker RS0622 could not be used to explain the genetic mechanism of WSSV resistance in *P. monodon* originated in Sri Lanka in our experiment.

Conclusion

This study verified the clarity of the IQ2000^{TM} WSSV Detection and Prevention System. Three markers, namely, 457bp SCAR, KM 19/20 andRS0622 were tested with the genomic DNA of *P. monodon* cultures. However, any association between marker genotype and the infection phenotype was not observed in DNA samples obtained from *P. monodon* cultures of Sri Lankan origin. Therefore, further studies are necessary to develop a linked marker for the detection of cultures resistant to WSSV.

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