Polymerase Chain Reaction for the Detection of White Spot Syndrome Virus in Post-larvae of Penaeid Shrimps

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White spot syndrome is the most deadly viral disease of penaeid shrimps and has devastated shrimp aquaculture in many Asian countries. Affected shrimps are characterised by the presence of white spots on the carapace. A two step polymerase chain reaction (PCR) was used for the detection of latent state of white spot syndrome virus in post-larvae of *Penaeus monodon* and *P. indicus* from hatcheries. The one step PCR gave an amplification product of 1447 bp and two step PCR a 941 bp product. The two step amplification with nested primers was found to be more sensitive than one step amplification. The sensitivity of two step amplification was 1000 times higher than the one step amplification. Of the post-larvae tested, 47% were found to be positive for white spot syndrome virus by two step PCR while only 22% were positive by one step PCR.

Key words : Polymerase chain reaction, white spot syndrome, post-larvae, *Penaeus monodon*, *Penaeus indicus*

About two dozen diseases of shrimp occur in cultured farms. Out of these white spot syndrome virus (WSSV) is the most deadly and causes mortality in different species, *viz*, *Penaeus monodon*, *P. japonicus*, *P. chinensis* and *P. pencillatus* (Peng *et al.*, 1995; Wang & Liu, 1996). The disease caused by the virus was first observed in East Asia, during 1992 (Huang *et al.*, 1994; Chen, 1995). The disease spread very rapidly and in India it was first reported in 1996 (Hameed *et al.*, 1998). The affected shrimp is characterised by white spots on the carapace. (Chou *et al.*, 1995; Wang *et al.*, 1995). The virus can induce 100% mortality in infected shrimp within 3 to 5 days (Nakano *et al.*, 1994). WSSV is a member of the genus *Whispovirus* within a new virus family called *Nimavirida* (van Hulten *et al.*, 2001).

The economic viability of shrimp farms has been greatly affected by WSSV infection. Outbreaks caused by WSSV infection can be reduced to

a great extent by screening the brooders for the presence of WSSV infection. The post-larvae used for aquaculture in the Kerala state are brought from neighbouring states and hence screening of brooders is rarely possible. The next best possible alternative is to screen the seeds for the presence of WSSV. In recent years, a number of researchers have developed polymerase chain reaction (PCR) technique for the detection of WSSV (Kim *et al.*, 1998; Lo *et al.*, 1996). The two step PCR is found to be more sensitive than the one step PCR to the order of 10^3 to 10^4 times (Lo *et al.*, 1996). In this paper, the comparative efficiency of two step PCR and one step PCR for the screening of WSSV in post-larvae of penaid shrimp is evaluated.

Materials and Methods

PCR DNA template

Post larvae (20-30 mg) were homogenised and were used as DNA template for the first round of PCR amplification. The amplified PCR product from the first round of PCR amplification was used as the template for the second round of PCR amplification.

PCR primers

Nested PCR assay was performed using the WSSV primer sets developed by Lo *et al.*, 1996. The first set of primers amplify a 1447 bp fragment and the second internal set of primers amplify a 941 bp fragment of the WSSV genome. To eliminate the incidence of false reactions, a template free reagent control, a known negative control and known positive control were run in all the reactions.

Agarose gel electrophoresis

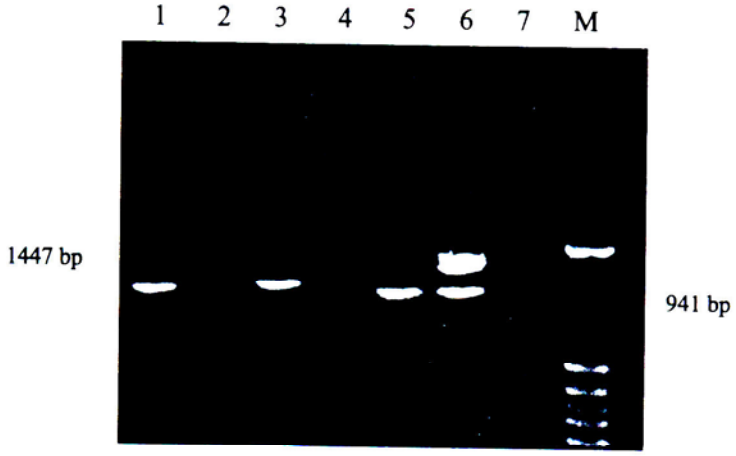
A 10 µl of the amplified DNA product was analysed on a 1% agarose gel, stained with ethidium bromide (0.5 $mg.ml^{-1}$) and run on 1X TAE. (Sambrook & Russel, 1999).

DIG DNA labelling and detection was done as per manufacturers instructions to confirm the specificity of the PCR product.

Results and Discussion

PCR has become the method of choice for the screening of shrimp for the presence of WSSV because of its high sensitivity compared to the classical methods like southern blot or dot blot hybridization. An expected

fragment of 1447 bp was amplified in the first step of amplification and 941 bp in the second step (Fig. 1). The specificity of the PCR reaction for detection of WSSV was already well recorded. (Lo *et al.*, 1996). The DIG labelled PCR product hybridised with DNA extracted from infected *P. monodon* while it did not react with healthy shrimp. This result demonstarted the specificity of the PCR product. Using the WSSV specific primers used in the study all the DNA samples consistently yielded a product of 1447 bp in the first PCR reaction and 941 bp in the second PCR reaction.



Lanes 1, 3, 5 are positive by nested PCR. Lane 6 is positive by both steps and M is molecular weight marker: pBR 322 DNA digested with HinfI.

Fig. 1. Detection of WSSV by nested PCR

To evaluate the sensitivity of two step PCR over single step PCR, serial dilutions of the extracted DNA was used to run the PCR reaction. The sensitivity of the two step PCR was 10^3 to 10^4 times higher than the one step PCR. DNA samples that yielded 1447 bp PCR product in the first step usually gave a 1447 bp as well as 941 bp product in the second step (Fig. 1). This was because of the presence of certain amount of primers left behind after the first amplification. Of the post-larvae tested, 47% were found to be positive for white spot syndrome virus by two step PCR while only 23% were positive by one step PCR (Table 1).

Various methods such as Cetyltrimethylammonium bromide treatment followed by phenol chloroform extraction and ethanol precipitation (Lo *et al.* 1996), use of TESP buffer with 1% Triton X 100 (Yang *et al.*, 1997)

Table 1. Comparison of one-step and two-step WSSV diagnostic PCR with post-larvae of *Penaeus monodon* and *P. indicus*

Post-larvae	One-step PCR			Two-step PCR		
	No. of post-larvae tested	No. tested positive	% positive	No. of post-larvae tested	No. tested positive	% positive
P. monodon	75	20	26.7	75	40	53.3
P. indicus	56	10	17.9	56	22	39.3
Total	131	30	22.9	131	62	47.3

or DNAzol reagent (Magbanua *et al.*, 2000) have been used by different authors for the extraction of WSSV DNA from shrimp. These methods are complicated, time consuming, and there is risk of contamination and loss of DNA during extraction process.We have used a simple and fast method for extraction of WSSV DNA as described by Kiatpathomchai *et al.*, 2001. This method gave very satisfactory results for the assay conditions used.

The lack of a reliable supply of disease free post-larvae contributes to the uncertainty and economic losses of shrimp farmers' worldwide. Many of the shrimp farms in Kerala rely on wild stock for brood, seed and to stock ponds and most hatcheries rely on ready to spawn adult females from seas as a source of nauplii. This tendency has led to an increase in the number of disease outbreaks due to WSSV in recent years. If this trend has to be reversed the industry has to depend on captive stocks that are screened for diseases.

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