DEVELOPMENT OF VISUAL DETECTION METHOD FOR DETECTION OF WHITE SPOT SYNDROME VIRUS

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INTRODUCTION

- White spot syndrome virus (WSSV) is a rapid replicating and virulence virus that has emerged globally as one of the prevalent and lethal disease that affect the shrimp population and has caused major loss in the shrimp industry worldwide.
- According to the International Committee on Taxonomy of Viruses (ICTV), WSSV is the only member of the genus *Whispovirus* within the *Nimaviridae* family and the virions of WSSV are enveloped, rod-shaped and they are 70-170 nm in width and 210-420 nm in length.
- Shrimp productions are starting to decline due to serious infection of white spot syndrome virus towards the shrimps that caused white spot disease. Current available detection methods such as ShrimpKit, LAMP and immunoassay are time consuming and require costly materials and machines.
- Therefore, this study focuses on developing a new visual detection method for white spot syndrome virus where it is not time consuming, more sensitive and less expensive procedure compared to other available methods.

OBJECTIVES

1. To construct positive control for the visual detection of WSSV.
2. To convert the obtained DNA concentrations into copy number using ThermoFisher DNA copy number calculator.
3. To optimize Polymerase Chain Reaction (PCR) to determine minimum copy number of WSSV DNA that can be detected for SybrGreen staining.

LITERATURE REVIEW

- The prevalence of WSSV is due to the virus spread rapidly and as a highly contagious virus, it is able to manifest to other non-affected neighboring areas (Chuah *et al.*, 2006).
- Although mechanism of nucleic acid binding of SybrGreen dye is not known, the wide spectrum of its application is due to its excellent properties such as temperature stability, favourable photophysical properties, high selectivity and sensitivity for double stranded DNA (Zipper *et al.*, 2004)

RESULTS AND DISCUSSION

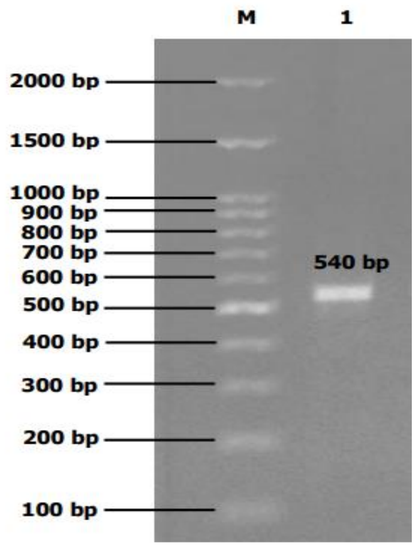
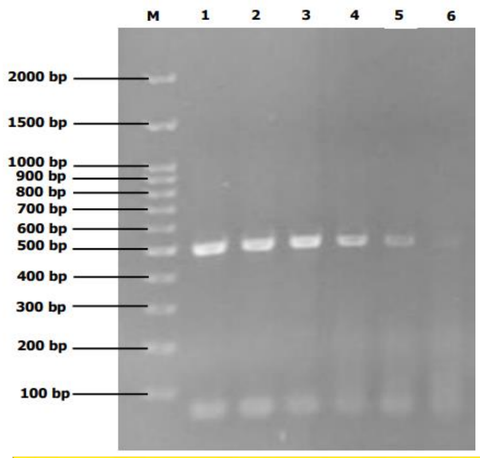
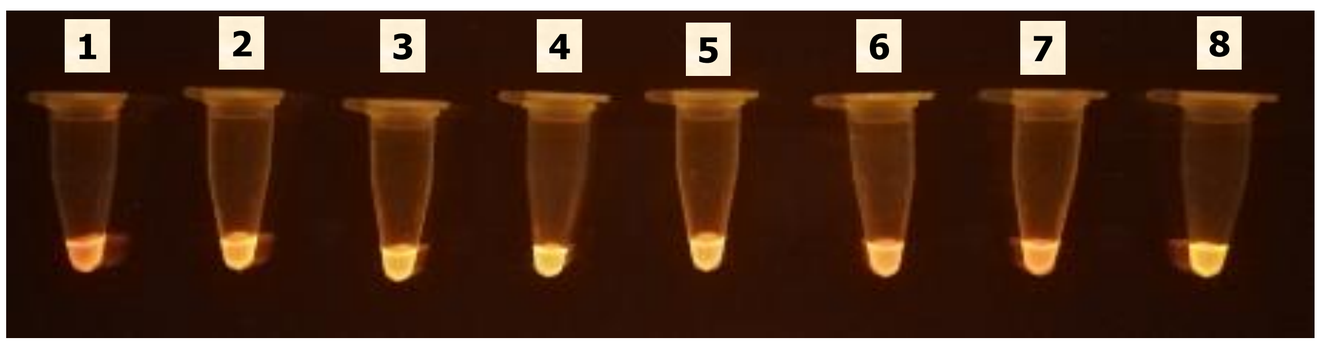


Figure 1 Gel electrophoresis of positive control for WSSV 500. Lane 1 represented the PCR products for WSSV 500.Lane M indicates the 100 bp DNA ladder.



Lane M: 100bp DNA ladder
Lane 1: 2 x 10^6 CN
Lane 2: 2 x 10^5 CN
Lane 3: 2 x 10^4 CN
Lane 4: 2 x 10^3 CN
Lane 5: 2 x 10^2 CN
Lane 6: 2 x 10^1 CN

Figure 2 Gel electrophoresis of first round nested PCR. The WSSV 500 Forward and WSSV 500 Common Reverse primers are able to amplify the WSSV-DNA as the band appeared at the position of 500 bp at all copies number. The primers are sensitive enough as they are able to detect the WSSV-DNA even at the lowest copy number which is 2 x 10^1 copies/µl. Detection at low amount of virus particles is crucial in shrimp farming industry as it can prevent great losses for the farmers in the future.



No 1: Negative control
No 2: 2 x 10^6 CN
No 3: 2 x 10^5 CN
No 4: 2 x 10^4 CN
No 5: 2 x 10^3 CN
No 6: 2 x 10^2 CN
No 7: 2 x 10^1 CN
No 8: Positive control

Figure 3 Visual staining of conventional PCR products using Vivantis Viva SybrGreen Nucleic Acid Stain. in the presence of WSSV-DNA, the sample changed color from orange to green and this amount of fluorescence produced is corresponding to the amount of WSSV-DNA present. Tube 2 shows the highest fluorescence intensity as it contains the highest amount of WSSV-DNA that allows more stain to bind to the DNA and Tube 7 shows the lowest fluorescence intensity as it contains the least amount of WSSV-DNA.

METHODOLOGY

DNA extraction from infected shrimp → Amplification of DNA sample using PCR (Primers: WSSV 500 CF and WSSV 500 CR) → DNA quantification of diluted PCR product using nanophotometer → Conversion of DNA concentration into copy number for WSSV 500 bp → Construction of positive control using diluted PCR product for WSSV 500 → Performing serial dilution that acts as the DNA template for each copy number → Optimization of PCR and analyzation using agarose gel electrophoresis to determine minimum copy number that can be detected → Staining of PCR products using Vivantis Viva Sybrgreen nucleic acid stain

CONCLUSION

- The minimum copy number of WSSV-DNA that can be detected by WSSV 500 Forward and WSSV 500 Common Reverse primers is 2 x 10^1 copies/µl and in the presence of WSSV-DNA in shrimp, the stain changed color from orange to green under blue-light transilluminator.
- These primers used may be applied for future diagnostic use to detect the white spot syndrome virus primarily in shrimp farming industry due to its sensitivity in which it is capable of detecting the WSSV-DNA in a sample even at a low copy number or concentration.

REFERENCES

Chuah, T.T., Oseko, N., Maeno, Y., Kua, B.C. & Palanisamy, V. (2006). Examination for Viral Inactivation of WSSV (White Spot Syndrome Virus) Isolated in Malaysia Using Black Tiger Prawn (*Penaeus monodon*). *Japan Agricultural Research Quarterly*, *40*(1), pp 93-97.

Zipper, H., Brunner, H., Bernhagen, J. & Vizthum, F. (2004). Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications', *Nucleic Acids Research, 32*(12), pp 1-10.