Detection of White Spot Syndrome Virus (WSSV) of Shrimp (*Litopenaeus vannamei*) in UPT Pengembangan Budidaya Air Payau Bangil , Pasuruan District , East Java

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Abstract

Vanname shrimp (Litopenaeus vanname) is still a favourite in shrimp culture production in Indonesia, since it has faster growth advantages. However, the White spot syndrome virus (WSSV) attack is still a big threat for farmers, because it can proliferates rapidly and cause mass mortality of 90-100%. The UPT Pengembangan Budidaya Air Payau in Bangil maintains vanname and controls virus infection by detecting through a molecular approach. Detection using the Nested PCR method with IQ 2000 kit with molecular weight marker 848 bp, 550 bp, 296 bp. The sample will be tested positive if the bands that appear are in the size 296 bp and / or 550 bp . The results of the detection in shrimp rearing ponds were negative (-) because only the band 848 bp that showed, it was mean the shrimp free from WSSV infection. Water quality data shows that the shrimp rearing environment is in optimal conditions.

Keyword : *Litopenaeus vanname, WSSV*

Introduction

The white leg shrimp *Litopenaeus vannamei* is native of the coastal Eastern Tropical Pacific, from Mexico to Peru L. vannamei is the most important species for global crustacean aquaculture, representing over 50% of production). *Litopenaeus vannamei* is the most cultured shrimp throughout the world representing about 90% of shrimp production (Lightner et al., 2012). In Indonesia Shrimp is still a leading commodity, contributing 40% of total national fishery product exports. Since the government issued a policy to introduce it in an effort to overcome the decline in shrimp production (KKP, 2001). This shrimp has several advantages over other penaeid shrimp, such as a higher growth rate and allows it to be maintained at a high stocking density (Yanti, 2017).

Cultivation of Vannamei Shrimp (*Litopenaeus Vannamei*) currently the main commodity because it is increasingly being developed in Pasuruan Regency. This has been intensified by the Pasuruan Regency Government through the Department of Marine and Fisheries (DKP) as an alternative when the cultivation of Tiger Shrimp (*Penaeus Monodon*) has encountered many problems with death due to disease and decreased carrying capacity of the pond environment. Until the end of 2017, Vannamei Shrimp was developed in four districts, namely Bangil, with a potential land area of 991.5 hectares. UPT PBAP conduct Super intensive vaname cultivation with a stocking density of 198 / m^2.

White spot syndrome Virus (WSSV) is a major shrimp diseases, which has cause high mortality rate and economic losses to the major shrimp farming Indonesian. Shrimp production in Indonesia decreased 20% to 555,138 tons due to WSSV (Suriawan, 2019). WSSV caused high mortality rates of up 90–100% within 3–7 days after infection, since it can proliferates rapidly (Zheng, 2019). WSSV is member of the family Nimaviridae, enveloped virus with a large double strands DNA. The virions are ellipsoid to bacilliform in shape with 250–380 nm in length and 80–120 nm in diameter. Double stranded circular DNA virus with a genome of approximately 300 Kb (Wang,2020). Recently, there is a lack of effective prevention and treatment methods for this disease (Cheng, 2020).

WSSV multiplies due to environ-mental stress, temperature, pH, salinity, plankton blooming, melting and spawning (Sekar, 2019). Shrimp of severe WSSV infection showed white spots with 0.5- 2 mm diameters at exoskeleton and epidermis, lack of appetite, death. UPT PBAP Bangil controlled the WSSV infection with detection using *Nested PCR* continually as preventive action.

Materials dan Method

Sample (code: W1 and W2) take in from shrimp cultured pond at UPT PBAP Bangil, east java and detection of virus at UPT PBAP molecular laboratory.

Detection of WSSV

WSSV detection use kit IQ 2000 (GeneReach Biotechnology Corp) has been certified by OIE (World Organization of Animal Health)

DNA Extraction

Add 500µl lysis buffer in a 1.5 ml tube, Put tissue shrimp sample into the tube and grind with disposable grinder, Incubate the prepared sample at 95ºC for 10 minutes, then centrifuge at 12000 g (12000 rpm r = 5~7 cm) for 10 minutes. Transfer 200 µl of the upper clear solution to fresh 1/5 ml tube with 400 µl 95% ethanol. Vortex briefly, centrifuge at 12000 g for 5 minutes, then decant the ethanol and dry the pellet. Dissolve the pellet by ddH2O or TE buffer. 200 µl TE buffer.

Amplification

DNA amplification or propagation is done by mixing DNA template (DNA isolate test sample) with reagent from IQ plus WSSV Kit. DNA amplification is carried out with a thermocycler or PCR device.

The following amplification conditions apply to 0.2 ml thin- wall tube or 96- well plate. And prepare first and nested PCR reaction reagent mixture like in table 1 and table 2.

First PCR

Puppet 8µl of the first PCR reaction reagent mixture into each 0.2 ml reaction tube with proper label. Add 2 µl of the extracted sample DNA or standard into each reaction mixture. Cover each reaction mixture with 20 µl mineral oil unless your thermal cycler is equipped with oil-free design. Perform PCR Reaction

Table 1. reagent mixture first PCR

First PCR reaction reagent mixture : 8 µl / reaction	
First PCR PreMix	7.5 µl
IQzyme DNA Polymerase	0.5 µl

Table 2. reagent Nested PCR reaction

Nested PCR reaction reagent mixture : 15 µl / reaction	
Nested PCR Pre Mix	14 µl
IQzyme DNA Polymerase	1 µl

Nested PCR

Add 15 µl of nested PCR reaction reagent mixture to each tube after first PCR was completed. Be sure that the reagents go through oil overlay. Perform nested PCR reaction, after nested reaction is completed, add 5µl of 6X loading dye to each reaction tube and mix well

Electrophoresis

Prepare 2 % agarose gel, add 2 g agarose into a glass- made wide out bottle or flask with 100 ml TBE buffer, heat in microwave . Cool down the clear agarose around 50ºC and slowly pour into gel box, carefully remove the plastic comb and blockers at the both sides of the gel box when agarose gel is completely coagulated. Add 1x TBE buffer until covering the gel. Load 5 µl PCR product-loading dye mixture into each well and 5 µl DNA marker for reference PCR product size. When samples are loaded, connect gel box to the power

supply before switching on. Constant voltage between 100 V ~ 150 V.

Gel Staining

Pour Ethidium Bromide (EtBr) into plastic tray or zip- lock bag with electrophoresis-finished gel. The solution must cover the whole gel. Shake lightly at room temperature for 10 minutes. Then, distain the gel in another plastic tray with distilled water for another 10 minutes to eliminate the background, Lay the gel o UV transilluminator to read the final result.

Water Quality Measurement

Water quality data obtained from UPT PBAP laboratory Bangil, East Java

Result and Discussion

WSSV Detection

The test results using nested PCR show that the samples W1 and W2 are negative because lane 4 and lane 5 are seen only with bands with an estimated size of 848 bp. The sample will be tested positive if the bands that appear are in the size 296 bp and / or 550 bp. It is suspected that 296 bp and 550 bp are envelope proteins virus that have a major role in infection, they usually play a crucial roles in virus entry, assembly and budding. To our knowledge this is the first report on WSSV envelope proteins found to be involved in gene regulation (Zuo,2011)

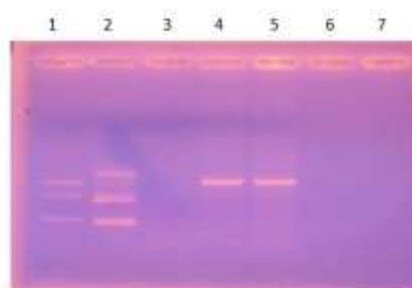

Figure 1. The Electrophoresis WSSV diagnosed, Lane 1 : Molecular weight marker, 848 bp, 630 bp, 333 bp; Lane 2 : WSSV P (+) standard, 2000 copies/ reaction ; Lane 4: WSSV negative sample; Lane 5 : WSSV negative sample

There are several type envelope protein were detected, namely VP28 with size of 615 bp (Megahed, 2015) which could be used directly as a passive immune strategy to control the WSSV infection in shrimp (Wu et al, 2005), dan vial envelope protein VP41B with size 308 bp (Navarro et al, 2018). VP41B was previously reported to be multi-merized to participate the assembly of virus particles and is predicted to o have potential transmembrane domain (Zuo,2011)

Water Quality Measurement

One of the causes of disease attack is a decrease in water quality, therefore control must be carried out every day to maintain the stability of the water quality of the Vanname Shrimp cultivation environment. Data on the quality of water for vanname shrimp culture at the UPT PBAP in Bangil, East Java can be seen in table 3.

The temperature is in the range 27.6 - 31.1^{o} C, Priatna (2004) states that the temperature range for vanname shrimp cultivation is 23.5-30.8 ^{o}C, so that the temperature range in UPT is still in the appropriate range. An increase in temperature will cause oxygen consumption by shrimp to increase so that the metabolic rate of shrimp also increased (Ulaje, 2020). An increase in temperature of 10° C can cause an rise in oxygen consumption of two to three times (Budiarti et al, 2005), the allowable amount of temperature fluctuation is 1,5 C so that the shrimp does not stress and their metabolism is maintained. Research conducted by Zulfikar (2016) at temperature of 7.5-8.5 ^{o}C had no effect on WSSV

Salinity on the plots was in 8- 16, salinity values that too high (> 40 ppt) or too low (<5 ppt) made shrimp more susceptible to disease attacks (Santiago, 2014). According to Effendi (2000), the factor that causes changes in salinity is rainfall. Furthermore, Baliao (2000) states that the ideal salinity for shrimp culture is 15-25 ppt. Salinity is directly related to shrimp osmoregulation, so it can be a water limiting factor (Wadidjah, 1998). Changes in salinity that occur quickly can cause shrimp mortality. Salinity below 15 ppt causes the shrimp immune system to be vulnerable to WSSV attack (Zulfikar, 2016).

The pH data from the Vanname shrimp rearing plots in the UPT was in the range of 6.83- 8.16. Wardoyo (1997) stated that the ideal pH for shrimp cultivation is in the range of 6.8-9.0. A pH higher than 8.5, a water change must be performed because it indicates an increase in toxic ammonia. Previous research by (Zulfikar, 2019) showed a pH range of 7.5- 8.5 had no effect on WSSV infection

Table 3. Water quality data Vannamei Shrimp

No	Parameter	Unit	Value
1	Temperature	°C	27-30
2	Salinity	‰	8-16
3	pH	-	6,83-8,16

Conclusion

Detection carried out on shrimp samples cultivated by the UPT PBAP Bangil showed that they were not infected with WSSV and the water quality of the shrimp culture environment was still in the optimal range for shrimp growth.

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