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EXPERIMENTAL INFECTION MODELS FOR THE SHRIMP VIRUS WHITE SPOT SYNDROME VIRUS STUDIES (WSSV): REVIEW

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Abstract - White spot syndrome Virus (WSSV) is a major pathogen in shrimp aquaculture, and its rampant spread has resulted in great economic loss. WSSV is the most devastating Vibriosis threatening the shrimp culture ponds usually lead to the outbreak of the White spot syndrome Virus (WSSV). The enveloped virus particle has an ovoid- to bacilliform shape with a tail like appendage. The Virus replicates and assembles in the nucleus of infected cells. The review presents the usefulness of survey on the Infection models for shrimp virus (WSSV) for Crustaceans experiments.

Key Words: White spot syndrome virus, Crustacean, WSSV, Economic loss, Penaeid shrimp, Outbreak.

1. INTRODUCTION

The crustacean farming industry has been suffering solmen problems and enormous economic losses from an outbreak of White Spot Syndrome Virus (WSSV) is an enveloped Virus With a circular double strand DNA of about 300 Kb belonging to a new virus family Nimiviridae (Van Hulten , et al .,2001;Yang et al., .,2001). Shrimp Cultivation is often affected by outbreaks of deadly infectious diseases caused by mainly by viruses. In the maintenance of substantial production of farmed Shrimp, an understanding of the shrimp immune system would allow for the development of management strategies to control virulent or problematic pathogens encountered on shrimp farms. The virus can infect many kinds of freshwater shrimp. Conventional identification of the Environmental factors for shrimp. Field survey showed that in seasons with temperature lower than 20^0 C or higher than 30^0 C the outbreak of WSSV is abated; and temperatures between 22^0C to 30^0 C allow WSSV to replicate at much high rate (Withyachumnarkul.,2003; Mosker et al. ,2012). Studies in laboratory also verified the above pheromone that temperature is crucial in determining WSSV proliferation (Du et al .,2008) Gao et al .,2011;You et al.,2010).

Viral diseases and associated mortalities are emerging as the major threat to Penaeid shrimp culture (Sindermann, 1990). Though just a few nanometers in diameter and when laid side by side, 310 million of them stretch a little less than half an inch, they can virtually wipe out the farm raised shrimp crop and cause anxiety to shrimp aquaculturists about the future (Otta et al., 1998).WSD causes severe mortality and kills the cultured shrimps during 3-10 days (Lightner, 1996). The outbreak of WSD was first reported from Marsu Penaeus japonicus shrimps are susceptible to be infected by the White Spot Syndrome virus (WSSV) (Rodriguez et al .,2003). In South East Asian Countries, WSSV was reported in Kuruma prawn, black tiger shrimp P.monodon red tail shrimp P.penicillatus and Chinese prawn (Chou et al., 1995; Lightner, 1996). Methodological approach to evaluate the Disease Diagnosis and control measures will be presented in this review.

1.1 Risk factors for WSSV outbreaks in Penaeus monodon

Total alkalinity during both production cycles was above 80 ppm and suitable for P.monodon culture (Chanratchkool, 1995; Tookwinas, 2000). Further probiotics encourage the proliferation of bacteria that inhibit the colonization of pathogens (Das et al., 2006; Ganguly et al., 2010). Vibrio infection due to poor environmental conditions make shrimp susceptible to WSSV (Hettiarachi et al., 1999). The poor conditions may have been attributable to sub optimal temperature and salinity. Temperature and salinity affect immune response of crustaceans (Vargas-Albores et al., 1998; Le Moullac and Haffner ,2000). Salinity affects the immune response of Marus peneaus japonicus the further from the original salinity the shrimp are maintained the weaker their immune response of Marus Penaeus japonicus ; (Yu et al.,2003). The ideal salinity for shrimp culture is 15-25 ppt (Baliao, 2000). Acute salinity stress is more significant at low salinity than that high affecting the immuno concern of P.monodon and resulting in increased susceptible to WSSV infection Shrimp maintained at 15 ppt did not completely eliminate virus particles from circulation and thwart infection (Joseph and Phillip. 2007).

1.2 Occasional white spot syndrome Virus Outbreak in Traditional Paddy Cum Prawn Fields in India.

Shrimp Production has increased almost exponentially since the mid -1970 s and now accounts for about 58 Percent of aquaculture production from brackish water (Bosock et al., 2010).

Isolation and Identification of Total Heterotrophic Bacterial Isolates. (Secondary bacterial infections in WSSV infected P.monodon).

After recording the morphological characters and pigmentation on representative types constituting at least 20-40 numbers of colonies were selected from each plate and resteaked on to TSA,1/2 ZMA or ZMA plates to ensure purity. All the purified TSA or ½ ZMA or ZMA slants further characterization and identified to generic level using the taxonomic key for identification by (Muroga, 1987) and (Barrow et al.,1993). The proteinase genomic DNA from mammalian tissue (Strauss, 1994). The PCR products were analyzed in 1% agarose gel containing ethidium bromide at a concentration of 0.05 µg/ ml and visualized under ultraviolet Transillumination (Lo et al., 1996).

In the shrimp ponds and the feeder canal, TAN values were between 0.0039-1.87 mg/l. The nitrate level recorded during the study are very low and were well below the safe level (1.28 mg/l) of nitrite recommended for Penaeus monodon by (Law, 1988). Bottom soil quality is an some of it (Boyd, 1989). A significant decrease in haemolymph OP seen as the water P^H decreased from 7.0 to 6.5 (Lemmonnier ,et al.,2004). Many studies have reported growth of Penaeus monodon in fresh water and low salinity as low as 2ppt. Shrimp farmers have often expressed concerns over the polluted water surrounding their farms ,as pollution by industrial commercial and urban contaminations (Hirono,1992). Can slow growth increase disease Outbreaks and accelerates the mortality rate of shrimps. Low levels of DO (< 3.7 mg/L) when combined with high NH3 –N (0.5 mg/L) has shown to be harmful for P.semisulcatus (Wajsbort, et al., 1990). (Burnett and Burnett 2000) suggested hypoxia results in a depression of the generalized innate immune response in Paleo monetes pugio, Penaeus vannamei on the basis of measurements of circulating haemocytes and survival of shrimp exposed to vibrio. (Guerrero – Galvan et al., 1998). Have reported that during the rainy season, dissolved oxygen tend to decrease, as the feeding rates and shrimp and phytoplankton biomass were increasing until harvest. Several studies have proved the contribution of settled sludge that is not removed produces 44% more TAN than in tanks, where they are removed. High levels of TAN were recorded in the Perennial ponds and the seasonal ponds. It was 2-180 times more than the safe level (0.01 mg/L NH3 – N) Calculated by (Chin and Chen, 1987). For P.monodon larvae Bio agumentation of the ponds with Bacteria that can Oxidize ammonia and nitrite Could be used to improve water quality, as (Rao, et al ., 2000).

1.3 WSSV infection in P.monodon is facillated by housekeeping by molecules

P.monodon is the commercially important species. In recent years production of P.monodon interactions and unevil the underlying mechanisms involved in WSSV entry and pathogenesis in shrimp. Due to its serious impact on shrimp farming, there is an urgent need to understand virus-host partners of WSSV in P.monodon WSSV interacting proteins from subcuticular epithelial tissue of P.monodon using direct Protein- Protein- interaction approach.

Protein identification by MALDI-TOFMS

Spots corresponding to the proteins of interest were excised from 1D and 2D SDS PAGE gels and were subjected to alkylation followed by in gel digestion with trypsin. The virus overlay Protein binding assay has been employed successfully to identify a number of putative virus interacting Proteins (Upanan et al., 2008; Liang et al., 2010; Paingankar et al., 2010). A number of different Proteins that were able to bind to WSSV were identified using VOPBA and MALDI-TOF analysis HSP70 has been shown as host interacting partner of various animal viruses. (Xu et al., 2009). The level of HSP 70 has been showed to increase in heamolymph of WSSV-infected crab Scylla serratae (Liu et al., 2011). Whereas it has been also shown that HSP 70 levels decreases after WSSV infection in subcuticular epithelium of P.monodon (Wu et al., 2007).

1.4 DNA Extraction and Detection of WSSV by PCR

The hemolymph, stomach, gonad, receptaculum seminis and whole body of juveniles were individually homogenized and digested with ISOGEN (Japan Gene Co). The total DNA was amplified using two specific primer sets P1/P2 and P3/ P4. After 30 cycles amplification for each primer set at 93 ^{0}c (60 sec) 57 ^{0} c (90 sec) and 72 ^{0}c (60 sec) the amplified products were analyzed by agarose gel electrophoresis. A known WSSV-infected P.japonicus was processed as a positive control. The development stage in which WSSV was first detected by PCR was in eggs followed by PL5 and PL10, when the water temperature was 22.1-29.4^{0}c WSSV was detected IN PL 29 and PL 51 at the nursery stage. High mortality rates reaching 50-100% Occurred within 10days in the nursery facillates after detection of WSSV. WSSV was detected in the receptaculum seminis at higher rates after spawning than before spawning and its prevalence increased rapidly from june onwards. The stomach is not suitable target organ for WSSV was detected in receptaculum seminis at a higher rate after spawning than in ovaries before spawning (Mushiake et al., 1999). Therefore the use of receptaculum seminis is recommended as the target organ for WSSV detection for brood stock solution. There is no data identifying a similar Phenomenon in Kuruma prawn however. The Possibility might exist because the spawning season of Kuruma prawn ranges between April and October and this species repeats copulates and spawning several times in one season.

1.5 Polymerase chain reaction (PCR) detection of white spot syndrome virus in cultured and wild crustaceans in India

The WSSV carriers are common in natural shrimp stocks and other crustaceans in India. The inner surface (Takashi et al., 1994). In outbreak shrimp ponds, Cumulative mortality may reach 80 to 100% within 7 to10 d post-

infection (Nakano et al ., 1994., Karunasagar et al., 1997). Molecular methods such as gene probes (Durand et al., 1996). (Lo et al., 1996a) Wongteerasupya et al., 1996). (Nunan and Lightner 1997) and polymerase chain reaction (Takashi et al.,1996, Lo et al,1996 a33) have also been found useful in diagnosis WSSV infection , Particularly with carrier animals showing no gross signs of disease. For nested 2-step PCR (Lo et al .,1996 b). IPI of a post PCR mixture using the primer pair PMI /PM2 was used as the template for a second PCR reaction with the primer pair PM3/ PM4. The results showed that a significant proportion of grossly healthy Penaeus monodon post larvae from Indian hatcheries carry WSSV. Thus there is a need to screen apparently healthy for WSSV by PCR before stocking in ponds.

Diagnostic and Preventive Practices for WSSV

The study focused on the epizootiology of this viral disease in both seed production and nursery cultures farms from 1996 to 1999. It was found that elimination of eggs from WSSV –Positive spawners by polymerase chain reaction (PCR) based technique was an effective control measure against WSSV in seed production. Brood stocks were captured in five different areas. Samples of haemolymph, stomach and gonad of wild female and male prawn were submitted to WSSV detection by PCR as described. The mean body weight of females and males were 78%-10.5 and 44.7-63.9g, respectively the stomach and gonad samples were aseptically extracted at volume of 100µg, and stocked at 80 ^{0}c until the PCR analysis.

1.6 RNA i technology promising solution to shrimp virology

RNA i technology is for therapeutic intervention treatment of viral infections, dominant disorders ,neurological disorders and many types of cancers Recent discoveries show that a class of RNA molecule (small RNAS) operates many of the cells controls (Elbashir et al., 2001., Bentic,2007) provided a biochemical understanding of RNA i pathway. The study showed that the functional units of RNA i are likely represented by ds RNAs shorter than 30 base pairs (MC Caffery et al., 2002). The initial studies of RNA i focused on cellular RNA targets (Rand et al ., 2003) but present studies are on targeting sequence Specific RNAs (Xu et al., 2007). Reported the use of both exogenously synthetic long ds RNAs to induce an antiviral response in shrimp Double -stranded RNA is Recognized by Toll pathway in shrimp antiviral immunity in a sequence independent manner (Arts et al .,2007). Alternative approaches are use of edible ds RNA producing bacteria (Sarathi et al., 2008).

CONCLUSIONS

From this review, it should be evident that some black tiger shrimp P.monodon, P.penicillatus and Chinese Prawn P.chinesis (Chou et al., 1995. Peng et al., 1995). Risk factors poor environmental conditions have been attribute to sub optimal temperature and salinity. Immune response of crustaceans (Vargas-Albores et al.1998). Occasional WSSV Outbreak in Traditional Paddy Cum Prawn fields. Aquaculture production from brackish water (Bostock et al., 2010). PCR detection of WSSV cultured and wild Penaeus crustaceans outbreak shrimp cumulative mortality post infection (Nakano et al., 1994; Karunasagar et al., 1997).

RNA i technology at shrimp virology recent discoveries show that the a class of RNA molecules operates many cells of control by using variety of the shrimps. To identifies the viral diseases (Elbashir et al., 2001; Bentic, 2007). Biochemical understanding in RNA pathway. The immune system of shrimp is of great research interest and the use of different immunostimulants has proved effective in improving the immune system of shrimp and thereby reducing mortality. However, such practices may not help in heavily contaminated areas due to various reasons. It is to be expected that in the long run, immunostimulants will help sustain shrimp aquaculture. There is a need for reproducible and standardized experimental models in WSSV virus at many shrimp species. As pointed out this review ,standardization of each step of pathogenicity test is different environmental parameters, RNA i technology WSSV outbreaks reason for shrimp factors may influence the results of viral diseases experiment.

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