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Susceptibility of *Macrobrachium rosenbergii* to Local White Spot Syndrome Virus Isolate Using Immersion Assay

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Abstract: White spot syndrome virus (WSSV) continue to be one of the leading causes of serious epizootic in cultured shrimp. *Macrobrachium rosenbergii* or giant freshwater prawns are found to be resistant in White spot syndrome virus (WSSV). However, conflicting studies have shown that pathogenicity of WSSV to *M.rosenbergii* may vary according to the life cycle of prawn, strain of WSSV and the source of the virus during passaging. On this preliminary study, the susceptibility of *M. rosenbergii* stocks to local strain of WSSV as exposed via immersion assay will be determined. Likewise, the pathogenicity of WSSV will be identified using the median lethal dose (LD50). The confirmation for the presence of WSSV on giant freshwater prawns will be done using nested PCR. The preliminary study will provide valuable data for the succeeding experiments.
Key Words: WSSV, *Macrobrachium rosenbergii,* immersion assay

1. INTRODUCTION

Infectious diseases continue to be one of the limiting factors to the expansion and sustainable growth of different aquaculture. Most of these diseases are associated with infectious agents such as bacteria and viruses. Among the known viruses of crustaceans, white spot disease caused by the White Spot Syndrome Virus (WSSV) greatly affects crustacean aquaculture and continues to be an obstacle toward sustainable farming (Walker *et al.,* 2011). The WSSV has a wide spectrum of host which includes crayfish, lobsters, crabs, shrimps and freshwater prawns (Escobedo-Bonilla *et. al.,* 2007; Rajendran *et al.,* 1999). Some of these species will not die from the virus, but act as carriers which pose a serious threat in transmitting infection to cultured host (Flegel, 2007).

WSSV was first reported in the Philippines in 2000 wherein 72% (51 out of 71) of the cultured *Penaeus monodon* samples were tested positive through PCR detection (Magbanua *et al*., 2000). In 2007, WSSV has already become established in the local marine environment and in wild populations of P. monodon in the Philippines. Thus, broodstock collected during the dry season could serve as the main source of WSSV contamination in shrimp farms due to vertical transmission of the virus in hatcheries (De la Peña *et al.,* 2007). Immunostimulants (Andrino *et al.,* 2014; Amar & Faisan Jr., 2012; Genio *et al.,* 2014) and molecular techniques (Alenton *et al.,*

2016; Maningas & Tare, 2015; Lazarte & Maningas, 2015) are being tested in the Philippines to prevent and lessen the burden of the pathogen.

Macrobrachium rosenbergii or giant freshwater is the most widely culture freshwater prawn species with the annual yield of 30000 t (FAO, 2009). In comparison to vulnerability of diseases in culture, the *M. rosenbergii* is less susceptible than *P. monodon* (Sahul Hameed *et al.,* 2000). However, conflicting reports have found when it comes to the resiliency of the prawns to WSSV. Some studies identified that certain life stages of *M. rosenbergii* are more susceptible to WSSV. Kiran, Rajendran, Jung and Oh (2002) found out that larval and postlarval stages were susceptible but older prawns were quite refractive to acute infection and mortality. Studies showed that difference on the mortality of giant freshwater prawns occur when exposing to different strains of WSSV (Corteel *et al.,* 2012). This due to the different parameters such as onset and severity of disease and median lethal dose, lethal time and infection dose which are specific for each viral strain (Escobedo-Bonilla *et al.,* 2006). Differential host passaging of WSSV also shows variability on genes that may be related to the virulence of the virus (Waikhom *et al.,* 2006). *Macrobrachium rosenbergii* shows different mortality based on its life stages. Reference showed that except the post-larval and juvenile stages, all other life-stages of *M. rosenbergii* are tolerant to WSSV using intramuscular injection assay (Kiran *et al.,* 2002).

Aside from infecting the host through oral assay, another way of simulating the natural route of virus entry will be through immersion assay (Escobedo-Bonilla *et. al.,* 2007). On this study, the susceptibility of *M. rosenbergii* stocks to local strain of WSSV as exposed via immersion assay will be determined. Likewise, the pathogenicity of WSSV will be identified using the median lethal dose (LD50). As a preliminary study, this will be a valuable data for the succeeding experiments.

2. METHODOLOGY

A. Prawns

Post-larvae 20 (PL20) *Macrobrachium rosenbergii* will be imported from BFAR Muñoz, Nueva Ecija. Prior to the challenge, prawns will be acclimatized and reared for a period of 7 days after the arrival. It will be fed with a commercial pelleted food twice daily. Water temperature will be kept at 27-29°C throughout the acclimatization period.

i. Bacterial Analysis

Three randomly selected *M. rosenbergii* will be tested for the bacterial count (cfu/g). Prawns will be weighed and homogenized using sterilized normal saline solution (NSS) (0.1g/ml). Homogenized solution will be serially diluted (10^{-1} to 10^{-6}) and 100 µl for each dilution will be transferred in nutrient agar plates using conventional spread plate method. Inoculated plates will be incubated at room temperature for 18-24h.

ii. WSSV Diagnosis

Ten randomly selected *M. rosenbergii* will be weighed and preserved to 95% ethanol. Prawns will be sent to SEAFDEC Tigbauan Main Station, Tigbauan, Iloilo. Animals will be tested for WSSV by nested PCR to confirm the absence of the virus and to ensure that prawns to be used for the experiments are free from WSSV.

B. WSSV

Isolate of WSSV from *Litopenaeus vannamei* moribund tissues will be provided by the SEAFDEC Tigbauan Main Station, Tigbauan, Iloilo. Infected tissues will be stored in -20°C environment.

C. Production of WSSV stock

Viral amplification will be done by injecting the diluted WSSV into healthy *Litopenaeus vannamei* juveniles (Escobedo-Bonilla *et al.,* 2005). The inoculated shrimp will be collected at 48-hour post inoculation (hpi) and will be frozen at -20°C. Gills and head tissues from the carcasses of shrimps will be minced and homogenized in ice-cold phosphate buffer solution (PBS). The homogenate will then be centrifuged at 9000 rpm for 10 mins at 4°C and the supernatant will be filtered through filter syringe. This will be done for three times and the last passage will be used for the challenge. The stock inoculum will be divided into aliquots and later will be stored at -20°C.

D. Immersion Assay

Twenty prawns will be immersed for 2h in 3 l of water with 10-fold serial dilutions of WSSV (10^{-1} to 10^{-5}). Negative control prawns will be immersed in 30ml PBS. After immersion, the prawns will be returned to aquaria.

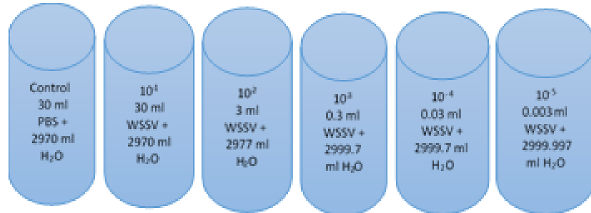

Figure 1. WSSV immersion assay set-up for each dilution.

Sixty prawns (20 prawns in each 3 replicates) per dilution will be tested. For each set-up, 18 20 liters of aerated and covered aquaria will be used. Following the challenge test, the test prawns will be observed for 14d. At the end of the experiment, surviving prawns will be sacrificed. Moribund tissues will be preserved and sent for the presence of WSSV and histopathology.

E. Determination of Lethal Dose (LD50)

Prawns will be examined at 24 h intervals (24, 48, 72, 96, 120 and 144 hpi) for gross disease signs and to record deaths and moribund pawns. The challenged will last up to 14 days. The mortality (LD50 ml^{-1}) will be calculated using the method of Reed & Muench (1983).

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