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- Brief Communication -

Experimental Infection of White Spot Syndrome Virus (WSSV) in Wild Crab, *Gaetice depressus*

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To confirm the possibility of a wild marine crab, *Gaetice depressus*, as a carrier for white spot syndrome virus (WSSV) and to develop an alternative experimental model for WSSV in winter season, the susceptibility of the crab to WSSV was assessed by artificial challenge and subsequently tested for infection by PCR assay. The results revealed that the crabs were as highly susceptible as penaeid shrimps. WSSV caused 100% mortality in *G. depressus* within 16 days after intramuscular injection. The presence of WSSV in the moribund crabs was confirmed by PCR and was found in gills and muscle tissue. These results suggest that *G. depressus* can be naturally infected by WSSV via moribund shrimps, and can act as a potential carrier of WSSV. In addition, *G. depressus* can be used as an alternative experimental animal for WSSV.

Key words : WSSV, *Gaetice depressus*, Susceptibility, Carrier, Alternative experimental animal

White spot syndrome virus (WSSV) is the causative agent of a severe shrimp viral disease, and is characterized by white spots on the exoskeleton and epidermis of affected shrimp. WSSV first appeared in northeast Asia in 1992-93, and rapidly spreaded to shrimp farms in many countries in Asia, Indo-Pacific and Western hemisphere. Since its appearance in Asia, WSSV has caused major losses to the shrimp culture industries in several countries, including Korea.

A most interesting feature of WSSV is its broad host range (Flegel 1997). It can infect a wide range of arthropods including several species of penaeid shrimp (Lu *et al.* 1997, Corsin *et al.* 2001), crabs (Chen *et al.* 2000, Sahul Hameed *et al.* 2001), fresh water crayfish (Huang *et al.* 2001, Jiravanichpaisal *et al.* 2001) and Artemia (Li *et al.*, 2003).

Gaetice depressus is a crab species found commonly along the coastal areas including shrimp-rearing ponds of Korea. In the present study, to confirm the possibility of this crab as a carrier for WSSV and to develop an alternative experimental animal for WSSV in winter season, the susceptibility of the crab to WSSV was assessed by artificial challenge and subsequently tested for infection by PCR assay.

Wild marine crab, *Gaetice depressus* (3-4 g body weight) was collected from the Dong-baek island of Pusan, where there have been no shrimp cultures, in Korea. After acclimation for a week, five crabs were randomly selected and screened for WSSV by polymerase chain reaction (PCR). For WSSV challenge experiment, the crabs were randomly divided into 2 groups of 8 crabs in each group. Each crab was placed in individual small cages to prevent cannibalism. During acclimation and experimental periods, crabs were fed with minced marine fish muscle. The water temperature and salinity were adjusted to 26 ± 1℃ and 33 ‰, respectively.

Deep-freezed WSSV infected shrimps, *Penaeus*

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chinensis, which were collected from a shrimp farm, were thawed and about 3 g of muscle tissue was ground using a homogenizer (ART-Moderne Labortechnik, Germany) in 10-fold volume of phosphate buffered saline (PBS) and centrifuged at 12,000 × g at 4 ℃ for 10 min. The supernatant was collected and divided into 1 ml aliquote. The presence of WSSV in the stock solution was confirmed by PCR.

The crabs were injected intramuscularly with the WSSV viral solution at a dose of 0.05 ml of the stock solution. Crabs in the control group were intramuscularly injected with 0.05 ml PBS. After injection, the dead crabs were recorded every day, and were stored immediately at -80℃ until WSSV diagnosis by PCR.

Total DNA was extracted from the crab muscle and gill with AccuPrep® Genomic DNA Extraction Kit (Bioneer, Korea). The PCR primers (VPexpF26, 5'-GGGAATTCATGGAATTTG-GCAACCTAAC-3' and VPexpR26, 5'-GGTC-GACCTTTACTTCTTGATTTCGTCC-3') were used to amplify a 650 base pairs fragment of WSSV DNA. The amplified products were electrophoresed in 2 % agarose gel incorporated with ethidium bromide at a concentration of 0.5 ug/ml in 1 × Tris-acetate-EDTA (TAE) buffer and the gels were analysed using Gel-Doc system (Bio-Rad).

By the PCR analysis of the 5 crabs randomly sampled before challenge experiment, the wild marine crab, *Gaetic depressus*, was proved to be initially free of any natural WSSV infection.

In the challenge experiment, the group of crabs intramuscularly injected with WSSV showed 100% mortality by days 16 post injection. In contrast, no mortality was observed from the control crabs, which were injected with PBS only, during the experimental period.

PCR analysis on dead crabs injected with WSSV revealed a distinct band of amplified DNA of 650 bp after electrophoresis of the PCR product, and the band was observed for extracts from both gills and muscle tissue (Figs. 1 & 2). However, no band was observed in live crabs of the control group.

The present study showed that the wild marine crab, *Gaetic depressus*, was highly susceptible to WSSV infection. This suggests that *G. depressus* may be naturally infected by WSSV via moribund shrimps, and can act as potential carrier of WSSV. Several studies have confirmed that many suspected decapod carriers of WSSV can transmit the virus to penaeid shrimps (Wongteerasupaya *et al.* 1996, Chang *et al.* 1998, Wang *et al.* 1998). Therefore, WSSV transmitting possibility of wild arthropods inhabiting near the shrimp farms and those used as raw feeds for shrimps should be studied further to

Fig. 1. PCR detection of WSSV-DNA in the gill of the dead crab by intramuscular injection of WSSV. M, marker; 1, positive control; 2, negative control; 3-10, died crab at 1, 5, 6, 8, 13 and 16 days after the injection, respectively. M, 100 bp marker.

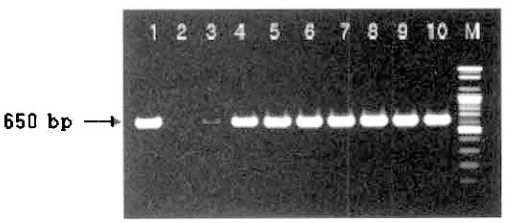

Fig. 2. PCR detection of WSSV-DNA in the muscle of the dead crab by intramuscular injection of WSSV. 1, positive control; 2, negative control; 3-10, died crab at 1, 5, 6, 8, 13 and 16 days after the injection, respectively. M, 100 bp marker.

development of control measures against WSSV disease in shrimp farms.

The present results also suggest that *G. depressus* can be used as an alternative experimental animal for WSSV. As this crab can be collected easily through year, experiments related to WSSV can be conducted stably, especially, in winter, which period has been a bottleneck for WSSV experiments using penaeid shrimps in Korea.

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