J. Grad. Sch. Biosp. Sci.,
Hiroshima Univ. (2003), **42** : 44~45

Studies on quasi-immune response of kuruma shrimp (*Penaeus japonicus*) against white spot syndrome virus (WSSV)

Jin Lu Wu

Graduate School of Biosphere Science, Hiroshima University,
Higashi-hiroshima 739-8528, Japan

White spot syndrome (WSS) has caused mass mortalities among cultured penaeid shrimp throughout the world since 1993. Because of the broad host and geography range of the causative agent, white spot syndrome virus (WSSV), the disease is a serious threat not only to the shrimp culture industry but also to marine crustacean ecology. WSS can be controlled in hatcheries by selecting WSSV-free spawners based on the detection of WSSV by PCR, but a total system for prophylaxis and control of the disease in grown-out ponds has not been established. In the present study, effects of rearing density of kuruma shrimp *Penaeus japonicus* on the mortality in experimental WSS, quasi-immune response of *P. japonicus* against WSSV, and application trials of the quasi-immune response were studied.

In the first step of the study, a method of preparation of WSSV inoculum for challenge tests in kuruma shrimp was developed. The virus concentration in the hemolymph drawn from moribund shrimp was quantified as 1.5×10^{7} genome copies /μL hemolymph by competitive PCR. The LD_{50} of the inoculum prepared from the hemolymph was calculated as $10^{-4.2}$ μL hemolymph /g shrimp by IM-challenges, or 9.5×10^{2} genome copies/g shrimp. Virulence testes by IM-injections with the virus preparations stocked at -80℃ demonstrated that the virus preparation can be preserved for 16 months without significant reduction of the virulence.

To investigate the effects of shrimp density on mortalities of *P. japonicus* in experimental WSSV-injected shrimp were reared at different densities. The cumulative mortalities of WSSV-injected shrimp for 14 days in the high (260 shrimp/m^2), middle (135 shrimp/m^2) and low (73 shrimp/m^2) density groups were 72, 46 and 18%, respectively. In the next experiment, injected shrimp were reared collectively in 3 tanks (A, B and C) at the same high density (260 shrimp/m^2); A: dead shrimp were immediately removed to avoid transmission of the pathogen through cannibalism and the waterborne route, B: dead shrimp were removed at scheduled times but were separated from living shrimp by a net partition to avoid cannibalism, C: dead shrimp were removed twice a day at scheduled times. Resulting cumulative mortalities for 20 days in groups A, B and C were 4, 24, and 64%, respectively. These results show that the higher mortalities occur in *P. japonicus* reared at the higher densities in experimental WSSV infection, and this phenomenon is mainly caused by a higher opportunity of horizontal transmission of the virus through cannibalism and the waterborne route.

Our previous researches have shown that the survivors of *P. japonicus* from natural or experimental WSS acquired the protection against WSSV challenge. In the present chapter, the onset and duration of the resistance in experimental survivors of *P. japonicus* produced by an IM injection with WSSV were surveyed by re-challenge tests. Re-challenge tests of the survivors conducted at Weeks 1~4 PIE revealed that the resistance commenced at Week 3 (relative percent

survival, RPS: 39%) and almost fully developed at Week 4 (RPS: 58%). Re-challenges at Months 1~3 PIE resulted in RPS values at 67, 54 and 6%, respectively, indicating the resistance persisted until Month 2. RPS values in neutralization tests performed at Weeks 1~4 and Months 1~3 PIE were -5, 14, 36, 50, 100, 38 and 6%, respectively, which coincided with the RPS values in each re-challenge test conducted in parallel. These results demonstrated that resistance of *P. japonicus* against the viral pathogen developed 3 or 4 weeks after an exposure to the virus, and it persisted for another month at 24℃. The resistance was paralleled by a humoral neutralizing factor(s) in the plasma of shrimp.

To investigate the specificity of the quasi-immune response, experimental WSS-survivors on Day 30 were IM-challenged with *Vibrio penaeicida*, the causative bacterium of vibriosis in *P. japonicus*. WSS-survivors showed the resistance to WSSV (RPS: 83.3%) but not to *V. penaeicida* (RPS: 11.8%).

Protein profiles of the WSS-survivor plasma were analyzed by gel filtration, cation exchange chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). No significant differences in protein profiles were found by the gel filtration and SDS-PAGE analyses between the WSS-survivor and control plasmas. However, a distinct fraction was observed only in the WSS-survivor plasma by cation exchange chromatography.

DNA fragmentation was demonstrated to occur in experimentally WSSV-infected *P. japonicus* by TdT-mediated dUTP nick-end labeling (TUNEL) assay. The DNA fragmentation was frequently observed in the hematopoietic tissue, lymphoid organ, stomach epithelium and cuticular epithelium. The apoptosis-sensitive cells are relatively abundant in lymphoid organ compared with the stomach.

Potency of inducing the resistance to WSSV infection by IM-injection with inactivated WSSV with or without glucan or killed *V. penaeicida* was tested in kuruma shrimp. Formalin-inactivated WSSV presented a potency for inducing the protection on Day 10 post-vaccination with RPS values of 50% but heat-inactivated WSSV did not. No protection was observed on Day 30 in both formalin and heat-inactivated WSSV injected groups. When shrimp were vaccinated with formalin-inactivated WSSV with β-1,3-glucan (50 μg/ g shrimp) or with formalin-killed *V. penaeicida* (0.15 mg/ g shrimp), RPS values after an IM-challenge test carried out on Day 30 were 31 and 36%, respectively.