Study on VP15 of the White Spot Syndrome Virus for a Protective Effect against White Spot Disease in Kuruma Shrimp

(Doctoral qualification by coursework, Form 7)

Abstract of Dissertation

Department : Bioscience Name : Jirayu Boonyakida

Dissertation title : Study on VP15 of the White Spot Syndrome Virus for a Protective Effect against White Spot Disease in Kuruma Shrimp

Abstract : WSSV is one of the devastating shrimp pathogenic viruses causing a huge annual loss in shrimp aquaculture industries worldwide. Therefore, developing an efficient procedure for controlling the disease is required. WSSV contains at least five major structural proteins, including VP15, VP19, VP24, VP26, and VP28. These proteins can interact with host proteins or receptors to initiate a systemic infection and induce host immune responses. Later the concept of 'trained immunity' or 'immune priming' hints that the invertebrate species may possess a 'quasi-specific immunity' that can be primed to modulate the immune response against the interest causative agent. Here, the five major VPs (VP15, VP19, VP24, VP26, and VP28) from WSSV were cloned and expressed using the *E. coli* expression system and were further purified using the GST-affinity technique. The purified proteins were employed as candidate immunizing agents against WSSV in kuruma shrimp (Marsupenaeus japonicus). The strategy used for shrimp immunization was the prime-and-boost approach. The relative percent survival (RPS) values after the WSSV challenge were 78%, 27%, 56%, 44%, and 50% in the groups injected with VP15, VP19, VP24, VP26, and VP28, respectively. In vivo antiviral assay in this study newly revealed that the major nucleocapsid protein VP15, a DNA-binding protein, provided the most substantial protection against viral infection in immune-primed shrimp.

From the above result, we further investigated to identify the antigenic domain of VP15 by designing several truncated VP15 constructs; $VP15_{(1-25)}$, $VP15_{(26-57)}$, $VP15_{(58-80)}$, and $VP15_{(1-25,\ 58-80)}$. These truncated VP15s were expressed in E. coli and purified via the GST-affinity technique. Next, the proteins were used to immunize kuruma shrimp against the viral infection with the prime-and-boost strategy. Among the truncated VP15s, the $VP15_{(26-57)}$ gave the highest RPS value of 48% and was the only construct that provided a statistically significant protective effect. Based on the in vivo animal experiment, we hypothesized that the domain might contain a peptide-level antigenic determinant that could confer protection in shrimp against the virus. Four peptides named KR11, SR11, SK10, and KK13 were derived

from the secondary structure of VP15(26-57) and applied in vivo to investigate the effect of each peptide on the survival rate of the tested animal. While the groups receiving KR11, SK10, or KK13 showed survival rates and RPSs similar to the PBS control group, the group receiving SR11 showed an improvement in survival rate. The RPS value was similar to the group receiving VP15(26-57). Moreover, a gC1qR homolog from kuruma shrimp (MjgC1qR), a multifunctional protein of innate immunity, was cloned and expressed using a silkworm expression system. GST pull-down assay revealed that MjgC1qR could interact with VP15, VP15(26-57), and SR11. These results suggested that the peptide SR11 derived from VP15 could be a potential antigenic determinant enhancing shrimp resistance against WSSV.

Due to the high protein production capacity and post-translational processing of the silkworm (Bombyx mori) expression system. The system was applied to produce VP15 and VP19. The silkworm-produced VP15 and VP19 were then purified from the fat bodies using the Flag-tag affinity technique. Subsequently, the purified proteins were used for immunization in shrimp. The group immunized with silkworm-produced VP15 showed an RPS of 33.3%, while the silkworm-produced VP19 gave a survival rate similar to the PBS control group. This result verified that the VP15 produced by silkworm could also provide a protective effect on kuruma shrimp. The biological activity of the silkworm-produced VP15 was not significantly different from the E. coli-produced VP15.

Despite all the known countermeasures against WSSV, administering an anti-WSSV agent is considered the most effective measure despite the invertebrate species' lack of adaptive immunity. Among the mode of administration, oral administration of an immunizing agent is deemed to be the best and most manageable approach. As the *B. mori* pupae have been directly applied in farmed animal industries as feed or as a food supplement, the pupae have also been used as a protein expression platform. Combining these concepts, the silkworm pupae were used as a vector for delivering immunizing agents to shrimp in this study. The VP15, VP15(26-57), and SR11 were expressed as GST-fusion proteins using silkworm pupae. Next, the pupae with expressed recombinant proteins were mixed with a commercial feed and used as feed for kuruma shrimp. Shrimp fed with pupa/VP15, pupa/ VP15(26-57), or pupa/SR11 improved survival rate with an RPS of 81%, 100%, and 72%, respectively, compared to the control. Moreover, we have determined the changes in mRNA transcript of immune-related genes. Our findings indicate that oral vaccination of shrimp using pupa/VP15-derived products is an attractive preventive measure against WSSV infection and is applicable in the field.