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The first description of the blue swimming crab (*Portunus pelagicus*) transcriptome and immunological defense mechanism in response to white spot syndrome virus (WSSV)

Nattanicha Tribamrung [a b] ✉, Anurak Bunnoy [a b] ✉, Niti Chuchird [c] ✉, Prapansak Srisapoome [a b] ✉

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Highlights

- Interaction of blue swimming crabs and WSSV was intensively investigated *via* various high potential techniques.
- Gills were found as a major organ that exhibited highly copy number of WSSV and severely nuclear hypertrophy in this organ.
- RNA-seq techniques were used to create 12 transcriptomic libraries in the gills of WSSV infected crabs at 0-, 24- and 96-hpi.

- Various immune-related genes associated with anti-viral infection were discovered at 24-hpi and 96-hpi compared with 0-hpi.
- At 24-hpi and 96-hpi, several WSSV genes involving viral replication were highly observed.

Abstract

In the global shellfish farming industry, white spot syndrome virus (WSSV) is a major cause of mortality and a significant factor in economic losses. However, information on molecular immune responses to WSSV in blue swimming crabs (*Portunus pelagicus*) has never been reported. First, viral loads were measured in the gills, hepatopancreas, intestines, subcuticular epithelium and hemocytes of blue swimming crabs (50±10g) (n=4) after WSSV induction at 0, 24, 48 and 96h post injection (hpi). A significant increase in WSSV particles was observed in gills at 48 and 96 hpi, as supported by histopathology. To further investigate the acute immune response to WSSV, total RNA from the same gill tissues at 0, 24, and 96 hpi was used to construct 16 high-quality RNA-seq cDNA libraries. In summary, 162,740 unigenes were discovered in these transcriptomic libraries analyzed with the GO, KO, KOG, NR, NT, PFAM and SwissProt databases. Intensive sequence analysis against control crabs using three major categories of gene oncology (GO) of DEGs, biological processes (BPs), molecular functions (MFs), and cellular components (CCs), indicated that induction of WSSV in blue swimming crabs strongly affected the immune responses of the target animals significantly during the early stages of infection from 24 to 96 hpi. Furthermore, KEGG identified approximately twenty biological pathways of gene expression that were both downregulated and upregulated. Interestingly, at 24 and 96 hpi, several immune-related genes involved in virus defense in the blue swimming crab, particularly crustin 2, chitinase, anti-lipopolysaccharide, proteinase inhibitor, and lysozyme, were highly expressed during the WSSV early infection stages. At the same time, viral mRNA transcripts, including WSV289, WSV343, WSV306, deoxyuridine 5' triphosphate nucleohydrolase, RING finger containing E3 ubiquitin-protein ligase WSV403 and WSV404, were recorded in the top twenty upregulated genes. Moreover, some immune-responsive genes related to growth development, such as chitinase, tubulin alpha and beta chains, trypsin, and the cathepsin family, were also differentially expressed during these periods. Expression validation of 20 upregulated and 11 downregulated immune-related genes using qRT–PCR showed similar patterns with transcriptome information. Overall, the data showed that during WSSV infection, a number of immune-, metabolism-, and growth-related pathways were activated, and several of the pathways involved differed depending on the stage of virus

invasion. These findings could effectively help us better understand the impact of WSSV on the physiology of blue swimming crabs and serve as a valuable reference for future research on the immune system and disease control in this target species.

Introduction

The blue swimming crab (*Portunus pelagicus*, Linnaeus 1778) is an economically important crustacean found along the Pacific and Indian Ocean coasts. Its widespread habitats include mangroves, seagrass, algal beds, and near reefs at sandy and sand-muddy depths of 10–50 m, respectively. According to data from 2020, the global capture was 251,914 tons, while an additional 30.22 tons were produced in aquaculture around the world [1].

In recent years, blue swimming crab culture has grown in popularity, but it has not been as successful as it should have been due to a low survival rate, which results in low crop yields [2]. Several factors contribute to the unsuccessful cultivation of blue swimming crabs. This is caused by a number of issues, such as cannibalism, insufficient food, inefficient management of water quality and pond environment, and diseases. Vibriosis is a major bacterial infection caused by *Vibrio parahaemolyticus*, *V. harveyi*, *V. alginolyticus*, and *V. natrigens* [3], as well as parasites such as *Epistylis* sp., *Zoothamnium* sp., *Carcinonemertes* sp., and *Octolasmis* sp [4].

Furthermore, white spot disease (WSD), caused by white spot syndrome virus (WSSV), is one of the most damaging diseases that can result in 100% mortality and has a significant impact on shrimp and various crustacean production [[5], [6], [7]]. WSSV is a double-stranded DNA (dsDNA) virus that belongs to the genus *Whispovirus* in the family *Nimaviridae* and infects a wide range of crustaceans [6,7]. WSSV is most commonly found in penaeid shrimp and crab [6,7]. To date, the interaction of WSSV and several hosts has been intensively reported and is well known for the molecular mechanisms by which the crucial WSSV proteins VP28 and VP26 initially interact with the host receptor, facilitating virus attachment to host cells, cytoplasmic translocation, and nucleocytoplasmic translocation [8].

On the other hand, information on the infectious mechanisms involved in WSSV infection in blue swimming crabs is very limited. Since 1998, WSSV transmitted from black tiger shrimp (*Penaeus monodon*) has been experimentally demonstrated to kill 100% of blue swimming crabs within 8 days at very diluted concentrations, and the gills and subcuticular epithelium are severely affected, as indicated by histopathology [9]. In turn, WSSV from infected blue swimming crabs could also kill black tiger shrimp and could be an effective source for viral

transmission to black tiger shrimp and various crustaceans [10]. In the other crab species, WSSV was shown to severely infect mud crab (*Sylla serrata*) by specifically destroying subcuticular epithelium, gills, lymphoid organs, hematopoietic tissues, the antennal glands, and connective tissues [11], similar to those found in some freshwater crabs (*Paratelphusa hydrodomous* and *P. pulvinata*) [12].

In crustaceans, the majority of immune responses to particular pathogens rely on the innate immune system. Infection with viruses is mediated by cellular and humoral immune responses [13]. Cellular processes include phagocytosis, encapsulation, and nodule formation, whereas the humoral response includes a variety of immunological proteins. The gills are an integral part of the immune system, which also includes other organs. Its primary functions include respiration and regulation of ions, but it also contributes to immune responses and protection against pathogens [14]. In addition, gills are regarded as a key infection location, and systemic WSSV infections can spread to the gills [15].

To date, research on the immune defense mechanisms of blue swimming crabs in response to WSSV is limited. Transcriptome analysis is currently extensively used in aquaculture to identify and analyze the expression of interesting genes, such as growth genes, immune-related genes, and genes involved in disease pathogenesis. The purpose of this research was to characterize and investigate the immune response mechanisms, transcriptional profiles, and histological changes in different tissues of blue swimming crabs upon WSSV infection. The findings may contribute to a greater understanding of the response processes in WSSV infection and could be applied to WSSV prevention and treatment, which could profit the sustainable future of the blue swimming crab farming industry.

Section snippets

Experimental animals

A total of 110 healthy blue swimming crabs (50±10g) were obtained from the Phetchaburi Coastal Aquaculture Research and Development Center, Phetchaburi Province, Thailand. The

crabs were acclimated in 3000-L fiberglass tanks in 30 ppt seawater for a week with 24-h aeration. During acclimation, all crabs were fed three times per day with commercial shrimp feed, and the water was changed every three days. Three major serious pathogens, including WSSV, yellow head virus (YHV), and Vibrio…

Analysis of virally affected dose

In this experiment, WSSV particles at 1×10^3–1×10^6 DNA copy number were used to infect the blue swimming crabs. WSSV at a 1×10^5 DNA copy number was classified as an optimal viral dose for further experiments, since at 96-hpi in all injected crabs, they showed abnormal behaviors with no mortality, such as loss of appetite and remaining still in the basket. A few crabs in groups injected with 1×10^3–1×10^4 DNA copies exhibited similar behaviors with no mortality. Mass mortality of more…

Discussion

White spot syndrome virus (WSSV) is a leading cause of mortality in farmed crustaceans and a major source of economic losses in the global shellfish farming industry. However, to date, no information regarding the immune response of blue swimming crabs (*P. pelagicus*) to WSSV has been reported. Exploring the immune defense mechanisms of blue swimming crabs against WSSV is of great significance for the prevention of WSSV disease. It is necessary to gain a deeper knowledge of the viral-host…

Conclusion

In summary, the data showed that during WSSV infection, a number of immune-, metabolism-, and growth-related pathways were activated, and the pathways involved differed depending on the stages of viral invasion. Furthermore, this study suggests that the most abundant uncharacterized hypothetical proteins found in crustaceans during WSSV infection can be important indicators of immune function and health and that they play an important role in crustacean immune defenses. These proteins were…

CRediT authorship contribution statement

Nattanicha Tribamrung: Visualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Anurak Bunnoy:** Methodology, Formal analysis, Writing – review & editing. **Niti Chuchird:** Writing – review & editing. **Prapansak Srisapoome:**

Conceptualization, Resources, Supervision, Funding acquisition, Writing – review & editing, All authors reviewed and agreed to publishing the manuscript.…

Declaration of competing interest

The authors report no conflicts of interest in this work.…

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