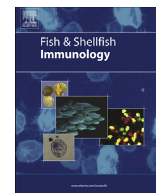
Fish & Shellfish Immunology 44 (2015) 257–264

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi



Full length article

Comparison of immune response of Pacific white shrimp, *Litopenaeus vannamei*, after multiple and single infections with WSSV and *Vibrio anguillarum*

Guo Qiao [a], De-Hai Xu [b], Zishen Wang [a], In-Kwon Jang [c], Zhitao Qi [a], Mingming Zhang [a,*], Su-Kyoung Kim [c,*]

[a] Department of Ocean Technology, College of Chemistry and Biology Engineering, Yancheng Institute of Technology, Yancheng 224051, China
[b] U.S. Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832, USA
[c] West Sea Fisheries Research Institute, National Fisheries Research & Development Institute, Incheon 400-420, Republic of Korea

A R T I C L E I N F O

Article history:
Received 20 November 2014
Received in revised form
3 February 2015
Accepted 6 February 2015
Available online 17 February 2015

Keywords:
Litopenaeus vannamei
Immune response
Multiple infections
White spot syndrome virus (WSSV)
Vibrio anguillarum

A B S T R A C T

Our previous study demonstrated that Pacific white shrimp (*Litopenaeus vannamei*) infected by multiple pathogens showed higher mortality and death occurred more quickly than those infected by a single pathogen (Jang et al., 2014). For better understanding the defense mechanism against white spot syndrome virus (WSSV) and *Vibrio anguillarum*, immune responses of shrimp were evaluated in this study. The mRNA expression levels of five immune-related genes were analyzed by quantitative reverse real-time PCR, which included proPO-activating enzyme 1 (PPAE1), PPAE2, proPO activating factor (PPAF), masquerade-like serine proteinase (Mas) and ras-related nuclear gene (Ran). Results demonstrated that the transcription was suppressed more intensively in the multiple infection group than those in single infection groups. The transcriptional suppression was directly related to the higher mortality. The hypoimmunity could benefit pathogen invasion, replication and release of toxin *in vivo*. Results in this study will help to understand immune defense mechanism after shrimp were infected by multiple pathogens in aquaculture.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

White spot syndrome virus (WSSV) infects various hosts and spread rapidly, which results in serious loss of the shrimp production in the world [1–4]. The WSSV can be detected in the apparently healthy shrimp [1,5–7] and the WSSV load in *Fenneropenaeus chinensis* without apparent syndrome was found to be 10^4 copies mg^{-1} tissue on average by qPCR [5,7]. Meng et al. [6] assayed WSSV infection in *Litopenaeus vannamei* and found a high infection rate (98.89%) with load of less than 10^5 copies mg^{-1} tissue. WSSV load in surviving *L. vannamei* can range from 10^2 to 10^6 copies ng^{-1} DNA [8]. Jang et al. [1] detected the WSSV load in the apparently healthy *L. vannamei* by qPCR and found 80% of sampling *L. vannamei* were WSSV-positive with a mean load of 9.48×10^3 copies mg^{-1} tissue. Durand & Lightner [8] reported that the moribund juveniles contained 10^5–10^6 copies ng^{-1} DNA. A minimum of 10^5 WSSV copies are required to transmit WSSV in shrimp by immersion and results in disease outbreak. *L. vannamei* with load of less than 10^5 copies mg^{-1} tissue could survive to harvest [6]. However, the pre-infected shrimp with WSSV could make shrimp more susceptible to environmental stress or other pathogens, such as *Vibrio* spp. and virus [9–12]. Under the field conditions, multiple infections by more than one pathogen can cause much greater losses in shrimp culture than any single infection. However, there is limited information available on multiple infections. Our previous study demonstrated that shrimp in multiple infection groups with WSSV and *Vibrio anguillarum* showed higher mortality, and death occurred more quickly than those infected by a single pathogen. The WSSV load increased rapidly during a time-course replication analysis by TaqMan real-time PCR [1]. In order to better understand why multiple infections caused higher mortality and led to greater losses in aquaculture, the defense mechanism of shrimp against multiple infections is evaluated.

* Corresponding author. Tel.: +86 515 88298190.
E-mail addresses: zhangmingm44ok@163.com (M. Zhang), isagemang@naver.com (S.-K. Kim).

http://dx.doi.org/10.1016/j.fsi.2015.02.009
1050-4648/© 2015 Elsevier Ltd. All rights reserved.

Shrimps, like other crustaceans and insects, are lack of adaptive immunity and entirely depend on innate immune responses to resist pathogen invasion [13]. Melanization, which is performed by phenoloxidase (PO) and controlled by prophenoloxidase (proPO) activation cascades, plays an important role in the crustacean immune system allowing a rapid response to pathogen infection [14]. The activation of the proPO cascade requires the proteolytic steps of serine proteinase (SPs) [15]. The terminal Clip-SPs that converts the proPOs to POs is termed proPO-activating enzyme (PPAE). PPAEs have been cloned from shrimps *Penaeus monodon*, *F. chinensis* and *L. vannamei*, and mainly expressed in the hemocytes. PPAEs have been demonstrated to be an immune-response gene since its transcript expression level is up-regulated after *Vibrio harveyi* infection [16]. Several members of serine proteinases are essential in the regulation of the proPO activating cascade including proPO activating factors (PPAFs) [17–21]. PPAFs may participate in the conversion of inactive proPO into active PO [22] and play important roles in shrimp immune system. PPAFs have been cloned and their regulations have been studied in a number of penaeid shrimps [23] and brachyuran crabs [22]. Another important immune response of hemocyte in Crustacea is phagocytosis besides PO immune system [24]. The ras-related nuclear gene (Ran gene), one of subfamilies of small GTPases, is highly conserved in eukaryotes from yeast to humans [24,25]. Like other Ras family members, Ran alternates between its GDP- and GTP-bound states and is required in the phagocytosis process [26]. Ran gene is involved in the shrimp antiviral immune response [27] and able to regulate the hemocytic phagocytosis against bacterial and viral infection in shrimp [24,28]. There is limited information available on molecular immune response in shrimp between single infection and multiple infections. For better understanding the defense mechanism against multiple pathogens, transcriptional level of several well-known immune-related genes in shrimp were investigated in this study, including PPAE1, PPAE2, PPAF, Mas and Ran.

2. Materials and methods

2.1. Experimental animals and conditions

Pacific white shrimp, *L. vannamei* (body weight of 1.5 ± 0.2 g, mean ± standard deviation) was obtained from the Taean Center at West Sea Fisheries Research Institute, National Fisheries Research & Development Institute (NFRDI), Republic of Korea. All experimental shrimp were maintained in a static aquaria at a water temperature of 27–29 °C, 30 practical salinity unit (psu), and pH 7.8–8.2 for 1 week prior to the experiments. The shrimp were fed four times per day with commercial diets (CJ Feeding Company, Korea), and 50% of the water was exchanged daily throughout the experimental period.

2.2. Water quality

Water temperature, DO, salinity and pH were measured with thermometer YSI85 (Yellow Springs Instrument, OH, USA). The concentrations of ammonia-N and nitrite-N were measured following the procedures of the American Public Health Association [29].

2.3. Disease diagnosis

In order to validate the pathogen free status in shrimp, six individuals were sampled and examined for bacterial and viral infection. For bacteria isolation, the surface of shrimp was sterilized with 70% alcohol. Shrimp hemolymph was withdrawn with sterilized syringe and streaked onto tryptic soy agar (ST) supplemented with 2% NaCl and thiosulphate-citrate-bile salt-sucrose agar (TCBS). The shrimp was then cut aseptically. The tissues from hepatopancreas and heart were collected with a sterilized loop and streaked onto ST and TCBS plates. All samples were incubated at 28 °C for 24–48 h and bacterial colonies were examined. For viral infection diagnosis, WSSV was checked by universal PCR using primers listed in Table 1 [30]. DNA extraction from whole body tissues was performed as described by Jang et al. (2009) and PCR amplification was conducted as follows: 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 57 °C for 45 s, and 72 °C for 1 min; followed by 72 °C for 10 min. The PCR products were checked by electrophoresis on a 1.5% agarose gel.

2.4. Challenge test

Challenge tests were performed as previously described [1], including single infection by one pathogen only or multiple infections by two pathogens concurrently. For single challenge test, shrimp were injected with 20 μL of *V. anguillarum* (3.3×10^6 colony-forming units mL^{-1}) or WSSV viral suspension (2×10^2 copies μL^{-1}) in the ventral sinus between the 4th and 5th pleopods using a 0.3-mL insulin syringe (BD Medical-Diabetes Care, USA). The control group was injected with equal volume of physical saline (PS). For multiple infection group, the shrimp were injected simultaneously with 20 μL of cocktail suspensions containing *V. anguillarum* (3.3×10^3 CFU mL^{-1}) and WSSV (2×10^2 copies μL^{-1}). These concentrations could cause shrimp disease, induced immune response, but kept most of shrimp survive so tissues could be collected for immune response study. Each group included 70 shrimps and the experiments were conducted in triplicate. Five shrimps were sampled at each time point (0, 3, 6, 12, 24, 48, 72 and 168 h) post injection (hpi) from each group. These samples would be used for the immune-related genes expression analysis.

2.5. Immune-related genes expression analysis by real-time PCR

Time-course immune-related genes mRNA expression in the whole body of shrimp was analyzed. Total RNA isolation, cDNA

Table 1
Primers and probes used in this study.

Target	Name	Nucleotide sequence (5′–3′)	Note
WSSV	Forward	CTTTCACTCTTTCGGTCGTGTC	[30]
	Reverse	TACTCGGTCTCAGTGCCAGA	
β-actin[a]	Forward	GTCAYCAGGGTGTGATGGTC	
	Reverse	ATCTTCTCCATGTCRTCCCAG	
PPAF	Forward	CCAGAAGACGTACGAGCGGTAT	Present study
	Reverse	GTAGAGAGCGCCTGAGTTGTAATTAG	
	Probe	CACCAGGACAGGAACGTCATCAGCG	
PPAE1	Forward	AGTTCCTACGACACGACCACCTA	[16]
	Reverse	TCGACGTTGAAGTTGGTGCTT	
	Probe	AACGACATCGCCATCATCAAGCTGC	
PPAE2	Forward	GTTGTGCCTCGAACGTAA	[43]
	Reverse	TTGCTGGTGGGCGTTATAGTT	
	Probe	CCAACGCCGTCACCAACACCC	
Mas	Forward	CGGCTGCGCTCAAAGG	[51]
	Reverse	TCGGATGAAGTTGGCATACG	
	Probe	TCCAGGTGTCTACGTCAACGTGGCC	
Ran	Forward	CCAAGAGAAATTGGGAGGTCTTC	
	Reverse	GGGAACATTCTTGTACGTGACTCTAG	
	Probe	ATGGTTACTACATCCAGGCCCACTGTGC	
β-actin[b]	Forward	CGAGGTATCCTCACCCTGAAAT	[16]
	Reverse	GTGATGCCAGATCTTCTCCATGT	
	Probe	CGAGCACGGCATCGTCACCAA	

[a] β-actin was used in multiplex PCR for WSSV detection.
[b] β-actin was used as an internal reference gene in the mRNA expression analysis of immune-related genes. PPAF: proPO activating factor; PPAE: proPO-activating enzyme; Mas: masquerade-like serine proteinase; Ran: ras-related nuclear gene.

Download English Version:

https://daneshyari.com/en/article/2431280

Download Persian Version:

https://daneshyari.com/article/2431280

Daneshyari.com