Fish & Shellfish Immunology 33 (2012) 324–331

Contents lists available at SciVerse ScienceDirect

Fish & Shellfish Immunology

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

Modulation of the innate immune system in white shrimp *Litopenaeus vannamei* following long-term low salinity exposure

Yong-Chin Lin [a], Jiann-Chu Chen [a,*], Chang-Che Li [a], Wan Zabidii W. Morni [a], Awangku Shahrir N. A. Suhaili [a], Yi-Hsuan Kuo [a], Yu-Hsuan Chang [a], Li-Li Chen [b], Wen-Ching Tsui [a], Yu-Yuan Chen [a], Chien-Lun Huang [a]

[a] *Department of Aquaculture, College of Life Sciences, National Taiwan Ocean University, Keelung 202, Taiwan, ROC*
[b] *Institute of Marine Biology, College of Life Sciences, National Taiwan Ocean University, Keelung 202, Taiwan, ROC*

A R T I C L E I N F O

Article history:
Received 26 February 2012
Received in revised form
20 April 2012
Accepted 7 May 2012
Available online 14 May 2012

Keywords:
Litopenaeus vannamei
Salinity
Immune parameters
Lifespan of haemocytes
Gene expression

A B S T R A C T

Immune parameters, haemocyte lifespan, and gene expressions of lipopolysaccharide and β-glucan-binding protein (LGBP), peroxinectin (PX), integrin β, and α2-macroglobulin (α2-M) were examined in white shrimp *Litopenaeus vannamei* juveniles (0.48 ± 0.05 g) which had been reared at different salinity levels of 2.5‰, 5‰, 15‰, 25‰, and 35‰ for 24 weeks. All shrimp survived during the first 6 weeks. The survival rate of shrimp reared at 2.5‰ and 5‰ was much lower (30%) than that of shrimp reared at 15‰, 25‰, and 35‰ (76%∼86%) after 24 weeks. Shrimp reared at 25% grew faster. Shrimp reared at 2.5‰ and 5‰ showed lower hyaline cells (HCs), granular cells (GCs), phenoloxidase activity (PO) activity, respiratory bursts (RBs), superoxide dismutase (SOD) activity, and lysozyme activity, but showed a longer haemocyte lifespan, and higher expressions of LGBP, PX, integrin β, and α2-M. In another experiment, shrimp which had been reared at different salinity levels for 24 weeks were challenged with *Vibrio alginolyticus* (6×10^6 cfu shrimp^{-1}), and WSSV (10^3 copies shrimp^{-1}) and then released to their respective seawater. At 96–144 h, cumulative mortalities of shrimp reared at 2.5‰ and 5‰ were significantly higher than those of shrimp reared at 15‰, 25‰, and 35‰. It was concluded that following long-term exposure to 2.5‰ and 5‰ seawater, white shrimp juveniles exhibited decreased resistance against a pathogen due to reductions in immune parameters. Increases in the haemocyte lifespan and gene expressions of LGBP, integrin β, PX, and α2-M indicated that shrimp had the ability to expend extra energy to modulate the innate immune system to prevent further perturbations at low salinity levels.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

White shrimp *Litopenaeus vannamei* has become the dominant species currently being cultured worldwide. However, shrimp farms have experienced viral diseases like monodon baculovirus virus (MBV), white-spot syndrome virus (WSSV), and Taura syndrome virus [1–3], and vibriosis caused by *Vibrio alginolyticus*, Vibrio *harveyi*, etc. [4,5]. WSSV is considered to be an extremely virulent pathogen, and may cause 100% mortality within 3–10 days [6]. The bacterium *V. alginolyticus* isolated from diseased white shrimp which exhibited whitish musculature and lethargy is considered to be a secondary and opportunistic pathogen [7]. Disease outbreaks are associated with increases in *Vibrio* populations in culture pond water [8].

In penaeid shrimp, three types of circulating haemocytes are recognized: hyaline (HCs), semi-granular, and large granular cells (GCs) [9,10]. They are involved in a pattern-recognition system, phagocytosis, prophenoloxidase (proPO)-activating system, encapsulation, nodule formation, and the release of antimicrobial peptides and lysozyme [11,12]. The proPO cascade is triggered by the presence of several microbial cell wall components through the recognition and binding of pattern-recognition proteins (PRPs) with pathogen-associated molecular patterns (PAMPs) [13–15]. Lipopolysaccharide and β-glucan-binding protein (LGBP), an important PRP, recognizes and responds to invaders, and initiates the proPO cascade [16,17]. Proteinase inhibitors, such as α2-macroglobulin (α2-M), play important roles in preventing overactivation of the proPO cascade and in entrapping protease produced by pathogens [14,18].

During the course of phagocytosis, the host's NADPH-oxidase is activated which in turn enhances oxygen consumption, and causes molecular oxygen to be reduced to form superoxide anions ($O_2^{\cdot-}$)

* Corresponding author. Tel./fax: +886 2 2462 0295.
E-mail address: jcchen@mail.ntou.edu.tw (J.-C. Chen).

1050-4648/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2012.05.006

that subsequently react and produce several other reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) [19]. Superoxide dismutase (SOD) catalyzes superoxide anions to molecular oxygen and hydrogen peroxide and provides antioxidant protection [20,21]. Superoxide anions are the first product released during this process known as respiratory bursts (RBs) [22]. Peroxinectin (PX) and integrin are involved in the proPO cascade and post-phagocytosis leading to the generation of cytotoxic products [15,16].

Wild white shrimp *L. vannamei* inhabits ranges of salinity of around 1‰–40‰ [23]. It exhibits hyper-osmotic regulation at low salinity levels, and exhibits hypo-osmotic regulation at high salinity levels with an iso-osmotic point of 718 mOsm kg^{-1} (equivalent to 25‰) [24]. White shrimp juveniles maintained at salinity levels of 15‰–20‰ or maintained at salinities of >20‰ and at water temperatures of 20 and 30 °C were reported to have better survival and growth [25,26]. However, farmers are likely to add fresh water to adjust the salinity levels to much lower than these levels; since they know that the growth of shrimp in brackish water is better than that in seawater. Low salinity has been reported to affect physiological responses including increased oxygen consumption and ammonia excretion, and decreased ammonia and nitrite resistance in shrimp [27–29]. However, little is known about the immune parameters and resistance against pathogens in shrimp following long-term culture in low salinities [30,31].

Modulation of the immune system of fish by their environmental conditions including salinity has been studied and was reviewed by Bowden [32]. We assumed that following long-term culture in low salinities, white shrimp may exhibit immune perturbation, leading to reduced resistance against pathogens, and subsequently result in modulation of the immune system. Accordingly, this study attempted to measure 1) growth, 2) resistance against *V. alginolyticus*, 3) resistance against WSSV, 4) immune parameters, 5) lifespan of haemocytes, and 6) transcripts of LGBP, PX, integrin β, and α2-M in white shrimp *L. vannamei* which had been reared at different salinity levels (2.5‰, 5‰, 15‰, 25‰, and 35‰) for 24 weeks. Important immune parameters of the HC count, GC (including semi-granular cells) count, total haemocyte count (THC), phenoloxidase (PO) activity, RBs, SOD activity, and lysozyme activity were examined [33].

2. Materials and methods

2.1. White shrimp L. vannamei and the experimental design

White shrimp *L. vannamei* post-larvae (PL_{5-6}) obtained from a hatchery farm in Kaohsiung, Taiwan were released into fibreglass tanks filled with filtered natural 35‰ salinity seawater at room temperature, and aerated to maintain the dissolved oxygen level at >5.8 mg L^{-1}. The post-larvae were fed live *Artemia* nauplii, and later an artificial diet until they grew to a weight of about 0.4 g. They were then separated into five tanks with final acclimated salinity levels of 2.5‰, 5‰, 15‰, 25‰, and 35‰. Salinity in each tank was adjusted by about 2.5‰–5‰ daily until the salinity in each tank had reached the desired level. It took 4, 8, 14, and 17 days to reach the 25‰, 15‰, 5‰, and 2.5‰ levels. For the growth, and immune parameter, and haemocyte lifespan experiments, 300 juveniles (with a mean weight 0.48 ± 0.05 g) were divided into five equal groups, each consisting of 6 replicates (10 shrimps/replicate) in five tanks (1 ton). Shrimp in each replicate were reared in cages that were suspended in water with different salinity levels. For the challenge tests with *V. alginolyticus* and WSSV, and the gene expression study, another 1125 juveniles were released separately into another fifteen tanks with different salinity water. There were three groups, and each group had five tanks. Each tank (1 ton) with 900 L respective seawater was stocked with 75 shrimp. One group was for the challenge test with *V. alginolyticus*, another group was for the challenge test with WSSV, and the other was for the gene expression study. Shrimp were fed twice daily at a rate of 5% of body weight at 9:00 and 18:00 with a formulated shrimp diet (Tairoun Feed Company, Taipei, Taiwan). Feces were removed daily before meal by siphoning.

Six experiments were conducted. They are 1) growth, 2) resistance against *V. alginolyticus*, 3) resistance against WSSV, 4) immune parameter assays, 5) lifespan of haemocytes, and 6) gene expressions. For the growth experiment, weight of shrimp was measured every 3 weeks up to 24 weeks during the period of May 1–October 15, 2011. For the rests of experiment, shrimp were sampled after 24 weeks. Water temperature fluctuated 20–32 °C (Fig. 1). Tanks received continuous aeration, and water flow was 1.3 L min^{-1}. During the experimental period, pH ranged 6.9–7.9 and dissolved oxygen (DO) concentration 6.20–8.20 mg L^{-1}. Concentrations of ammonia-N and nitrite-N which were measured twice a week by the phenolhypochlorite [34] and sulfanimide methods [35], were 0.07–0.19 and 0.02–0.10 mg L^{-1}, respectively. Only shrimp in the intermoult stage were used for the above-described experiments. The moult stage was determined by examining the uropoda in which partial retraction of the epidermis could be distinguished [36].

2.2. Preparation of V. alginolyticus

A pathogenic strain of *V. alginolyticus* isolated from diseased *L. vannamei*, which displayed symptoms of anorexia, lethargy, and whitish musculature was used for the study [7]. The bacterium was cultured in tryptic soy broth (TSB supplemented with 2% NaCl, Difco) for 24 h at 28 °C, and then centrifuged at 7155× *g* for 20 min at 4 °C [37]. The supernatant was removed, and the bacterial pellet was resuspended in a phosphate-buffered saline (PBS) solution at 3×10^8 colony-forming units (cfu) ml^{-1} as the bacterial suspension for the *Vibrio* challenge test.

2.3. Preparation of WSSV inoculum

The WSSV inoculum and test solution were prepared based on a previously described method [38]. Briefly, 100 μl of haemolymph

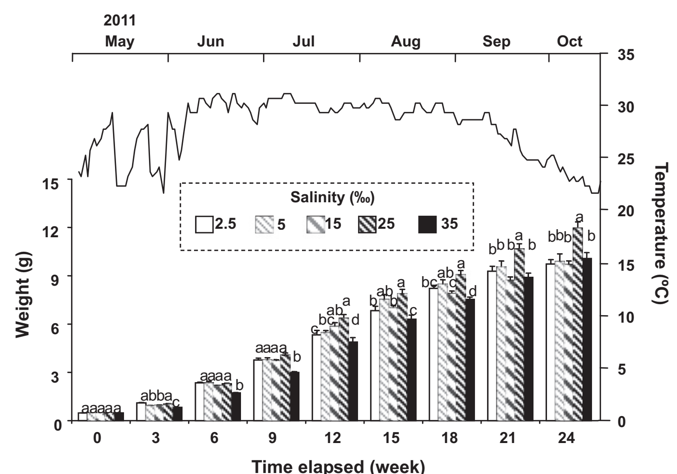

Fig. 1. Water temperature during the period of May 1–October 15, 2011, and mean (±SE) weight of white shrimp *Litopenaeus vannamei* reared at different salinity levels of 2.5‰, 5‰, 15‰, 25‰, and 35‰ after 0, 3, 6, 9, 12, 15, 18, 21, and 24 weeks. Bars with different letters in the same time period significantly differ among different salinity levels ($p < 0.05$).

Download English Version:

https://daneshyari.com/en/article/2432180

Download Persian Version:

https://daneshyari.com/article/2432180

Daneshyari.com