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Immune and physiological responses in Pacific white shrimp (*Penaeus vannamei*) to *Vibrio alginolyticus*

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Abstract

The susceptibility, physiological and immune responses of the Pacific white shrimp *Penaeus vannamei*, under challenge with *Vibrio alginolyticus* were investigated for a 72-h period. The survival of shrimp challenged with *V. alginolyticus* was 86.7, 75.6, 57.8, 54.4, 48.9 and 44.4% after 12, 24, 36, 48, 60 and 72 h, respectively. No significant differences in immune parameters were observed among the control shrimp injected with saline during 72 h. However, the total hemocyte count (THC), phenoloxidase (PO), superoxide dimutase (SOD) activities, and respiratory bursts were decreased with *V. alginolyticus* challenged with *V. alginolyticus* after 12 h. The physiological parameters of hemolymph glucose, lactate, and lipid contents increased, and peaked at 36 h after infection. The challenge with *V. alginolyticus* augmented the magnitudes of the hyperglycemic, hyperlacticemic, and hyperlipidemic responses, following the same trends of the changes in the control shrimp. The observations of this study show that the Pacific white shrimp *P. vannamei* immune parameters of THC, phenoloxidase activity and respiratory burst, and physiological parameters of glucose, lactate, and lipids were changed due to infection by *V. alginolyticus*. In addition, hemolymph glucose and lipid were only significantly correlated with SOD activities, while the hemolymph lactate was found to be highly correlated with all the immune parameters examined, except the THC.

Keywords: Physiological; Immune; *Vibrio alginolyticus*; Challenge; *Penaeus vannamei*

1. Introduction

The white shrimp *Penaeus vannamei* (Perez Farfante and Kensley, 1997), which is distributed along the Pacific coast of Central and South America, was introduced into Taiwan in 1985, and spontaneous spawning and larval rearing have been conducted successfully for several generations (Lin et al., 1990). Farmed production of *P. vannamei* in Taiwan reached 12,109 t in 2005. However, the shrimp culture industry has been limited by outbreaks of infectious diseases, particularly those caused by viruses and bacteria (Lightner and Redman, 1998). Thus, it is important to improve the immune ability of *P. vannamei* in order to increase resistance toward pathogen infection.

Decapod crustaceans exhibit many different physiological responses to environmental stressors (Mangum, 1983; McMahon and Wilkens, 1983; Fingerman et al., 1996). For example, physiological responses to temperature changes and hypoxia include changes in ventilation, circulation, haemocyanin oxygen-binding properties, and in aerobic and anaerobic metabolism. Glucose and lactate are two of the most common

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physiological parameters currently used as sensitive and reliable indicators for physiological stress responses (Haux et al., 1985). Hyperglycemia, in aquatic animals, is a typical sublethal response to environmental stressors such as disease, hypoxia and heavy metals (Radford et al., 2005). Lactate is the major anaerobic endproduct in crustaceans under hypoxic and anoxic conditions (Hill et al., 1991). During recovery from hypoxia, lactate levels are initially elevated. Since lactate is not excreted, it must be slowly remetabolised through the process of gluconeogenesis. Wachter et al. (1997) showed that only part of the increased oxygen uptake associated with recovery from anoxia is utilized for the removal of lactate. In addition, total plasma lipids have been proposed as an important component in crustacean metabolism as suggested by the percentage of lipid present in the fresh weight of some organs and tissues, especially of the midgut gland (O'Connor and Gilbert, 1968; Armitage et al., 1972; Chang and O'Connor 1983).

In decapod crustaceans, hemocytes are involved not only in phagocytosis, but also in the production of melanin via the prophenoloxidase (proPO) system (Johansson and Söderhäll, 1989; Söderhäll et al., 1996). Phagocytosis is a common cellular defense reaction, and is generally recognized as a central and important way to eliminate micro-organisms or foreign particles. Once a pathogen enters the hemolymph, the host's NADPH-oxidase is activated, which in turn reduces oxygen molecules and subsequently produces several reactive oxygen intermediates (ROIs), such as superoxide anion (O_{2-}), hydroxyl radical (OH), singlet oxygen ($1O_2$), and hydrogen peroxide (H_2O_2). This process is known as respiratory burst, and plays an important role in microbicidal activity (Muñoz et al., 2000). The production of superoxide anion has been reported in the hemocyte of *Penaeus japonicus* (Bachère et al., 1995), *Penaeus monodon* (Song and Hsieh, 1994), *Litopenaeus stylirostris* (Le Moullac et al., 1998), and *P. vannamei* (Muñoz et al., 2000; Liu and Chen, 2004; Liu et al., 2004; Tseng and Chen, 2004). In addition, phenoloxidase activity has been detected in many species of penaeid shrimps such as São Paulo shrimp *Farfantepenaeus paulensis* (Perazzolo and Barracco, 1997), yellowleg shrimp *Fafantepenaeus californiensis* (Hernández-López et al., 1996), tiger shrimp *P. monodon* (Sung et al., 1998), blue shrimp *L. stylirostris* (Le Moullac et al., 1997), and white shrimp *P. vannamei* (Liu and Chen, 2004; Liu et al., 2004; Tseng and Chen, 2004; Mercier et al., 2006).

The purpose of this study was to examine several physiological (glucose, lactate and triglycerides) and immune (total hemocyte count, phenoloxidase activity, respiratory burst, superoxide dismutase activity) parameters to determine the susceptibility of *P. vannamei* to *V. alginolyticus* infection.

2. Materials and methods

2.1. Shrimp

Pacific white shrimp (*P. vannamei*, Perez Farfante and Kensley, 1997) juveniles (12.69±1.5 g) were obtained from King Car Shrimp Inc., (Pingtung, Taiwan) and were acclimated in the laboratory for 2 weeks before experimentation. For the susceptibility experiment, test and control groups were comprised of 10 shrimps each in triplicate. For the determination of immune parameters, tests were carried out on five replicate test groups consisting of three shrimp each in 20 l PVC tanks containing 10 l of aerated test solution.

In all tests, the shrimps were fed twice daily with a formulated shrimp diet (Hsin-Da Feed Co., Pingtung, Taiwan), and no significant changes in weight were observed during the course of the study. The water temperature in the tanks was maintained at 27±1 °C, pH 7.8–8.0, and salinity was 20‰ throughout.

2.2. Valginolyticus

A known pathogenic strain of *V. alginolyticus* (CH003), which had been isolated from diseased *P. vannamei* in Pingtung, Taiwan, was used for the study. Stocks were cultured on tryptic soy agar (TSA supplemented with 2% NaCl, Difco) for 24 h at 28 °C and then transferred to a 10 ml tryptic soy broth (TSB supplemented with 2% NaCl, Difco) for 24 h at 28 °C. The broth culture was centrifuged at 7155 ×*g* for 20 min at 4 °C. The supernatant fluid was removed and the bacterial pellet was re-suspended in saline solution (0.85% NaCl) at 1×106 colony-forming units (cfu) ml^{-1} for the susceptibility test.

2.3. Susceptibility of P. vannamei to V. alginolyticus

Challenge tests against *V. alginolyticus* were conducted in triplicate, with 10 shrimp per group following the protocol described by Liu and Chen (2004). The mortalities of experimented shrimp were monitored at 12-h intervals for a 72-h period, following a single injection of bacterial suspension at a dose of 1×105 cfu (g shrimp)$^{-1}$ into the ventral sinus of the cephalothorax. The stock solution of the bacterial suspension was prepared at a concentration of 5×107 cfu ml^{-1}, and the injection volume was determined by the body weight of the experimented shrimp, i.e., 20 µl of the bacterial solution was given to each 10 g of shrimp. Shrimp in the unchallenged control group were injected with 20 µl of saline solution only (Table 1). Experimental and control shrimp (10 animals per aquarium) were maintained in 60-l glass aquaria at a salinity of 20 ppt.

2.4. Immune parameters of P. vannamei to V. alginolyticus

Hemolymph samples were collected at the beginning of the test (0 h), and at 12, 24, 36, 48, 60 and, 72 h. Hemolymph (100 µl) was withdrawn from the ventral sinus of each shrimp into a 1 ml sterile syringe (25 gauge) containing 0.9 ml anticoagulant solution (30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA, 0.12 M glucose (pH 7.55), osmolality 780 mOsm kg^{-1}).

2.4.1. Total hemocyte count (THC)

A drop of the anticoagulant-hemolymph mixture was placed on a hemocytometer to measure THC (total hemocyte count) using an inverted phase contrast microscope (Olympus IX 71, Japan). The remainder of the mixture was used for subsequent tests. All hemocytes in both top and bottom fields (1×1 mm) of the hemocytometer were counted.

2.4.2. Phenoloxidase (PO) activity

Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA)(Ashida and Soderhall, 1984; Hernández-López et al., 1996; Liu and

Table 1
Susceptibility of white shrimp *Penaeus vannamei* challenged with *Vibrio alginolyticus*

Bacterial dose (cfu $shrimp^{-1}$)	Survival (%) after time elapsed (h)						
	12	24	36	48	60	72	
Saline	100	100	100^{x}	100^{x}	100^{x}	100^{x}	100^{x}
1×10^{6}	100	86.7± 3.61	75.6± 2.52^{y}	57.8± 3.06^{y}	54.4± 1.15^{y}	48.9± 2.08^{y}	44.4± 2.52^{y}

The letters of x,y indicate the differences among the sampling times are significant ($p<0.05$) for *Penaeus vannamei*. Values are mean±SE ($n=30$ shrimp in each case).