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The immune response of tiger shrimp *Penaeus monodon* and its susceptibility to *Photobacterium damselae* subsp. *damselae* under temperature stress

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Abstract

Tiger shrimp *Penaeus monodon* held in 25‰ seawater and 26 °C seawater were injected with *Photobacterium damselae* subsp. *damselae* grown in TSB at a dose of 8.48×10^4 colony-forming units (cfu) shrimp^{-1}, and then reared onward at water temperatures of 22, 26 (control), 30 and 34 °C. Over 24–96 h, the cumulative mortalities of *P. damselae* subsp. *damselae*-injected shrimp held in 22 and 34 °C were significantly higher than those for injected-shrimp held in 26 and 30 °C. In a separate experiment, shrimp held in 25‰ seawater and 26 °C, and then cultured onward at 22, 26 (control), 30 and 34 °C were examined for immune parameters, phagocytic activity and clearance efficiency of *P. damselae* subsp. *damselae* at 12–96 h. THC (total hemocyte count), DHC (differential hemocyte count), phenoloxidase (PO) activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency in shrimp decreased significantly after 24–96 h transfer to 22 and 34 °C. It was concluded that transfer of tiger shrimp *P. monodon* from 26 °C to 22 and 34 °C reduced their resistance against *P. damselae* subsp. *damselae* infection.

Keywords: *Penaeus monodon*; *Photobacterium damselae* subsp. *damselae*; Temperature; Susceptibility; Immune parameters; Phagocytic activity; Clearance efficiency

1. Introduction

Tiger shrimp *Penaeus monodon*, which is naturally distributed from the east coast of Africa, Red Sea to Pakistan, Malay Archipelago, Philippines and Australia, is commercially important in several Pacific rim countries. This species is euryhaline, and has a tolerance for salinity ranging from 3‰ to 45‰ with an iso-osmotic point of 750 mOsm kg^{-1} which is equivalent to 25‰ (Cheng and Liao, 1986). Salinities in the range of 10–35‰ and temperatures in the range of 25–35 °C were suitable levels for growth of *P. monodon* (Liao, 1986; Chen, 1990); seasonal ranges of water temperature at shrimp farms may vary from 15 to 32 °C in Taiwan (Chen, 1990).

Commercial farming of tiger shrimp *P. monodon* has been badly hit by an epidemic of viruses, monodon baculoviros virus (MBV), white spot syndrome virus (WSSV), yellow head virus (YHV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Lo et al., 2003).

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The bacteria, *Vibrio alginolyticus*, *Vibrio harveyi* and *Photobacterium damselae* subsp. *damselae* (also known as *Vibrio damsela*) are considered to be secondary and opportunistic pathogens, and have been demonstrated to cause disease outbreaks of vibriosis associated with poor environmental conditions (Lee and Chen, 1994; Lee et al., 1996; Liu et al., 1996). These diseases have also been reported to be associated with increases of *Vibrio* population in culture pond waters (Sung et al., 2001).

Decapod crustaceans have three types of circulating hemocytes: hyaline cell (HC), semi-granular cell (SGC) and granular cell (GC) (Hose et al., 1990). They are involved not only in phagocytosis, an important process in eliminating microorganisms or foreign particles (Bayne, 1990), but also in the production of melanin via the prophenoloxidase (proPO) system which is an important component of the cellular defense reaction (Söderhäll and Cerenius, 1998). Phenoloxidase is the terminal enzyme in the proPO system and is activated by cell polysaccharides like β-1,3-glucan, lipopolysaccharide or peptidoglycan from microorganisms through pattern recognition proteins (Smith et al., 1984).

Once bacteria or foreign particles are engulfed by hemocytes, the host's NADPH-oxidase is activated, which in turn increases oxygen consumption and subsequently produces several reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$) (Roch, 1999). These oxidants including superoxide anion can cause cytotoxic problems (Warner, 1994). Superoxide dismutase (SOD) catalyses the rapid two-step dismutations of the toxic superoxide anion, and scavenges it to molecular oxygen and hydrogen peroxide through the alternate reduction and oxidation of the active-site metal ion (Zelick et al., 2005).

It is known that temperature affects the development of WSSV in kuruma shrimp *Marsupenaeus japonicus* (Guan et al., 2003), in white shrimp *Litopenaeus vannamei* (Vidal et al., 2002) and in freshwater crayfish *Pacifastacus leniusculus* (Jiravanichpaisal et al., 2004). It is also known that changes in temperature affect the phagocytic activity of freshwater prawn *Macrobrachium rosenbergii* against *Lactococcus garvieae* (Cheng et al., 2003), and white shrimp *L. vannamei* against *V. alginolyticus* (Cheng et al., 2005).

It is assumed that changes in temperature may weaken the immune system of *P. monodon*, and lead to its susceptibility to vibriosis. Accordingly, this study examined 1) changes in temperature on the susceptibility of *P. monodon* to *P. damselae* subsp. *damselae*, and 2) changes in temperature on the immune responses of *P. monodon*.

2. Materials and methods

2.1. P. monodon

P. monodon were obtained from a commercial farm in Iilan, Taiwan, and acclimated in the laboratory for two weeks; only shrimp in the intermolt stage were used for the study. The molt stage was determined by the examination of uropoda in which partial retraction of the epidermis could be distinguished (Robertson et al., 1987). In all tests, the shrimp were fed twice daily with a formulated shrimp diet (Tairoun Feed Company, Taipei, Taiwan). The shrimp ranged from 15.7 to 23.2 g, averaging 18.75±3.60 g (mean±SD) with no significant size difference among the treatments. During experiments, salinity was maintained at 25‰, pH 7.9 to 8.2 while temperature was maintained at 26±1 °C.

2.2. P. damselae subsp. damselae

P. damselae subsp. *damselae* obtained from Bioresources Collection and Research Center, Food Industry and Development Institute (Hsinchu, Taiwan) was used for the study. This species was demonstrated to cause high pathogenicity in *P. monodon* (Lee and Chen, 1994). Stocks were on tryptic soy agar (TSA supplemented with 2.5% NaCl, Difco) for 24 h at 26 °C, and transferred to 10 ml tryptic soy broth (TSB supplemented with 2.5% NaCl, Difco) for 24 h at 26 °C for use as a stock bacterial broth. Stock cultures were 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 4.24×10^6 cfu ml^{-1} for the susceptibility test, and 4.70×10^6 cfu ml^{-1} for phagocytic activity and clearance efficiency tests.

2.3. Effect of temperature change on the susceptibility of P. monodon to P. damselae subsp. damselae

Challenge tests were conducted in triplicate with ten shrimp per replicate following the methods described before (Liu and Chen, 2004). Into the ventral sinus of the cephalothorax of each shrimp, 20 μl of bacterial suspension (4.24×10^6 cfu ml^{-1}) was injected resulting in 8.48×10^4 cfu $shrimp^{-1}$. After injection, shrimp were kept in a separate 60 l glass aquarium (10 shrimp each) containing 40 l of aerated water (25‰) at 22, 26 (control), 30 and 34 °C. The experiment lasted 96 h. Shrimp injected with an equal volume of sterile saline solution and kept in 22, 26, 30 and 34 °C served as the unchallenged controls (Table 1).