ELSEVIER

Fish & Shellfish Immunology 23 (2007) 364–377

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Immune responses and gene expression in white shrimp, *Litopenaeus vannamei,* induced by *Lactobacillus plantarum*

Chiu-Hsia Chiu [a], Yuan-Kuang Guu [a], Chun-Hung Liu [b], Tzu-Ming Pan [c,**], Winton Cheng [b,*]

[a] *Department of Food Science, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*
[b] *Department of Aquaculture, National Pingtung University of Science and Technology, 1 Sheuh Fu Road, Nei Pu Hsiang, Pingtung 91201, Taiwan*
[c] *Institute of Microbiology and Biochemistry, National Taiwan University, Taipei 10617, Taiwan*

Received 15 September 2006; revised 22 November 2006; accepted 23 November 2006
Available online 5 December 2006

Abstract

The total haemocyte counts, phenoloxidase (PO) activity, respiratory bursts, superoxide dismutase (SOD) activity, and phagocytic activity and clearance efficiency to *Vibrio alginolyticus*, as well as prophenoloxidase (proPO), lipopolysaccharide- and β-1,3-glucan-binding protein (LGBP), serine protein (SP), and peroxinectin (PE) mRNA transcription of *L. vannamei*, and its susceptibility to *V. alginolyticus* when the shrimp were fed diets containing *Lactobacillus plantarum* at 0 (control), 10^7, and 10^{10} cfu (kg diet) $^{-1}$ for 48 and 168 h were evaluated. The results indicated that PO activity, SOD activity, clearance efficiency to *V. alginolyticus*, proPO and PE mRNA transcription, and the survival rate after challenge with *V. alginolyticus* all significantly increased, but the total haemocyte counts significantly decreased in shrimp fed a diet containing *Lac. plantarum* at 10^{10} cfu (kg diet)$^{-1}$ for 168 h. However, no significant differences in phagocytosis, LGBP, or SP mRNA expression of shrimp were observed among the different treatments. It was concluded that administration of *Lac. plantarum* in the diet at 10^{10} cfu (kg diet)$^{-1}$ induced immune modulation and enhanced the immune ability of *L. vannamei*, and increased its resistance to *V. alginolyticus* infection.

Keywords: *Litopenaeus vannamei*; *Lactobacillus plantarum*; Probiotics; Phenoloxidase activity; Respiratory bursts; Superoxide dismutase; Haemocyte count; Phagocytic activity; Clearance efficiency; Challenge; Immune gene transcription

1. Introduction

White shrimp, *Litopenaeus vannamei,* which is distributed along the Pacific coast of Central and South America, has been introduced to the Eastern hemisphere, and has become the primary species currently being cultured in Southeast Asian countries. During the past two decades, shrimp culture around the world has suffered problems linked to deteriorating pond environments due to development of intensification, subsequently resulting in stress-induced

* Corresponding author. Tel.: +88 68 770 3202x6224; fax: +88 68 774 0225.
** Corresponding author. Tel.: +88 62 3366 4519; fax: +88 62 2362 7704.
E-mail addresses: tmpan@ntu.edu.tw (T.-M. Pan), winton@mail.npust.edu.tw (W. Cheng).

1050-4648/$ - see front matter
doi:10.1016/j.fsi.2006.11.010

disease incidence. Many shrimp farms have been particularly affected by epidemics of viruses and vibriosis. Therefore, the health of shrimp and enhancement of their immunity are of primary concern.

In decapod crustaceans, circulating haemocytes are generally classified into three types, hyaline, semi-granular, and large granular cells [1]. Haemocytes are involved not only in coagulation but also in the production of melanin via the prophenoloxidase (proPO) system, which plays an important defence role [2,3]. Both semi-granular and granular cells carry out functions of the proPO system, which has a role in recognition and defence [2]. Conversion of proPO to phenoloxidase (PO) occurs through prophenoloxidase-activating enzyme (ppA), a serine protease [4]. PO is the terminal enzyme in the proPO system, and ppA is activated by several microbial polysaccharides, including β-1,3-glucan and lipopolysaccharide (LPS) from fungal cell walls through the non-self recognition system [5]. Specific recognition proteins, including β-1,3-glucan-binding protein, LPS-binding protein, peptidoglycan-binding protein, and LPS- and β-1,3-glucan-binding protein (LGBP), which recognise and respond to intruders, have been reported in several crustacean species including penaeid shrimp [6–8].

In addition, peroxinectin (PE), an associated protein of the proPO system, is synthesised and stored in secretory granules of semi-granular cells and granular cells in an inactive form, is released in response to stimulus, and is activated outside the cells to mediate haemocyte attachment and spread [9,10]. PE has multiple functions of cell adhesion [9], opsonification [11], degranulation [12], peroxidase activity [13], and encapsulation enhancement [14]. The biological activity of PE is generated concomitantly with activation of the proPO system [13]. PE of *L. vannamei* has been cloned and characteristic by Liu et al. [15].

Several reactive oxygen species are produced during phagocytosis. Beginning this process, the membrane-bound enzyme complex, NADPH oxidase, assembles after binding the cell to a foreign particle, and reduces molecular oxygen to the superoxide anion (O_2^-), subsequently leading to the production of hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^\bullet$), and numerous other reactive compounds [16]. The superoxide anion is the first product released from respiratory bursts, and plays an important role in microbicidal activity [17]. Although reactive oxygen intermediates (ROIs) play an especially important role in host defence, host cells can be damaged by the overexpression of ROIs. Most cells have also acquired the relevant protective mechanisms to maintain the lowest possible levels of ROIs inside the cell, including superoxide dismutase (SOD), catalase, and glutathione peroxidase.

Probiotics are defined as live microbial or cultured product feed supplements, which beneficially affect the host by producing inhibitory compounds, competing for chemicals and adhesion sites, modulating and stimulating the immune function, and improving the microbial balance [18–20]. They have been used in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds. A wide range of microalgae (*Tetraselmis*), yeasts (*Debaryomyces*, *Phaffia,* and *Saccharomyces*), and Gram-positive (*Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus*, and *Weissella*) and Gram-negative bacteria (*Aeromonas*, *Alteromonas*, *Photorbodobacterium*, *Pseudomonas,* and *Vibrio*) has been applied as probiotics in fish and shellfish. Applying probiotics to aquaculture ponds and feed to improve aquatic animal growth, survival, health, and disease prevention has been reviewed [20–22].

Administration of probiotics, such as *Thalassobacter ultilis* PM-4 and *Bacillus* sp., to elevate the survival rate in commercial shrimp hatcheries and farms has been evaluated [23,24] and reviewed [20]. Probiotics being used to stimulate the immune response of fish has also been considered [25]. However, the effect of probiotics on the immune response and gene expression of shrimp has not been clarified so far. *Lactobacillus plantarum* is an important species in the fermentation of various plant products [26], is known to produce antimicrobial substances, like plantaricin, that are active against certain pathogens, and is used as a probiotic [27].

The purpose of this study was to examine the immune parameters including total haemocyte counts, PO activity, respiratory bursts, SOD activity, phagocytic activity and clearance efficiency to *Vibrio alginolyticus*; the immune gene expression including proPO, LGBP, a serine protein, and PE mRNA transcription of *L. vannamei*; and its susceptibility to *V. alginolyticus* when the shrimp were fed diets containing *Lac. plantarum*.

2. Materials and methods

2.1. Preparation of the Lac. plantarum mixture

Lactobacillus plantarum 7-40 (NTU102) isolated from home-made Korean-style cabbage pickles were used in this study. Bacteria were cultured in a sterilised 2-L flask with de Man, Rogosa, and Sharpe (MRS) broth (Merck,