

# Effects of phosphatidyl serine on immune response in the shrimp *Litopenaeus vannamei*

Research Article

Liubing Yang, Luqing Pan\*

Department of Aquaculture, Key Laboratory of Mariculture,  
Ministry of Education, Ocean University of China,  
266003 Qingdao, China

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**Abstract:** Phosphatidyl serine plays an important role in animal innate immunity. Given its important functions, numerous investigations have been carried out on its immunological function in many animals. However, studies of phosphatidyl serine in the white shrimp *Litopenaeus vannamei*, an economically important animal, are rare. In this paper, we demonstrated influences of injecting phosphatidyl serine (PS) on immune response including some parameters from pro-phenol oxidase activating system (pro-PO system) and hemocyanin-derived phenol oxidase activity (Hd-PO) along with antibacterial and bacteriolytic activities in the white shrimp *Litopenaeus vannamei* with different PS concentrations (5, 10 and 20  $\mu\text{g mL}^{-1}$ ). The results showed that PS could affect immune response of *L. vannamei* significantly ( $P < 0.05$ ), including total hemocyte counts (THC), PO activity from hemocyte, phenol oxidase (PO) activity from plasma, hemocyanin concentration, Hd-PO activity as well as antibacterial and bacteriolytic activities in the plasma. Among the lines, 20  $\mu\text{g mL}^{-1}$  PS had the strongest effect on the above parameters, whereas 5  $\mu\text{g mL}^{-1}$  had the least effect. The experimental results indicated that PS was able to activate exocytosis of pro-PO and formation of Hd-PO in white shrimp after injection, further regulating the immune process reflected by variation of antibacterial and bacteriolytic activities in a certain way.

**Keywords:** Pro-phenol oxidase activating system • Hemocyanin-derived phenol oxidase activity • Antibacterial activity • Bacteriolytic activity

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## 1. Introduction

In recent years, several studies have focused on crustacean immunity in aquaculture and aquatic life. It is generally considered that crustacean immunity has two distinct pathways: humoral and cellular defence [1]. Hemocytes play a central role in mediating immune capability via phagocytosis, encapsulation and nodule formation, whilst humoral factors such as agglutinins, killing factors, lysins, precipitins, cytokine-like molecules and clotting agents make up the humoral defence system [2,3]. During an invertebrate immune reaction, a pro-phenol oxidase activating system (pro-PO system) plays a vital role against pathogens or parasites and mainly consists of proteinase, pattern recognition proteins and proteinase inhibitors. These are activated by small quantities of microbial cell wall extracts such as LPS, laminarin, and peptidoglycan,

that are regulated by a type of serine proteinase and its inhibitor *in vivo* [4-8]. In addition, there are some studies about the relationship between pro-PO activation and environment, in which different environmental conditions including circatidal rhythmicity, dissolved oxygen, pH, salinity and ammonia-N concentration could also affect activity of PO enzyme cascade in decapod crustaceans [9-11].

Hemocyanin is a respiratory protein that commonly exists within the open vascular system of arthropod and mollusks [12]. Hemocyanin is a major plasmatic protein, accounting for up to 93 per cent of the whole protein in the hemolymph in crustaceans [13,14]. Arthropod hemocyanin are considered to be multimeric, ( $n \times 6$ ) copper-containing proteins, with an average molecular weight for each subunit being approximately 75 kDa [15,16]. Recently, there has been a lot of evidence supporting the viewpoint that hemocyanin is a type

\* E-mail: panlq@ouc.edu.cn