Fish and Shellfish Immunology 81 (2018) 161–167

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Physiological and immune response in the gills of *Litopenaeus vannamei* exposed to acute sulfide stress

Yafei Duan, Yun Wang, Hongbiao Dong, Hua Li, Qingsong Liu, Jiasong Zhang*, Dalin Xiong

Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture and Rural Affairs, Key Laboratory of Fishery Ecology and Environment, Guangdong Province, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, 510300, PR China

ARTICLE INFO

Keywords:
Crustacean
Environmental pollutants
Osmoregulation
Respiratory metabolic
Immune

ABSTRACT

Sulfide is a harmful environmental pollutant that affects the survival and immunity of shrimps. The gill is important for shrimp respiratory and osmotic adjustment, the physiological and immune homeostasis of the organ can be influenced by sulfide. In this study, we investigated the acute toxicity of sulfide (5 mg/L) on the morphology, physiological and immune response in the gills of *Litopenaeus vannamei*. H&E stain showed that sulfide stress damaged the gills histological structure. Specifically, osmoregulation capacity including of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase activity was both increased at 6 h and 12 h, and decreased at 72 h; the contents of free amino acid including of Gly, Pro, Ser, Thr and Ala were decreased at 72 h. Respiratory metabolic enzymes, such as cytochrome *c* oxidase and succcinate dehydrogenase activity was decreased at 12 h-72 h, while fumarate reductase and lactate dehydrogenase activity kept a higher level at 12 h-72 h. Significant variations in the activities of immune enzymes (acid phosphatase, alkaline phosphatase, total antioxidant capacity and lysozyme). The expression of immune-related genes (heat shock protein 70, thioredoxin and caspase-3) was increased at first and then decreased, while hypoxia inducible factor 1α kept a higher level at 6 h-72 h. These results revealed that sulfide stress influenced the *L. vannamei* gills physiological and immune function by damaging histological structure, and confusing osmoregulation, respiratory metabolic and immune capacity.

1. Introduction

Pacific white shrimp *Litopenaeus vannamei* is an important shrimp species that supports a large aquaculture industry in China [1]. Recent years, shrimp aquaculture has suffered serious economic losses caused by diseases [2,3], for which environmental stress is often a crucial inducing factor. *L. vannamei* occurs in pond benthic zones, it is frequently affected by environmental pollutants such as sulfide. Sulfide poses highly toxicity to shrimp, including growth reduction, mortality, immune suppression, and pathogen susceptibility [4–6]. The 96 h-LC_{50} of sulfide is highly in crustaceans, including *L. vannamei* (4.25 mg/L), *Macrobrachium rosenbergii* (4.2 mg/L), *Eriocheir sinensis* (3.09 mg/L), and *Eohaustorus estuaries* (3.32 mg/L) [6–9]. Chronic sub-lethal exposure to sulfide reduce the survival of the shrimp through functional changes in gluconeogenesis, protein synthesis and energy metabolism [6]. The tolerance to sulfide seems to be species dependent and is also dependent on developmental stage [10]. If the sulfide concentration beyond specific tolerance levels, it may cause the shrimp loss equilibrium and death. Therefore, it is important to understand details of the toxicity of sulfide to shrimp.

Sulfide is generated from the anaerobic decomposition of the organic wastes in the bottom layer and sediments of shrimp pond [4,11]. Sulfide included of water soluble hydrogen sulfide (H_2S), bisulfide anion (HS^-) and sulfide anion (S^{2-}), and H_2S plays a decisive role in sulfide stress [12,13]. H_2S can inhibit the electron transport chain of cytochrome oxidase, inactivate glutathione through combining with the thiol of glutathione; thus affect the biological oxidation process, block the cell respiration, and induce hypoxia in vivo [14,15]. Although sulfide concentration in natural water environment is typically low, low dose exposure can cause the immune depression and increase the pathogen susceptibility of the shrimp [4,5].

The shrimp gill is important for respiratory and osmotic adjustment. It is continuously exposed to foreign substances, including water components, microbes, and toxins from environment, the physiological homeostasis of the organ clearly effects the shrimp health. Pathogens in hemolymph are mainly absorbed through the gills, and induce physiological response or injury to gills more than other organs; the gill is also involved in the immune response for eliminating pathogens in crustaceans [16,17]. Therefore, the aim of this study was to investigate the acute toxicity of sulfide on the morphologic, physiological and

* Corresponding author. South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 231 Xingangxi Road, Guangzhou, 510300, PR China.
E-mail address: jiasongzhang@hotmail.com (J. Zhang).

https://doi.org/10.1016/j.fsi.2018.07.018
Received 2 May 2018; Received in revised form 4 July 2018; Accepted 10 July 2018
Available online 12 July 2018
1050-4648/ © 2018 Elsevier Ltd. All rights reserved.

Table 1
Primer sequences used in this study.

Primer name	Sequence (5′-3′)	GenBank accession number
HSP70-F	AGGAGACCGCTGAGGCTTAC	AY645906
HSP70-R	AGCACATTCAGACCCGAGAT	
Trx-F	TTAACGAGGCTGGAAACA	EU499301
Trx-R	AACGACATCGCTCATAGA	
HIF-1α-F	GGAGTCTTTGAGAGAGAG	FJ807918
HIF-1α-R	GCCTCCTTCCGTGATCTTC	
Casp-F	CGAAGTCAAAGCCAGAAACA	EU421939
Casp-R	ACTGCTACTTCCCTGGTGAC	
β-actin-F	GCCCTGTTCCAGCCCTCATT	AF300705
β-actin-R	ACGGATGTCCACGTCGCACT	

immune responses in the gills of *L. vannamei,* including of histological structure, osmoregulation, respiratory metabolic activity, and immune capacity. These results will provide new insights on the gills barrier of *L. vannamei* to sulfide toxicity.

2. Materials and methods

2.1. Shrimp specimens and culture conditions

Healthy juvenile L. *vannamei*, averaging 5.4 ± 0.3 g in weight, were randomly collected from a local hatchery and reared in a semi-intensive culture pond at Shenzhen Base, South China Sea Fisheries Research Institute of Chinese Academy of Fishery Sciences (Shenzhen, China). The shrimp were acclimatized in filtered, aerated seawater (salinity 30‰, pH 8.3, temperature 30 ± 0.5 °C, oxygen 6.0 ± 0.2 mg/L) for one week before the beginning of the experiment, and fed daily at a ratio of 5% of body weight using commercial formulated pellet feed (Haida Feed, Jieyang, China). One-third of the water was changed daily. Water quality parameters including salinity, pH, dissolved oxygen and temperature were continually measured throughout the experiment using portable multiparameter meter (YSI, USA).

2.2. Sulfide exposure and sampling

After acclimation, the experimental shrimps were randomly divided into two groups (the control group and the sulfide stress group), and each group included three replicate tanks. The shrimp were housed at 100 shrimps per tank, and each tank contained 300 L of filtered aerated seawater. The tanks used in the sulfide exposure experimental stage were the same as those used in the acclimation stage, and the shrimp were not moved to new tanks. Before starting the experiment, the health status of the shrimps were examined for no signs of infection. The control group was exposed to regular seawater rearing conditions (sulfide 0 mg/L). Based on the 96-h 50% lethal concentration (LC50) of sulfide (4.3 mg/L) for *L. vannamei* reported by Li et al. [6], the sulfide concentration of the sulfide stress group in this study was set to 5.0 mg/L by adding sodium sulfide ($Na_2S{\cdot}9H_2O$) to regular seawater. The sulfide level was measured by the methylene blue spectrophotometric method, and the water was renewed every 4 h by adding $Na_2S{\cdot}9H_2O$ solution to maintain the constant concentration of sulfide at 5.0 ± 0.2 mg/L, according to the evaporation rate of sulfide. All tanks were constantly aerated, and water quality parameters were continually measured using portable multiparameter meter (salinity 30‰, pH 8.3, temperature 30 ± 0.5 °C, oxygen 6.3 ± 0.2 mg/L). After the experiment, the seawater waste was dealt safely to make it harmless for environment.

After sulfide exposure for 0, 6, 12, 24, 48 and 72 h, whole gills of shrimps from each tank were randomly sampled, and snap frozen in liquid nitrogen for biochemical assays and gene expression analysis. Additionally, gills of six shrimps were randomly sampled at 72 h, and frozen at −80 °C for free amino acid (FAA) content analysis.

2.3. Histological analysis

The gill of three shrimps from each tank were randomly sampled at 72 h post-sulfide exposure, and stored in 4% paraformaldehyde for 24 h. After rinsing with running water for 8 h, the tissues were dehydrated in series of ethanol solutions (70%, 80%, 90%, and 100%), made transparent with xylene, embedded in paraffin, and sectioned in a microtome (Leica, RM2016, Germany) to a 4 μm thickness. After stating with hematoxylin and eosin (HE) dye, sections were observed and photographed under light microscopy (Olympus, Japan).

2.4. Biochemical analysis

The gill of three shrimps from each tank at the same time period were homogenized with sterile 0.9% saline solution to prepare 10% (*w*:*v*) homogenates. The homogenates were centrifuged at 3500 rpm for 10 min at 4 °C, and the supernatants was analyzed for biochemical parameters using a microplate reader (Bio-Rad, USA). The total protein concentration in tissue homogenates was measured using a Coomassie brilliant blue protein assay kit (Jiancheng, Ltd., Nanjing, China) according to the manufacturer's protocol. The assays were all run in three replicate samples.

The activities of osmoregulation enzymes, such as Na^+/K^+-ATPase (NKA) and Ca^{2+}/Mg^{2+}-ATPase (CMA), were analyzed with commercial test kits (Jiancheng, Ltd., Nanjing, China) according to the manufacturer's protocol. NKA and CMA activity was expressed as micromole inorganic phosphate per mg protein per hour.

The activities of respiratory metabolic enzymes, such as cytochrome *c* oxidase (CCO) activity was measured according to the method of Affonso et al. [13]; fumarate reductase (FRD) was measured according to the method of Xiao et al. [18]; succcinate dehydrogenase (SDH) and

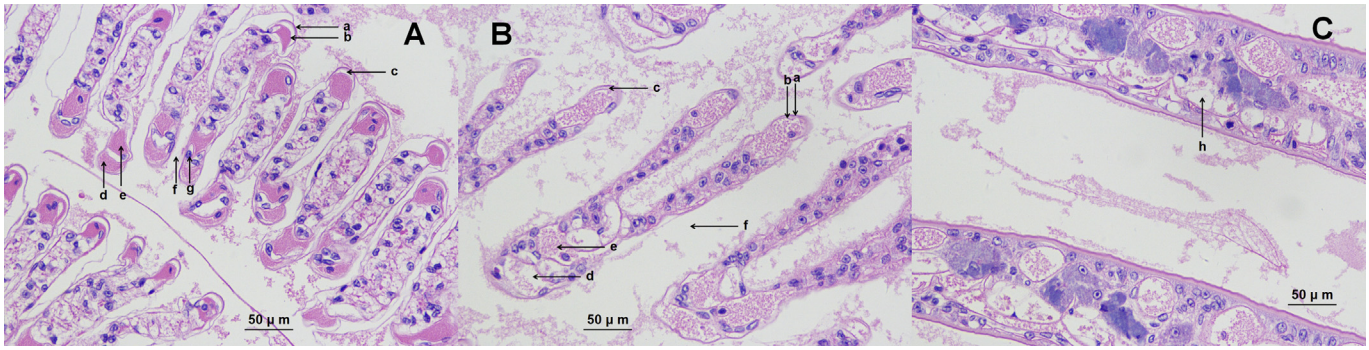

Fig. 1. Gills tissue structure of *L. vannamei* with HE stain after 72 h exposure to sulfide. (**A**) Gill from the control shrimp, × 400 magnification; (**B, C**) Gill from the shrimp exposure to 5.0 mg/L sulfide, × 400 magnification. The letters in the figure indicated that: (**a**) cuticle layer, (**b**) epithelium, (**c**) subcuticular space, (**d**) afferent vessel, (**e**) efferent vessel, (**f**) septum, (**g**) hemocyte, (**h**) vacuole.

Download English Version:

https://daneshyari.com/en/article/8498118

Download Persian Version:

https://daneshyari.com/article/8498118

Daneshyari.com