ELSEVIER

Fish & Shellfish Immunology 19 (2005) 127–138

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

The immunostimulatory effect of hot-water extract of *Gracilaria tenuistipitata* on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*

Wen-Ying Hou, Jiann-Chu Chen *

Department of Aquaculture, College of Life and Resource Sciences, National Taiwan Ocean University, Keelung 202, Taiwan, ROC

Received 12 August 2004; revised 16 November 2004; accepted 16 November 2004

Abstract

The total haemocyte count (THC), phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency to the pathogen *Vibrio alginolyticus* were examined in the white shrimp *Litopenaeus vannamei* (10.3 ± 1.5 g) injected individually with hot-water extract of *Gracilaria tenuistipitata* at 4 or 6 μg g^{-1}. *L. vannamei* receiving hot-water extract of *G. tenuistipitata* at either dose increased significantly its THC, phenoloxidase activity, and respiratory burst after 2 days. *L. vannamei* received hot-water extract of *G. tenuistipitata* at 6 μg g^{-1} increased its phagocytic activity and clearance efficiency to *V. alginolyticus* after 1 day. In another experiment, *L. vannamei* which had been injected with hot-water extract of *G. tenuistipitata* were challenged with *V. alginolyticus* at 2×10^6 colony-forming units (cfu) $shrimp^{-1}$ and then placed in seawater of 34‰. The survival of shrimp that received hot-water extract of *G. tenuistipitata* at 6 μg g^{-1} was significantly higher than that of shrimp that received saline and the control shrimp after 3 days, and at the termination of the experiment (6 days after the challenge). It is therefore concluded that *L. vannamei* receiving the hot-water extract of *G. tenuistipitata* at 6 μg g^{-1} or less increased its immune ability and resistance to *V. alginolyticus* infection.

Keywords: *Litopenaeus vannamei*; *Vibrio alginolyticus*; Hot-water extract of *Gracilaria tenuistipitata*; Total haemocyte count; Phenoloxidase activity; Respiratory burst; Superoxide dismutase activity; Phagocytic activity; Clearance efficiency

* Corresponding author. Tel./fax: +886 2 2462 0295.
E-mail address: jcchen@mail.ntou.edu.tw (J.-C. Chen).

1050-4648/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2004.11.009

1. Introduction

During the past 15 years, shrimp farming, mainly based on local species such as tiger shrimp, *Penaeus monodon*, kuruma shrimp, *Marsupenaeus japonicus*, and the exotic species white shrimp, *Litopenaeus vannamei*, have been particularly badly hit by epidemics associated with viruses [1], and *Vibrio* bacteria like *Vibrio alginolyticus* [2,3] and *V. parahaemolyticus* [4] due to deteriorated environment. Evidence indicates that shrimp pathogens are opportunistic agents. Disease outbreaks are also associated with increases in the proportion of potentially pathogenic species in the *Vibrio* population of cultured pond waters [4,5]. Therefore, the health of shrimp and enhancement of its immunity are of primary concern.

In decapod crustaceans, circulating haemocytes are generally classified into three types: hyaline, semi-granular and large granular cells [6]. Haemocytes are involved not only in phagocytosis but also in the production of melanin via the prophenoloxidase (proPO) system [7,8]. Both semi-granular and granular cells carry out the functions of the proPO system [7]. Phenoloxidase is the terminal enzyme in the proPO system and is activated by several microbial polysaccharides, including β-1,3-glucan from fungal cell walls [9].

Several reactive oxygen species are produced during phagocytosis. Starting this process, the membrane-bound enzyme complex, NADPH oxidase, assembles after binding the cell to a foreign particle, and reduces molecular oxygen to superoxide anion (O_2^-), subsequently leads to the production of hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^\bullet$) and numerous other reactive compounds [10]. Superoxide anion is the first product released from respiratory burst, and plays an important role in microbicidal activity [11].

The immunostimulatory effects of immunostimulants like levamisole, glucan, and other polysaccharides have been widely studied in fish [12,13] and crustaceans [14]. It is known that some red algae contain antitumour polysaccharides. For example, κ-carrageenan extracted from *Eucheuma cottonii* inhibits the growth of Ehrlich carcinoma in mice [15]. Porphyran extracted from red alga *Porphyra yezoensis* has been shown to have antitumour activity [15]. Hot-water extracts from several species of red algae including *Porphyra yezoensis* and *Gloiopeltis furcata* have been reported to increase the resistance of common carp *Cyprinus cyprinus* against *Edwardsiella tarda*, and yellowtail *Seriola quinqueradiata* against *Streptococcus* infection [16].

This study was undertaken to examine the immune response of *L. vannamei* and its resistance against *V. alginolyticus* when receiving a hot-water extract of *Gracilaria tenuistipitata*, a common red alga currently being cultured in Taiwan. Several immune parameters were examined including total haemocyte count, phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency of *L. vannamei*, and its susceptibility to *Vibrio alginolyticus* when the shrimp were injected with hot-water extract of *G. tenuistipitata*.

2. Materials and methods

2.1. Culture of V. alginolyticus

The bacterium *V. alginolyticus* (CH003) was isolated from diseased *L. vannamei* [3]. It was cultured on tryptic soy agar (TSA supplemented with 2% NaCl, Difco) for 24 h at 25 °C before being transferred to 10 ml of tryptic soy broth (TSB supplemented with 2% NaCl, Difco), where it remained for 24 h at 25 °C as stock culture for tests. The broth cultures were centrifuged at $7155\times g$ for 15 min at 4 °C. The supernatant fluids were removed and the bacterial pellets re-suspended in saline solution at 1×10^8 and 2×10^8 cfu ml^{-1} as stock bacterial suspensions for the susceptibility test, and phagocytic activity and clearance efficiency tests, respectively.