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The immunostimulatory effects of sodium alginate and iota-carrageenan on orange-spotted grouper *Epinephelus coicoides* and its resistance against *Vibrio alginolyticus*

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Abstract

The lysozyme activity, alternative complement activity (ACH50), respiratory burst, SOD (superoxide dismutase) activity and phagocytic activity of orange-spotted grouper *Epinephelus coicoides* were examined when the fish were injected intraperitoneally with sodium alginate at 10, 20, 30 mg kg^{-1} and ι-carrageenan at 10, 20, 30 mg kg^{-1}, respectively after 24, 72 and 120 h. Serum ACH50 increased directly with dose after 24 and 72 h for both sodium alginate and ι-carrageenan treatments. The fish that received sodium alginate at 20 mg kg^{-1} after 24 and 72 h, and the fish that received ι-carrageenan after 72 and 120 h showed significantly increased respiratory burst, SOD activity and phagocytic activity, respectively. In another experiment, *E. coicoides* which had been injected individually with sodium alginate and ι-carrageenan at 10, 20, 30 mg kg^{-1}, were challenged with *Vibrio alginolyticus* at 1.8×10^9 colony-forming units (cfu) $fish^{-1}$ and then placed in seawater of 33‰. The survival of fish that received sodium alginate at 20 mg kg^{-1}, and the fish that received ι-carrageenan at 30 mg kg^{-1} was significantly higher than that of fish which received saline and the control fish after 48 h as well as at the termination of the experiment (120 h after the challenge). It is therefore concluded that *E. coicoides* which received sodium alginate at 20 mg kg^{-1} or ι-carrageenan at 30 mg kg^{-1} increased the non-specific immune response and resistance from *V. alginolyticus* infection.

Keywords: *Epinephelus coicoides*; *Vibrio alginolyticus*; Sodium alginate; Carrageenan; Alternative complement activity; Lysozyme activity; Respiratory burst; Superoxide dismutase activity; Phagocytic activity

1. Introduction

Grouper culture has become an alternative and important activity since penaeid shrimp culture industry collapsed in the late eighties in Taiwan. Among the more than 150 species of grouper worldwide, orange-spotted grouper *Epinephelus coicoides* and malabia grouper *Epinephelus malabaricus* are the most commonly cultured. The production of

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farmed grouper has increased more than two times from 5052 tonnes in 2001 to 12,103 tonnes in 2004 in Taiwan. Vibriosis is a common problem in the intensive culture of grouper [1,2]. The pathogens *Vibrio alginolyticus* and *Vibrio carchariae* are causative agents of vibriosis with a gastroenteritis syndrome (swollen intestine containing yellow fluid) [3,4]. In addition, nodavirus and iridovirus are the causative agents of viral nervous necrosis and sleepy disease in hatchery-reared larvae and juveniles of grouper, respectively [5,6]. Therefore, prevention of disease and maintenance of fish health are of primary concern.

Some adjuvants and immunostimulants like β-glucans, chitins and bacterial polysaccharides are commonly used to prevent occurrence of disease and to enhance immunity of fish [7,8]. β-glucan administration has been reported to increase antibody production, complement activity, lysozyme activity, macrophage activity and respiratory burst of channel catfish *Ictalurus punctatus* [9], rainbow trout *Oncorhynchus mykiss* [10], Atlantic salmon *Salmo salar* [11,12], and gilthead seabream *Sparus auratus* [13].

It is known that κ-carrageenan extracted from red alga *Chondrus ocellatus* has been reported to increase the phagocytic activity of common carp *Cyprinus carpio* and its resistance against *Edwardsiella tarda* and *Aeromonas hydrophila* via intraperitoneal injection [14,15]. It is also known that sodium alginate extracted from brown algae *Undaria pinnatifida* and *Macrocystis pyritera* has been reported to increase the non-specific defense system of common carp *C. carpio* and its resistance against *E. tarda* infection [16,17]. An alginic acid (Ergosan) extracted from brown alga *Laminaria digitata* has also been reported to increase the non-specific defense response of snakehead *Channa striata* [18], rainbow trout *O. mykiss* [19], and sea bass *Dicentrarchus labrax* [20].

Presently little is known about the response of grouper to immunostimulants like sodium alginate or carrageenan. Accordingly, the aim of this work was to examine several innate immune parameters including lysozyme activity and alternative complement activity (ACH50) in serum, and respiratory burst, superoxide dismutase (SOD) activity, and phagocytic activity of head kidney leucocytes in grouper *E. coicoides* and its resistance against *V. alginolyticus* following injection of sodium alginate and carrageenan.

2. Materials and methods

2.1. Animals

Around three hundred grouper *E. coicoides* obtained from Kaohsiumg, Taiwan, were shipped to our laboratory and kept for two weeks in a 5000 L circular tank with recirculating aerated seawater (33‰) at 24 ± 1 °C. Fish were fed daily with a commercial diet (Grobset, Taiwan).

2.2. Experimental design

Sodium alginate (A2158, low viscosity, Sigma Chemical Co., Saint Louis, MO, USA) from kelp *Macrocystis pyritera*, and iota (ι)-carrageenan (C1138, Type II, Sigma) from Irish moss *Chondrus crispus* blended with various seaweeds was dissolved in sterile Hanks' balanced salt solution (HBSS, Sigma) to produce 10, 20, 30 mg ml^{-1}, respectively as test solutions before injection. Two studies were conducted. For the study of resistance of grouper to *V. alginolyticus*, test and control groups were comprised of five fish each in duplicate. For the studies of blood leucocyte count, serum lysozyme and alternative complement activity, and head kidney leucocyte respiratory burst, SOD activity and phagocytic activity, test and control groups were comprised of eight fish. The weight of the fish ranged from 686 to 815 g, averaging 756 ± 58 g (mean ± S.D.) with no significant size differences among the treatments.

2.3. Culture of V. alginolyticus

V. alginolyticus (ATCC17749) obtained from Bioresources Collection and Research Centre, Food Industry and Development Institute (Hinchu, Taiwan) was used for the study. It was cultured on tryptic soy agar (TSA supplemented with 3% NaCl, Difco) for 24 h at 28 °C before being transferred to 10 ml tryptic soy broth (TSB supplemented with 3% NaCl, Difco), where it remained for 24 h at 28 °C as stock culture for tests. The broth cultures were centrifuged at 7155 × *g* for 15 min at 4 °C. The supernatant was removed and the bacterial pellets were re-suspended in saline at 2.4×10^9 cfu ml^{-1} for the resistance test of grouper to *V. alginolyticus*.