The immune stimulatory effect of sodium alginate on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*


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**Abstract**

The total haemocyte count (THC), differential haemocyte count (DHC), phenoloxidase activity, respiratory burst (release of superoxide anion), superoxide dismutase activity, phagocytic activity and clearance efficiency to the pathogen *Vibrio alginolyticus* were measured when the white shrimp *Litopenaeus vannamei* (9.4–11.3 g) were injected individually with sodium alginate at 10, 20 or 50 μg g⁻¹. No significant differences in THC, DHC and superoxide dismutase activity were observed among the shrimp injected with saline and those injected with sodium alginate at 10, 20 or 50 μg g⁻¹. However, *L. vannamei* injected with sodium alginate at 20 μg g⁻¹ increased its phenoloxidase activity and respiratory burst after 2 days and one day, respectively. *L. vannamei* injected with sodium alginate at 50 μg g⁻¹ maintained a higher phagocytic activity and clearance efficiency to *V. alginolyticus* after 4 days. In another experiment, *L. vannamei* which had been injected with sodium alginate, were challenged with *V. alginolyticus* at 2 × 10⁵ colony-forming units (CFU) shrimp⁻¹ and then placed in seawater of 34‰. The survival of shrimp that received sodium alginate at either dose was significantly higher than that of control shrimp at the termination of the experiment (6 days after the challenge). It is therefore concluded that *L. vannamei* received sodium alginate at 10 μg g⁻¹ or more and increased its immune ability and resistance from *V. alginolyticus* infection.

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**Keywords:** *Litopenaeus vannamei*, *Vibrio alginolyticus*, Sodium alginate; Phenoloxidase activity; Respiratory burst; Phagocytic activity; Clearance efficiency

**1. Introduction**

During the past fifteen years, commercial shrimp farming, mainly based on local species such as the tiger shrimp *Penaeus monodon*, kuruma shrimp *Marsupenaeus japonicus*, and the exotic species white shrimp *Litopenaeus vannamei* have suffered disease outbreaks associated with bacteria like *Vibrio alginolyticus* [1],
V. damsela [2], V. harveyi [3], V. parahaemolyticus [4], and V. vulnificus [5], and viruses like monodon baculovirus (MBV) [6], white spot syndrome virus (WSSV) [7], infectious hypodermal and hematopoietic necrosis virus (IHHNV) [8], yellow head virus (YHV) [9] and Taura syndrome virus (TSV) [10] in Taiwan. Consequently, the total farmed production of penaeid shrimp declined from 82,598 ton in 1987 to 8878 ton in 2001.

In decapod crustaceans, circulating haemocytes are generally classified into three types: hyaline, semi-granular and large granular cells [11]. Haemocytes are involved not only in phagocytosis but also in the production of melanin via the prophenoloxidase (proPO) system [12,13]. Both semi-granular and granular cells carry out the functions of the proPO system [12]. 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 [14].

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 lead to the production of hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1O$_2$), hydroxyl radical (OH$^-$) and numerous other reactive compounds [15].

Superoxide anion is the first product released from respiratory burst, and plays an important role in microbicidal activity [16].

The immune stimulatory effects of glucan, chitosan and other polysaccharides have been widely studied in fish and crustaceans and reviewed by Sakai [17] and Song and Huang [18]. Sodium alginate extracted from wakame (a brown alga) Undaria pinnatifida has been reported to enhance the resistance of common carp Cyprinus carpio against Edwardsiella tarda infection [19]. Sodium alginate extracted from giant kelp Macrocystis pyrifera has been reported to increase the non-specific defense system of C. carpio [20]. Sodium alginate consists of β-1,4-D-mannuronate and α-1,4-L-guluronate, which is different from that of fungus and yeast-derived polysaccharides [21]. It is assumed that penaeid shrimp when receiving sodium alginate may increase their immune ability and enhance resistance to Vibrio infection. Accordingly, several immune parameters were examined including haemocyte count, phenoloxidase activity, respiratory burst, superoxide dismutase activity, phagocytic activity and clearance efficiency of L. vannamei, and its susceptibility to V. alginolyticus when the shrimp were injected with sodium alginate.

2. Materials and methods

2.1. Culture of V. alginolyticus

A strain of V. alginolyticus (NTOU-CH003) isolated from diseased L. vannamei, which displayed symptoms of anorexia, inactivity, poor growth and necrotic musculature was used for the study. The pathogen 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 fluids were removed and the bacterial pellets were re-suspended in saline solution at $1×10^7$ and $2×10^8$ cfu ml$^{-1}$ as stock bacterial suspensions for the susceptibility study, and for the phagocytic activity and clearance efficiency studies of L. vannamei to V. alginolyticus, respectively.

2.2. Experimental design

Sodium alginate (A2158, low viscosity, Sigma Chemical Co., Saint Louis, MO, USA) extracted from M. pyrifera was used in the study. Sodium alginate was dissolved in sterile saline to concentrations of 5, 10, 25 mg ml$^{-1}$ before injection.
About three hundred shrimp harvested from the University Marine Station adjacent to the coast of Keelung, Taiwan were shipped to our laboratory. Shrimp were placed in fiberglass tanks (5 m³), and acclimated to room temperature (27.0±0.5 °C) for 2 weeks. During the acclimation period, shrimp were fed twice daily with a formulated shrimp diet (Tairou Feed Company, Tainan, Taiwan). Only shrimp in the intermoult stage were used for the study. The moult stage was examined by the examination of uropoda in which partial retraction of the epidermis could be distinguished [22]. Three studies were conducted. For the study of susceptibility of shrimp to *V. alginolyticus*, test and control groups comprised of 10 shrimp. For the studies of haemocyte count, phenoloxidase activity, respiratory burst and superoxide dismutase activity, test and control groups comprised of 8 shrimp. For the studies of phagocytic activity and clearance efficiency, test and control groups composed also of 8 shrimp. All studies were conducted in triplicate. The shrimp ranged from 9.4 g to 11.3 g, averaging 9.21±3.7 g (mean±SD) with no significant size differences among the treatments.

### 2.3. Effect of sodium alginate on the susceptibility of *L. vannamei* to *V. alginolyticus*

*L. vannamei* was injected individually into the ventral sinus of the cephalothorax with 5, 10 and 25 mg ml⁻¹ sodium alginate solution (around 20 μl) to reach doses of 10 μg g⁻¹, 20 μg g⁻¹ and 50 μg g⁻¹, respectively on the first day. The shrimp injected with saline (20 μl) served as saline group. The challenge test was conducted on the second day by the injection of 20 μl of bacterial suspension (1×10⁷ cfu ml⁻¹) resulting in 2×10⁵ cfu shrimp⁻¹ into the ventral sinus of the cephalothorax. The shrimp that received no sodium alginate, and then received *V. alginolyticus* at 2×10⁵ cfu shrimp⁻¹ served as the challenged control. The shrimp that received no sodium alginate, and then received saline (20 μl) however, served as the unchallenged control (Table 1). Experimental and control shrimp (10 aquarium⁻¹) were kept in 60 l glass aquaria containing 40 l of water at 34°C. There were therefore a total of six treatments. Each treatment was conducted with 30 shrimp. Water was renewed daily, and the experiment lasted 6 days.

### 2.4. The immune parameters of *L. vannamei* injected with sodium alginate

*L. vannamei* was injected individually in the ventral sinus of the cephalothorax with sodium alginate solution to reach 10 μg g⁻¹, 20 μg g⁻¹ and 50 μg g⁻¹ on the first day. The shrimp that received saline (20 μl) and those with no injection served as the saline and control groups, respectively. There were five treatments (control, saline, 10 μg g⁻¹, 20 μg g⁻¹ and 50 μg g⁻¹) with four sampling times (1, 2, 4 and 6 days). Eight shrimp for each treatment and time were used for the studies. In addition, eight shrimp without any treatment were also used as the initial group.

<table>
<thead>
<tr>
<th>Bacterial (cfu shrimp⁻¹)</th>
<th>Sodium alginate (μg g⁻¹)</th>
<th>No. shrimp</th>
<th>Survival (%) after time elapsed (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Saline Control 30</td>
<td>100</td>
<td>70.0 ± 0.0b</td>
<td>60.0 ± 0.0b</td>
</tr>
<tr>
<td>2×10⁵ Control 30</td>
<td>86.77 ± 86.8a</td>
<td>80.0 ± 5.8b</td>
<td>63.7 ± 3.3b</td>
</tr>
<tr>
<td>2×10⁵ Saline 30</td>
<td>86.77 ± 86.8a</td>
<td>80.0 ± 5.8b</td>
<td>63.7 ± 3.3b</td>
</tr>
<tr>
<td>2×10⁵ 10 30</td>
<td>90.0 ± 5.8a</td>
<td>86.7 ± 8.8b</td>
<td>83.7 ± 6.8b</td>
</tr>
<tr>
<td>2×10⁵ 20 30</td>
<td>90.0 ± 5.8a</td>
<td>80.0 ± 10.0a</td>
<td>76.7 ± 6.7a</td>
</tr>
<tr>
<td>2×10⁵ 50 30</td>
<td>93.7 ± 6.8a</td>
<td>86.7 ± 6.7a</td>
<td>80.0 ± 5.8a</td>
</tr>
</tbody>
</table>

Data in the same column with different letters are significantly different (P<0.05) among different treatments. Values are mean ± S.E.
After 0, 1, 2, 4 and 6 days of injection, haemolymph (100 µl) was withdrawn from the ventral sinus of each shrimp into a 1 ml sterile syringe (25 gauge) containing 0.9 ml anticoagulant solution (trisodium citrate 30 mM, sodium chloride 0.34 M, EDTA 10 mM, pH 7.55, osmolality adjusted with glucose to 780 mOsm kg⁻¹). They were divided into three parts. A drop of the anticoagulant–haemolymph mixture (100 µl) was placed on a haemocytometer to measure THC and DHC using an inverted phase-contrast microscope (Leica DMIL, Leica Microsystems, Wetzlar GmbH, Germany). Another part of the anticoagulant–haemolymph mixture was used for the identification and numeration of hyaline cells, semi-granular cells and granular cells based on the methods described by Le Moullac et al. [23]. Haemocyte count in all test and control groups after 6 days however was not measured due to experimental failure. The remainder of the haemolymph mixture was used for subsequent tests.

Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) following the procedures of Hernández-López et al. [24]. The details of measurements were described previously [25]. The optical density of the shrimp’s phenoloxidase activity was expressed as dopachrome formation per 50 µl haemolymph.

Respiratory burst of haemocytes was quantified by the reduction of NBT (nitroblue tetrazolium) to formazan as a measure of superoxide anion (O₂⁻) as described previously [25,26]. The optical density at 630 nm was measured in triplicates using a microplate reader (Model VERSAmax, Molecular Devices, Sunnyvale, CA, USA). Respiratory burst was expressed as NBT-reduction per 10 µl haemolymph.

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical dependent reactions using the Ransod Kit (Randox, Crumlin, UK). Briefly, the reaction mixture (1.7 ml) contained 0.05 mM xanthine and 0.025 mM 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) dissolved in 50 mM CAPS (pH 10.2) and 0.94 mM EDTA. In the presence of xanthine oxidase (80 U l⁻¹, 250 µl), superoxide and uric acid were produced from xanthine. The superoxide radical then reacted with INT to produce a red formazan dye. The optical density was measured at 505 nm, 37 °C, and the rate of reaction was estimated from the absorbance readings 30 s and 3 min after adding xanthine oxidase. A reference standard SOD was supplied with the Ransod Kit. One unit of SOD was defined as the amount required to inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units ml⁻¹ [27].

2.5. Phagocytic activity and clearance efficiency of L. vannamei to V. alginolyticus

*L. vannamei* that received saline and sodium alginate were the same as that described above. Tests were carried out in eight replicate test groups with one shrimp in each treatment. For the phagocytic activity and clearance efficiency tests, 20 µl of bacterial suspension (2×10⁸ cfu ml⁻¹) resulting in 4×10⁶ cfu shrimp⁻¹ were injected into the ventral sinus. After injection, the shrimp were kept for 1 h in a separate tank containing 40 l of water at 27.5±0.5 °C. Then, 200 µl of haemolymph was collected from the ventral sinus and mixed with 200 µl of sterile anticoagulant (trisodium citrate 30 mM, sodium chloride 0.34 M, EDTA 10 mM, pH 7.55, osmolality adjusted with glucose to 780 mOsm kg⁻¹). This mixture was divided into two equal sub-samples, one to measure phagocytic activity and the other to measure clearance efficiency. The methods for the measurements for phagocytic activity and clearance efficiency were described previously [25].

2.6. Statistical analysis

A multiple comparison (Tukey) test was conducted to compare the significant differences among treatments using the SAS computer software (SAS Institute Inc., Cary, NC, USA). Before analysis, percent data (susceptibility study) were normalised using an arc sin transformation before analysis. For statistically significant differences, it was required that P<0.05.
3. Results

3.1. Effect of sodium alginate on the susceptibility of L. vannamei to V. alginolyticus

All the unchallenged control shrimp survived. By contrast, death occurred after one day for the challenged shrimp. After 2 days, the survival of shrimp that received sodium alginate at 50 µg g⁻¹ was significantly higher than that of challenged control shrimp. After 3–6 days of challenge, the survival of shrimp that received sodium alginate at 10, 20 and 50 µg g⁻¹ was significantly higher than the shrimp that received saline and the control shrimp (Table 1).

3.2. The immune parameters of L. vannamei injected with sodium alginate

No significant differences in THC and DHC were observed among the shrimp that received sodium alginate at 10, 20, 50 µg g⁻¹, the shrimp that received saline, and the control shrimp, at the beginning of the experiment, nor after 4 days of treatments. The hyaline cells constituted 52.4% to 53.0% of the THC, and varied from 174.2 ± 12.3 x 10⁵ (mean ± S.E.) to 204.0 ± 26.1 x 10⁵ cells ml⁻¹. The semi-granular cells contributed 32.3% to 33.2% of the THC, and varied from 110.2 ± 8.2 x 10⁵ to 124.6 ± 5.8 x 10⁵ cells ml⁻¹. The granular cells contributed 13.8% to 15.3% of the THC, and varied from 41.5 ± 10.5 x 10⁵ to 59.0 ± 9.6 x 10⁵ cells ml⁻¹. The mean (± S.E.) THC varied from 330.8 ± 21.8 x 10⁵ to 365.3 ± 10.3 x 10⁵ cells ml⁻¹ (Table 2).

Phenoloxidase activity of shrimp that received sodium alginate at 50 µg g⁻¹ after one day, and phenoloxidase activity of shrimp that received 20 µg g⁻¹ and higher was significantly higher than that of shrimp that received saline and the control shrimp after 2 days. However, no significant difference in phenoloxidase activity was observed among the five treatments after 4 to 6 days (Fig. 1).

Respiratory burst of shrimp that received sodium alginate at 20 µg g⁻¹ or higher was significantly higher than that of shrimp that received saline as well as the control shrimp after one day. Respiratory burst of shrimp that received 10 µg g⁻¹ and higher µg g⁻¹ was significantly higher than that of shrimp that received saline and the control shrimp after 2 days. Respiratory burst of shrimp that received sodium alginate at any

Table 2
Mean (± S.E.) THC (total haemocyte count), HC (hyaline cells), SGC (semi-granular cells) and GC (granular cells) in the L. vannamei which had been received sodium alginate at 10, 20 and 50 µg g⁻¹, and then challenged with V. alginolyticus

<table>
<thead>
<tr>
<th>Haemocyte</th>
<th>Sampling time (day)</th>
<th>Treatment</th>
<th>10 µg g⁻¹</th>
<th>20 µg g⁻¹</th>
<th>50 µg g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC (×10⁵ ml⁻¹)</td>
<td></td>
<td>Control</td>
<td>Saline</td>
<td>Control</td>
<td>Saline</td>
</tr>
<tr>
<td>0</td>
<td>365.3 ± 10.3a</td>
<td>365.3 ± 10.3a</td>
<td>365.3 ± 10.3a</td>
<td>365.3 ± 10.3a</td>
<td>365.3 ± 10.3a</td>
</tr>
<tr>
<td>1</td>
<td>353.3 ± 13.7a</td>
<td>351.4 ± 30.6a</td>
<td>359.6 ± 34.3a</td>
<td>354.0 ± 16.4a</td>
<td>365.6 ± 22.4a</td>
</tr>
<tr>
<td>2</td>
<td>355.3 ± 10.3a</td>
<td>349.0 ± 10.1a</td>
<td>358.3 ± 17.1a</td>
<td>355.0 ± 40.7a</td>
<td>338.8 ± 18.7a</td>
</tr>
<tr>
<td>4</td>
<td>348.8 ± 40.8a</td>
<td>330.8 ± 21.8a</td>
<td>334.8 ± 21.3a</td>
<td>342.5 ± 41.1a</td>
<td>338.8 ± 34.9a</td>
</tr>
<tr>
<td>HC (×10⁵ ml⁻¹)</td>
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<td>Control</td>
<td>Saline</td>
<td>Control</td>
<td>Saline</td>
</tr>
<tr>
<td>0</td>
<td>185.5 ± 5.6a</td>
<td>185.5 ± 5.6a</td>
<td>185.5 ± 5.6a</td>
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<td>1</td>
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<td>191.5 ± 12.5a</td>
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<td>194.5 ± 9.5a</td>
<td>182.2 ± 7.1a</td>
<td>190.2 ± 6.5a</td>
<td>195.3 ± 20.7a</td>
<td>177.7 ± 9.8a</td>
</tr>
<tr>
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<td>174.2 ± 12.3a</td>
<td>186.1 ± 13.7a</td>
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<td>Saline</td>
<td>Control</td>
<td>Saline</td>
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<tr>
<td>0</td>
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<tr>
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<td>114.4 ± 10.2a</td>
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<td>Control</td>
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<td>44.0 ± 4.9a</td>
<td>46.9 ± 6.3a</td>
<td>49.4 ± 5.7a</td>
</tr>
</tbody>
</table>

Data in the same row having same letter are not significantly different (P > 0.05) among treatments.
dose was significantly higher than that of shrimp that received saline and the control shrimp after 4 days. However, no significant difference in respiratory burst was observed among the five treatments after 6 days (Fig. 2A). Concerning the superoxide dismutase activity, no significant difference was observed among the five treatments (Fig. 2B).

3.3. Phagocytic activity and clearance efficiency of L. vannamei to V. alginolyticus

Phagocytic activity was significantly higher for the shrimp that received sodium alginate at 10 μg g⁻¹ than for the control shrimp after one day, and maintained to two days. Phagocytic activity was significantly higher for the shrimp that received sodium alginate at 10 μg g⁻¹, and higher than the shrimp that received saline as well as the control shrimp after 2 days. Phagocytic activity was 28.1%, 27.6%, 29.6%, 20.1% and 20.3% for the 50 μg g⁻¹, 20 μg g⁻¹, 10 μg g⁻¹, saline and control group, respectively after 2 days. Phagocytic activity was significantly higher for the shrimp that received sodium alginate at 50 μg g⁻¹ than that for shrimp that received sodium alginate at 20 μg g⁻¹, 10 μg g⁻¹, saline and the control group after 4 days. However, no significant difference in phagocytic activity was observed among the five treatments after 6 days (Fig. 3A).

Clearance efficiency was significantly higher for the shrimp that received sodium alginate at 50 μg g⁻¹, 20 μg g⁻¹ and 10 μg g⁻¹ than that of the control shrimp after 1 day. Clearance efficiency increased by 57.2%, 70.8%, 58.0% for the shrimp that received sodium alginate at 50 μg g⁻¹, 20 μg g⁻¹, 10 μg g⁻¹ after one day as compared to the control shrimp. Clearance efficiency was significantly higher for the shrimp that received 50 μg g⁻¹ sodium alginate than that for the shrimp that received saline as well as the control shrimp after 2 and 4 days. No significant difference in clearance efficiency, however, was observed among the five treatments after 6 days (Fig. 3B).
4. Discussion

Administration of β-1,3-1,6-glucan, extracted from yeast *Saccharomyces cerevisiae* by immersion has been reported to increase the resistance of *P. monodon* against *V. vulnificus* [28], and against WSSV [29]. Dietary administration of peptidoglycan derived from *Bifidobacterium thermophilum* has been reported to...
increase the resistance of *M. japonicus* against *V. penaeicida* and WSSV [30]. An oral administration of schizophyllan, a β-1,3-glucan extracted fungus *Schizophyllum commune* has been reported to increase the resistance of *M. japonicus* against *Vibrio* [31], and the resistance of *P. monodon* against *V. damsela* [32] and WSSV infection [33]. The survival rate of *P. monodon* brooder that were fed a diet containing β-1,3-glucan

![Fig. 3. Mean (±S.E.) phagocytic activity (A) and clearance efficiency (B) of *L. vannamei* received sodium alginate at 50 μg g⁻¹, 20 μg g⁻¹, 10 μg g⁻¹, received saline and the control shrimp. See Fig. 1 for statistical information.](image-url)
at 2 g kg\(^{-1}\) for 10 days, was significantly higher that that of shrimp brooder fed a diet without β-1,3-glucan [34]. In the present study, \textit{L. vannamei} injected with sodium alginate at a dose of 10 μg g\(^{-1}\) increased the resistance against \textit{V. alginolyticus}. Therefore, polysaccharides like fungus glucan, yeast glucan, and sodium alginate all showed positive effects on preventing penaeid shrimps against \textit{Vibrio} and WSSV infections.

\textit{P. monodon} which had been immersed in aerated yeast glucan (β-1,3-1,6-glucan) increased its phenoloxidase activity [28]. In another experiment, \textit{P. monodon} which had been immersed in viable cell suspension of \textit{V. vulnificus}, yeast glucan and zymosan (β-1,3-glucan–protein–lipid compound) all showed increased phenoloxidase activity in 24 h [35]. \textit{M. japonicus} following 7 days feeding of lipopolysaccharide (LPS) at a dose of 20 μg per kg shrimp body weight day\(^{-1}\), increased its phenoloxidase activity [36]. In the present study, phenoloxidase activity increased significantly for the shrimp injected with sodium alginate at doses of 20 μg g\(^{-1}\) and 50 μg g\(^{-1}\) increased significantly after 2 days. Therefore, yeast glucan, LPS, \textit{Vibrio} antigen and sodium alginate can trigger the phenoloxidase activity which indicates an increase in immune ability.

Nitroblue tetrazolium (NBT) staining has been used for both qualitative and quantitative analysis of superoxide anion which is the first product of the respiratory burst generated by haemocytes [37]. \textit{P. monodon} which had been immersed in viable cell suspension of \textit{V. vulnificus}, yeast glucan (β-1,3-1, 6-glucan) or zymosan, all stimulated the release of superoxide anion [35]. \textit{L. vannamei} which had been immersed in aerated β-1,6-glucan from \textit{S. cerevisiae}, sulphated polysaccharide from cyanobacteria strain Cyanothece sp. and laminarin from Laminaria digitata, all increased its release of superoxide anion in 6 h [38]. In the present study, \textit{Litopenaeus vannamei} that injected with sodium alginate at doses of 50, 20 and 10 μg g\(^{-1}\), maintained even higher activity after 4 days indicating an increase in immune ability. It is therefore determined that these polysaccharides including sodium alginate enhance the immune stimulatory abilities of \textit{P. monodon} and \textit{L. vannamei}.

A fungus glucan (β-1,3-glucan) has been reported to increase the phagocytic activity of \textit{M. japonicus} [31] and increased the phagocytic activity of \textit{P. monodon} when treated with fluorescently labelled latex beads [34]. In a similar study, Itami et al. [30] observed that an oral administration of peptidoglycan enhanced the phagocytic activity of \textit{M. japonicus}. Takahashi et al. [36] reported that \textit{M. japonicus} following 7 days of feeding of lipopolysaccharide (LPS) at a dose of 20 μg per kg shrimp body weight day\(^{-1}\), increased its phagocytic index. Adams [39] reported that more than 99% of heat-killed \textit{V. alginolyticus} were cleared from haemocytes of \textit{P. monodon} in 4 h of exposure. Sung et al. [35] observed that \textit{P. monodon}, which had been immersed in viable cell suspension of \textit{V. vulnificus} at 10⁷ cfu ml\(^{-1}\), is able to clear 94% of cells within 3 h. In the present study, phagocytic activity and clearance efficiency correlated well with the susceptibility of \textit{V. alginolyticus} when the shrimp were received sodium alginate. Therefore, fungus glucan, leptidoglycan, LPS, \textit{Vibrio} antigen and sodium alginate all showed the ability to have phagocytosis and clearance against \textit{Vibrio} incorporated.

In the present study, both phenoloxidase activity and respiratory burst of shrimp that received sodium alginate at a dose of 10, 20 or 50 μg g\(^{-1}\) increased within 2 days, but returned to normal level after 4 days of treatment. In addition to the higher activities of phenoloxidase and respiratory burst, the phagocytic activity and clearance efficiency of \textit{L. vannamei} to \textit{V. alginolyticus} still remained higher for the shrimp that received sodium alginate at a dose of 50 μg g\(^{-1}\) after 4 days, and returned to normal level after 6 days of treatment. It is considered that sodium alginate can be used as an immunostimulant, but additional as well as continuous doses are necessary to ensure the immune ability of \textit{L. vannamei}. Further research is needed to evaluate the immune stimulatory effect and the resistance against \textit{Vibrio} infection when the shrimp are fed sodium alginate containing diet.

In conclusion, the present study documents that \textit{L. vannamei} that received sodium alginate at 10 μg g\(^{-1}\), 20 μg g\(^{-1}\) or 50 μg g\(^{-1}\), increased its immune ability by increasing its phenoloxidase activity and respiratory burst. \textit{L. vannamei} that received sodium alginate at a dose of 10 μg g\(^{-1}\) or more increased its resistance against \textit{V. alginolyticus} by increasing its phagocytic activity and clearance efficiency. Sodium alginate can be used as an immunostimulant for \textit{L. vannamei}. 
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