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Sodium alginate from *Sargassum wightii* retards mortalities in *Penaeus monodon* postlarvae challenged with white spot syndrome virus

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ABSTRACT: Sodium alginate extracted from brown seaweed *Sargassum wightii* (16.35 ± 1.42%, mean [±SD] yield from 5 extractions) was prepared as a powder or beads and used to enrich *Artemia* nauplii at concentrations of 100, 200, 300 and 400 mg l^{-1}. The alginate-enriched nauplii were fed to *Penaeus monodon* shrimp postlarvae (PL) stage 15 (PL15, i.e. 15 d old) for 20 d. Mean weight gain and specific growth rate over this period were 0.24 g and 15.8%, respectively, in PL groups not fed alginate, and 0.20–0.28 g and 14.7–16.5%, respectively, in PL groups fed alginate. Amongst PL35 then challenged with white spot syndrome virus (WSSV) by immersion, all PL not fed alginate died within 9 d. However, amongst PL fed the 4 concentrations of alginate powder or beads, mortality rates reduced with increasing alginate concentration, and between 25 and 32% PL remained alive when the bioassay was terminated on Day 21. Amongst alginate-fed PL groups compared with the control group, mortality was reduced by 26.5 to 58.4%. Nested PCR detection of WSSV revealed sodium alginate concentration-dependent reductions in infection loads. The data indicate that sodium alginate extracted from brown seaweed and fed to *P. monodon* can retard progression of WSSV disease.

KEY WORDS: Tiger shrimp · WSSV · Brown seaweed · Algal extract · Microbeads · Viral disease · PCR

INTRODUCTION

Aquaculture of the major shrimp species, including giant tiger shrimp *Penaeus monodon*, Kuruma shrimp *Marsuspenaeus japonicus* and Pacific white shrimp *Litopenaeus vannamei*, has suffered in many countries owing to disease caused by viruses such as white spot syndrome virus (WSSV) (Chou et al. 1995), yellow head virus (YHV) (Wang & Chang 2000), Taura syndrome virus (TSV) (Tu et al. 1999) and monodon baculovirus (MBV) (Lightner et al. 1987). However, by far the most widespread and devastating disease problems in shrimp have been caused by WSSV (Moscardi 1999). The principal gross signs of white spot disease (WSD) are the development of white spots on the shrimp exoskeleton and carapace, rapid reductions in feed consumption, lethargy and reddening of appendages; the disease can result in 100% mortality of pond stocks within 3 to 10 d of the disease becoming evident (Flegel 1997).

The serious worldwide impacts of WSD and the broad host range of WSSV have prompted efforts to develop disease control strategies based on environmental management, quarantine procedures and immunostimulants as well as virus neutralization and immunization (Xiang 2001). Protective effects

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against WSSV infection have been reported for peptidoglycan- and lipopolysaccharide-based immunostimulants (Itami et al. 1998, Takahashi et al. 2000) and β-1,3-glucan (Song et al. 1997, Chang et al. 1999) delivered orally to *Marsuspenaeus japonicus* and *Penaeus monodon*. Protective responses against WSD have also been reported via oral delivery of herbal immunostimulants, such as an extract from *Cynodon dactylon* plants (Citarasu et al. 2006, Rameshthangam & Ramasamy 2007, Balasubramanian et al. 2008), and an acyclic nucleoside phosphonate ([S]-1-3-hydroxy-2-phosphonyl methoxy propyl cytosine) supplemented with extracts of the marine blue-green algae *Spirulina platensis* (Rahman et al. 2006). More recently, *Cereops tagal* mangrove extracts have been screened for anti-WSSV activity in *P. monodon* (Sudheer et al. 2011), and *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphyllum* var. *chinensis* powder showed increased protection against *Vibrio alginolyticus* and WSSV (Huynh et al. 2011).

Seaweeds are multicellular algae that can produce several substances found to have immunostimulatory activity, and brown seaweeds have recently been examined as a source of polysaccharides potentially useful as therapeutic agents and antibiotics. Fucoidan-containing extracts (Chotigeat et al. 2004) and hot water extracts (Immanuel et al. 2010) of *Sargassum* spp. provide *Penaeus monodon* with some resistance to WSD, as have fucoidan extracts from *Cladosiphon okamuranus* seaweed in *Marsuspenaeus japonicus* (Takahashi et al. 1998). Sodium alginate extracted from brown algae *Undaria pinnatifida* and *Macrocystis pyrifera* can enhance the nonspecific defense response of common carp *Cyprinus carpio* to *Edwardsiella tarda* (Fujiki et al. 1994, 1997). It can also enhance the migration of carp head kidney phagocytes to the peritoneal cavity to increase phagocytic activity (Fujiki & Yano 1997) and can enhance the survival of juvenile turbot *Scophthalmus maximus* challenged with *Vibrio anguillarum* (Skjermo et al. 1995). Similarly, alginate extracted from brown algae *U. pinnatifida* and *Lessonia nigrecans* increase *Litopenaeus vannamei* resistance to *V. alginolyticus* (Cheng et al. 2004).

Studies of methods to improve shrimp resistance to viral disease include immersion, injection, bioencapsulation and oral delivery routes of immunostimulants. For example, live *Artemia* have been used as a bioencapsulation system for nutrients (Watanabe et al. 1983), antimicrobial agents (Dixon et al. 1995) and antigens (Campbell et al. 1993). To further examine the potential protective properties of seaweed polysaccharides, we evaluated the ability of sodium alginate extracted from brown seaweed *Sargassum wightii* to protect *Penaeus monodon* postlarvae against WSD.

MATERIALS AND METHODS

Sodium alginate extraction from seaweed

Sargassum wightii seaweed was collected from coastal villages in the Kanyakumari District, Tamilnadu, India, washed thoroughly and dried under shade at room temperature. The dried seaweed was ground using a mixer grinder and nonpowdered material was removed by using a sieve (Immanuel et al. 2004). Sodium alginate was extracted from the ground seaweed by using a method modified from Torres et al. (2007). Briefly, 100 g of seaweed powder was soaked in 2% formaldehyde in an air-tight flask for 24 h, the formaldehyde was filtered off and the solid residue was washed 2 to 3 times with distilled water. The residue was placed in 0.2 M HCl at room temperature for 24 h, and the solid residue was again recovered by filtration and washed again 2 to 3 times in distilled water. The residue was extracted overnight in 2% Na_2CO_3 and filtered through muslin cloth. The solution was bleached with 2.5% sodium hypochlorite and liquid was then evaporated off in an oven at 60°C. The resulting residue was scraped from the container, made into a powder and weighed to calculate sodium alginate yield (%) as (sodium alginate weight/milled seaweed weight) × 100.

Physicochemical properties of sodium alginate

The colour, odour, taste and texture of extracted sodium alginate were evaluated using methodologies described previously (Kumar et al. 2011). A digital pH meter (Model 2001, Digisum Electronics System) was used to determine the pH of a 1% sodium alginate solution. Moisture content of the powder was determined using the Indian Standards Institution method (ISI 1984). Protein, carbohydrate, lipid, fucose and sulfate contents were estimated using standard methods (Seifter et al. 1950, Lowry et al. 1951, Dubois et al. 1956, Folch et al. 1957, Dodgson & Price 1962). Ash content was determined by combusting 1 g sodium alginate powder in a silica crucible in a muffle furnace at 600°C and, once cooled, weighing the ash. Ash prepared as described was boiled in 25 ml of 2 N HCl for 5 min, and any

insoluble ash was collected on ash-free filter paper and washed with hot water. This insoluble ash was transferred into a silica crucible, combusted and weighed as described above. The procedure was repeated to get an average weight to accurately determine the percentage of acid-insoluble ash. Ash was boiled similarly in 25 ml water for 5 min, and insoluble ash was collected and washed as above, transferred to a silica crucible, combusted for 15 min and weighed. The procedure was repeated to get an average weight of water-insoluble matter that was subtracted from total ash weights to determine the percentage water-soluble ash.

Preparation and *Artemia* enrichment of sodium alginate micro-beads

Micro-beads containing sodium alginate were prepared as described previously (Skjermo et al. 1995). Briefly, 1 g sodium alginate powder dissolved in 100 ml distilled water was sprayed through a nozzle (45 µm) into an aqueous solution of 50 mM $CaCl_2$ in 5% methanol, upon which beads formed instantly. Beads collected onto a sieve were washed gently in 500 ml of 3 mM $CaCl_2$ before being suspended in 200 ml of 3 mM $CaCl_2$. Bead diameters ranged from 10 to 35 µm. Before enrichment in *Artemia*, beads were washed in distilled water and collected by centrifugation at 1957 × *g* for 5 min.

Over a 12 h period, batches of *Artemia franciscana* nauplii (instar II stage) were fed (encapsulated) with either sodium alginate powder or beads at various concentrations of 100, 200, 300 and 400 mg l^{-1}. Nauplii were stocked at a density of 20 individuals (ind.) ml^{-1} in glass containers containing 5 l seawater that was aerated mildly to maintain oxygen levels and to disperse dietary particles uniformly. The encapsulated *Artemia* nauplii were collected in a sieve, washed carefully and stored. To assess whether alginate powder and beads had been encapsulated, some nauplii were examined under a microscope to confirm that their guts were full.

Shrimp and alginate feeding regimen

Penaeus monodon postlarvae (PL) stage 7 (PL7, i.e. 7 d old) were obtained from Matsyafed Hatchery, Quilon, Kerala. Upon arrival at the laboratory, PL were stocked into a 100 l fiberglass tank filled with filtered natural seawater (32 ± 1 ppt) at room temperature (27 ± 1°C) and aerated to maintain dissolved oxygen levels at >6 mg l^{-1}. PL were acclimated for 8 d on a diet of live *Artemia* nauplii.

After measuring their length and weight, uniformly sized PL15 *Penaeus monodon* (10.8 ± 3.6 mg, mean ± SD) were selected and stocked at a density of 10 ind. l^{-1} into small tanks containing 25 l of mildly aerated, filtered seawater (salinity, 32 ± 1) at 28 ± 1°C. Postlarval groups were fed diets of control *Artemia* or *Artemia* enriched by feeding them either sodium alginate powder or beads at the concentrations described above. *Artemia* were provided ad libitum at 06:00, 14:00 and 18:00 h at rates of 30, 30 and 40% daily ration, respectively. Any uneaten *Artemia* nauplii were collected before each feeding and half the water was exchanged daily. To maintain the nutritional quality of *Artemia* over the 20 d feeding trial (PL15 to PL35), enriched *Artemia* preparations were maintained with gentle aeration at 4 to 10°C (Leger et al. 1983). Each diet was assessed in triplicate groups of PL. After feeding for 20 d, PL weight gain was calculated for each group by deducting their initial weight from their final weight. The specific growth rate (SGR) over the period, expressed as percentage increase, was calculated by using the formula $SGR = [(\ln W_2 - \ln W_1)/(t_2 - t_1)] \times 100$, where W_1 = initial weight at time t_1 and W_2 = final weight at time t_2.

WSSV inoculum and challenge

WSSV-infected *Penaeus monodon* with prominent exoskeleton spots were collected from local shrimp farms. Cephalothorax soft tissues including gills were homogenized and clarified by low-speed centrifugation at 3000 × *g* for 20 min at 4°C. The clarified supernatant was centrifuged again at 8000 × *g* for 30 min at 4°C, and the final supernatant was filtered through a 0.4 µm membrane filter and stored at −20°C. The presence of WSSV in the inoculum was confirmed by nested PCR (Yoganandhan et al. 2003).

After feeding on the alginate diets, groups of PL35 *Penaeus monodon* (242.1 ± 28.0 mg) were challenged with WSSV by immersion. Each of the triplicate groups comprised 100 PL stocked at a density of 10 ind. l^{-1} in a 15 l plastic tank containing 10 l sterilized, aerated seawater. Before use, air stones and tubes were sterilized by immersion in 2.6% sodium hypochlorite and washed thoroughly with sterilized tap water. Tanks were covered to prevent cross-exposure and aseptic techniques were applied. The WSSV inoculum was added to the water at a dose of 1 ml l^{-1} (i.e. 0.1% v/v) as recommended previously

(Chen et al. 2000). During the challenge experiment, PL were fed with their respective control or alginate-enriched *Artemia* nauplii diets.

Postlarval survival was monitored at 8 h intervals and PL not reacting to gentle mechanical stimulation with a small, soft paintbrush were considered to be dead and were removed. Mortality observed at each time point was used to calculate mortality per day, and all trials weres terminated on Day 21 post-challenge. A cumulative mortality index (CMI) of each group was calculated by using the formula: $CMI = Dx_1 + Dx_2 + Dx_3 \ldots + Dx_n$ (final day), where D = number of dead PL on each day (x_1 to x_n). The higher the CMI value was, the lower the resistance was considered to be to WSSV. By using these CMI values, percentage reductions in mortality were calculated (Immanuel et al. 2001, 2004, 2007, 2010, 2012a).

WSSV PCR

WSSV loads in dead PL collected during the challenge trial were estimated by PCR. Dead PL preserved in 70% ethanol were rehydrated in distilled water for 1 h before DNA was extracted as described previously (Lo et al. 1996). Briefly, each PL was homogenized in NaCl-Tris-EDTA buffer (0.2 M NaCl, 20 mM Tris-HCl, 20 mM EDTA, pH 7.4) and centrifuged at 3000 × *g* at 4°C. The supernatant was mixed with a digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5% sodium dodecyl sulphate, 0.1 mg ml^{-1} Proteinase K) and incubated at 65°C for 2 h. Digests were extracted with phenol: chloroform:isoamyl alcohol (25:24:1) and DNA recovered by ethanol precipitation was dried and resuspended in Tris-EDTA buffer.

In the first step of PCR, 7.5 µl PCR PreMix and 0.5 µl of 2 U $µl^{-1}$ IQzyme DNA polymerase were added into each 0.2 ml reaction tube followed by 2 µl of each DNA sample. Primer details and sequences are given in Lo et al. (1996) and Yoganandhan et al. (2003). The thermal cycling conditions were 42°C for 30 min, 94°C for 2 min, 15 cycles of 94°C for 20 s, 62°C for 20 s and 72°C for 30 s, followed by 72°C for 30 s and 20°C for 30 s. Following this PCR, 15 µl nested PCR reagent mixture was added to each tube and this mixture was incubated for 30 cycles of 94°C for 20 s, 62°C for 20 s and 72°C for 30 s, followed by 72°C for 30 s and 20°C for 30 s. After addition of 5 µl 6× Loading Dye, PCR aliquots were analyzed by electrophoresis in 2% agarose gels containing ethidium bromide to visualize DNA bands using a UV transilluminator. WSSV DNA amounts in each PL from each group were categorized as high (2000 DNA copies), moderate (200 DNA copies) and low (20 DNA copies) based on the detection of DNA bands of 910, 550 and 296 bp in size, respectively. Amplification of shrimp actin gene DNA (848 bp) by the IQ 2000™ WSSV Detection and Prevention System (GeneReach Biotechnology) confirmed that each PCR performed as expected.

Statistical analysis

Data were expressed as mean ± SD and analyzed by using 2-way ANOVA and Student's *t*-test at a 5% significant level. A multiple comparison Tukey test was performed to determine the significance of differences among parameters assessed by using the Statistica 6.0 software (Statsoft).

RESULTS

Sodium alginate extraction yields and physicochemical properties

The yields of sodium alginate from 5 separate extractions of *Sargassum wightii* seaweed ranged between 14.6 and 18.1%, with a mean ± SD of 16.4 ± 1.4%.

The sodium alginate extracts were whitish-yellow in colour and odourless, and had a salty taste and powdery appearance. A 1% sodium alginate solution had a pH of 9.21. Moisture contents of the 5 sodium alginate extracts were 16.0 ± 1.1%, carbohydrate contents were 46.1 ± 2.1%, protein contents were 5.2 ± 0.9%, lipid contents were 4.1 ± 0.4%, fucose contents were 29.2 ± 1.4% and sulphate contents were 14.6 ± 0.6%. Total ash, acid-insoluble ash and water-soluble ash levels were 1.92 ± 0.05%, 0.13 ± 0.01% and 1.04 ± 0.04%, respectively.

Growth performance of *Penaeus monodon* postlarvae

After 20 d of feeding, the growth performance (weight gain) of *Penaeus monodon* PL15 fed *Artemia* nauplii was 243.2 mg, and PL15 fed *Artemia* nauplii enriched with sodium alginate powder or beads showed significant ($p < 0.05$) concentration-dependent variations. From the lowest to highest concentrations of sodium alginate powder and beads

tested, respectively, PL weight gains after feeding on *Artemia* nauplii enriched with 100 mg l^{-1} were 217.2 mg and 197.2 mg, with 200 mg l^{-1} were 244.2 mg and 209.2 mg, with 300 mg l^{-1} were 282.2 mg and 213.2 mg, and with 400 mg l^{-1} were 270.2 mg and 205.2 mg (Fig. 1). Two-way ANOVA analysis showed significant variations ($p < 0.05$) in weight gains between PL groups fed either sodium alginate powder or bead-enriched *Artemia* nauplii, whereas no significant variation ($p > 0.05$) was evident amongst PL groups fed with the various concentrations of alginate.

Specific growth rate

The specific growth rate (SGR) was similar amongst groups of *Penaeus monodon* PL15 fed control *Artemia* nauplii (15.8%) and nauplii enriched with any concentration of sodium alginate powder or beads (14.8 to 16.5%) (Table 1). However, *t*-test of the SGR showed significant differences ($p < 0.05$) between control PL15 and PL15 fed *Artemia* nauplii enriched with either 300 or 400 mg l^{-1} of sodium alginate powder or beads.

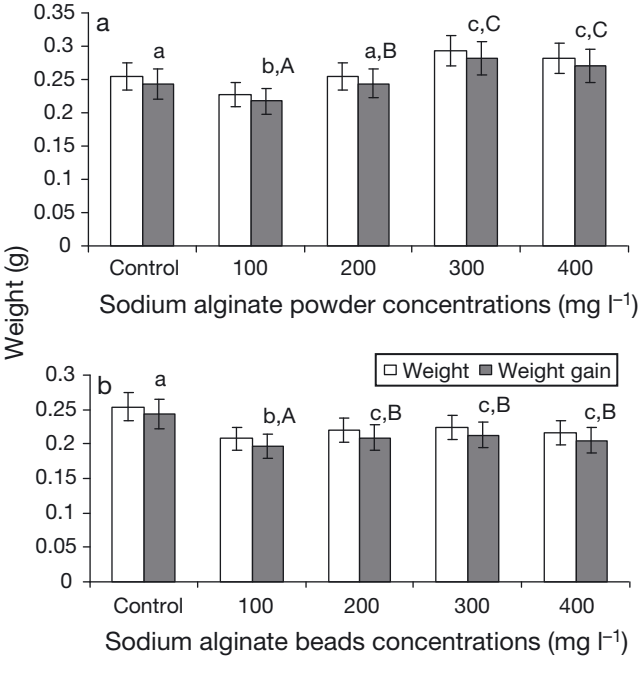

Fig. 1. *Penaeus monodon*. Final weight (g) and weight gain (g) of shrimp postlarvae (PL) fed *Artemia* nauplii enriched with different concentrations (100 to 400 mg l^{-1}) of sodium alginate (a) powder or (b) beads for 20 d (PL15 to PL35). Each value is the mean ± SD of 3 replicates. Within each group, bars accompanied by different letters are statistically different from the control (lower-case, *t*-test, $p < 0.05$) and each other (upper-case, post hoc Tukey test).

Table 1. *Penaeus monodon*. Specific growth rate (SGR) of postlarvae (PL) fed on *Artemia* nauplii enriched with different concentrations of sodium alginate powder or beads for 20 d (PL15 to PL35). Each value is the mean ± SD of 3 replicates. Within each group, values followed by different superscript letters indicate significant differences from the common control (lower-case, *t*-test, $p < 0.05$), and each other (upper-case, post hoc Tukey test); the 'Beads' group is distinct from the 'Powder' group and is designated as such by '1' following the letter

Concentration (mg l^{-1})	SGR (%)
0 (control)	$15.8 \pm 0.9^{a,a1}$
Powder	
100	$15.2 \pm 1.4^{a,A}$
200	$15.8 \pm 0.9^{a,A}$
300	$16.5 \pm 0.8^{a,B}$
400	$16.3 \pm 0.6^{a,B}$
Beads	
100	$14.8 \pm 0.7^{b1,A1}$
200	$15.1 \pm 1.2^{a1,A1}$
300	$15.2 \pm 1.4^{a1,A1}$
400	$15.0 \pm 1.4^{b1,A1}$

Mortality following WSSV challenge

WSSV challenge of *Penaeus monodon* PL35 fed control *Artemia* nauplii resulted in deaths from Day 3 post-challenge (pc); 50% cumulative mortality was reached on Day 6 pc and all shrimp had died by Day 9 pc (Fig. 2). Amongst *P. monodon* PL35 fed *Artemia* nauplii enriched with various concentrations of sodium alginate powder or beads, onset of mortality was delayed to Days 4 to 6 pc and 50% cumulative mortality was delayed to Days 12 to 17 pc. On Day 21 pc when the bioassay was terminated, 0, 9, 16 and 25% of the PL remained alive in the groups fed *Artemia* nauplii enriched with either 100, 200, 300 or 400 mg l^{-1} of sodium alginate powder, respectively, and 2, 14, 23 and 32% remained alive of those fed with *Artemia* enriched with 100, 200, 300 and 400 mg l^{-1} sodium alginate as beads, respectively. The CMI of the control group was 21537; it was considerably reduced in different concentrations of tested sodium alginate powder (by 26.5 to 52.4%) and beads (by 35.2 to 58.4%). When compared with the control group, the reduction in mortality rates for increasing concentrations of either sodium alginate powder or beads were statistically significant ($p < 0.05$) (Table 2).

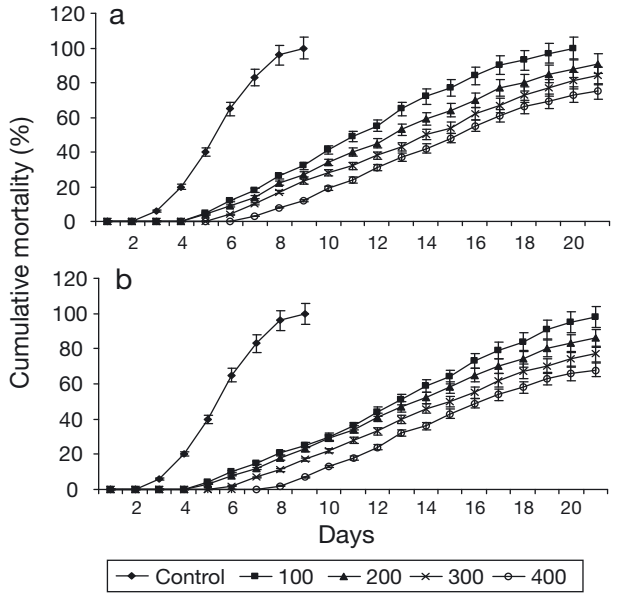

Fig. 2. *Penaeus monodon*. Percent (%) cumulative mortality over 21 d of shrimp postlarvae fed *Artemia* nauplii enriched with different concentrations (100 to 400 mg l^{-1}) of sodium alginate (a) powder or (b) beads after bath challenge with WSSV

Table 2. *Penaeus monodon*. Cumulative mortality index (CMI) and reduction in mortality (%) of shrimp postlarvae fed on *Artemia* nauplii enriched with different concentrations of sodium alginate powder or beads after challenge with WSSV. Each value is the mean ± SD of 3 replicates. Within each group, values followed by different letters are significantly different from the common control (*t*-test, $p < 0.05$, and subsequent post hoc Tukey test); the 'Beads' group is distinct from the 'Powder' group and is designated as such by '1' following the letter

Concentration (mg l^{-1})	CMI	Reduction in mortality (%)
0 (control)	21537 ± 244.9^{a,a1}	0 ± 0
Powder		
100	15827 ± 204.4^{b}	26.5 ± 0.11
200	13541 ± 170.6^{c}	37.1 ± 0.10
300	11886 ± 163.3^{d}	44.8 ± 0.13
400	10259 ± 122.5^{e}	52.4 ± 0.03
Beads		
100	13961 ± 146.9^{b1}	35.2 ± 0.05
200	12411 ± 138.8^{c1}	42.4 ± 0.01
300	10707 ± 122.5^{d1}	50.3 ± 0.00
400	8951 ± 97.9^{e1}	58.4 ± 0.02

WSSV infection loads detected by PCR analysis

WSSV loads in challenged groups of *Penaeus monodon* PL were assessed by IQ2000 PCR analysis (Table 3, Fig. 3). Amplification of a 910 bp product from DNA extracted from the WSSV inoculum confirmed the presence of high WSSV genomic DNA copy numbers. A 910 bp product was also amplified from challenged PL fed control *Artemia* nauplii. Amongst PL groups fed *Artemia* enriched with 100 mg l^{-1} sodium alginate powder or beads, a 910 bp product was also amplified, but only a 550 bp product and a 296 bp product were amplified primarily from PL fed *Artemia* enriched with moderate (200 mg l^{-1}) and higher (300 or 400 mg l^{-1}) concentrations of sodium alginate powder or beads, respectively.

Table 3. *Penaeus monodon*. Result of PCR analysis (see 'Materials and methods: WSSV PCR') on the range of infectivity of WSSV in control and experimental shrimps fed on *Artemia* nauplii enriched with different concentrations of sodium alginate powder or beads. Severe: 910 bp; Moderate: 550 bp; Slight: 296 bp

Concentration (mg l^{-1})	PCR result	
	WSSV	Infectivity range
WSSV inoculum	+ + +	Severe
Positive control	+ + +	Severe
Powder		
100	+ + +	Severe
200	+ +	Moderate
300	+	Slight
400	+	Slight
Beads		
100	+ + +	Severe
200	+ +	Moderate
300	+	Slight
400	+	Slight

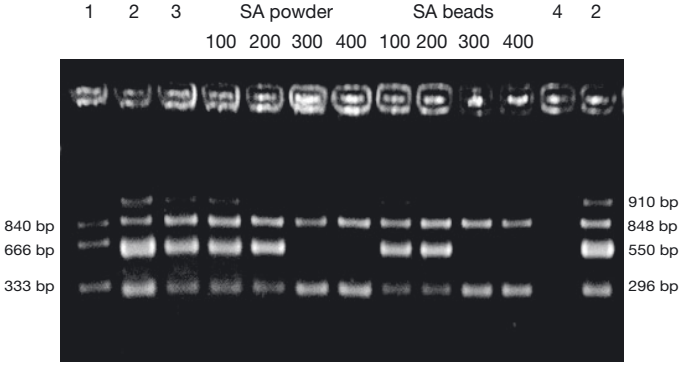

Fig. 3. *Penaeus monodon*. PCR analysis of WSSV infected control and experimental groups of shrimp postlarvae (PL). WSSV infectivity range: Severe: 910 bp; Moderate: 550 bp; Slight: 296 bp. Lane 1: marker; Lane 2: WSSV inoculum (from shrimp homogenate); Lane 3: positive control; Lanes 100 to 400: PL fed *Artemia* nauplii enriched with sodium alginate (SA) powder or beads at different concentrations (100 to 400 mg l^{-1}); Lane 4: negative control

DISCUSSION

Disease caused by WSSV remains a major threat to the shrimp aquaculture industry (Wyban & Sweeney 1991, Wilkenfeld 1992), even though recombinant proteins, dsRNA-based RNA interference and other sophisticated approaches have shown promise in limiting its impact (Witteveldt et al. 2004, Rout et al. 2007, Sarathi et al. 2008). Dietary polysaccharides, in particular alginates (Cheng et al. 2004) and fucoidans (Chotigeat et al. 2004, Immanuel et al. 2012b) derived from seaweeds, have also shown promise in protecting shrimp from infectious diseases.

In the present study, sodium alginate polysaccharide extracted from *Sargassum wightii* seaweed was fed to *Artemia*, and these alginate-enriched *Artemia* were fed to *Penaeus monodon* PL to assess their ability to protect the shrimp from infection and mortality following emersion challenge by WSSV. The average alginate yield from 5 extractions was 16.4 ± 1.4%, which compares favourably to yields of 16.9% reported for *S. vulgare* (Torres et al. 2007) and of 21.1 to 24.5% for *S. fluitans* and 16.3 to 20.5% for *S. oligocystum* (Davis et al. 2004). Somewhat higher alginate yields have been reported for *S. fluitans* (45%) and *S. oligocystum* (37%) (Davis et al. 2003), and for other seaweed species quite varied yields of 3.3% for *S. dentifolium*, 12.4% for *S. asperifolium* and 17.7% for *S. latifolium* have been reported (Larsen et al. 2003), suggesting that both species and extraction methods influence yields.

The physical characteristics of the *Sargassum wightii* sodium alginate extracts, including organoleptic properties, pH, moisture content and solubility, were generally consistent with those of highly purified algal and seaweed alginates described by Xiamen JieJing Biology Technology, Xiamen. Protein, carbohydrate, lipid, fucose, total ash, acid-insoluble ash, water-soluble ash and sulphate contents of the sodium alginate extracts were also measured. Protein content (5.2%) was somewhat higher than low-viscosity (LV, 1.1%) and high-viscosity (HV, 1.0%) alginates extracted from *S. vulgare* (Torres et al. 2007), but their moisture (14 and 16%) and total ash contents (2 and 1%) were comparable with the moisture (16%) and total ash (1.9%) contents of alginate powder prepared here from *S. wightii*, respectively. With regard to total carbohydrate (46.1%) and fucose (29.2%) contents determined for the *S. wightii* alginate, these varied considerably from those identified in alginates extracted from various algae harvested from the Egyptian Red Sea coast including *Cystoseira trinode* (74.9 and 11.6%, respectively), *S. dentifolium* (57.9 and 5.6%), *S. asperifolium* (32.2 and 4.2%) and *S. latifolium* (42.3 and 8.2%) (Larsen et al. 2003). The variations in biochemical compositions of seaweed polysaccharides mainly depend on the seaweed species, anatomical regions, growth conditions, extraction procedures and analytical methods (Immanuel et al. 2012b).

Over a 20 d feeding trial, the SGRs of PL15 *Penaeus monodon* fed *Artemia* nauplii enriched with *Sargassum wightii* alginate powder or beads supplied to the *Artemia* at concentrations between 100 and 400 mg l^{-1} ranged between 15.2 and 16.5% for powder and 14.8 and 15.2% for beads. In feeding trials using *Artemia* fed various concentrations of hot water extracts of *S. wightii* or *S. duplicatum* seaweeds, growth performance of *P. monodon* PL15 was higher (294 and 323 mg weight gain and 16.6 and 17.1% SGR, respectively) when *Artemia* had been fed an extract concentration of 750 mg l^{-1} compared with a lower concentration of 250 mg l^{-1} (274 or 295 mg weight gain and 16.3 or 16.6% SGR, respectively) (Immanuel et al. 2010). Consistent with this, improved growth performance has been noted in *P. indicus* juveniles fed *Artemia* nauplii enriched by feeding upon crude extracts of *Ulva lactuca* and *S. wightii* seaweeds (Immanuel et al. 2004) and in Atlantic halibut *Hippoglossus hippoglosus* larvae fed *Artemia* enriched by feeding upon alginate high in mannuronic acid (Skjermo & Bergh 2004).

Following the emersion challenge with WSSV, *Penaeus monodon* (PL35) fed *Artemia* nauplii enriched with sodium alginate powder or beads experienced reduced mortality rates progressively as alginate concentrations fed to the *Artemia* were increased from 100 to 400 mg l^{-1}. Immanuel et al. (2012b) stated that the dietary administration of fucoidan of *Sargassum wightii* increased resistance against WSSV in *P. monodon*, and observed that the percent mortality of experimental groups (0.1 to 0.3% concentration of fucoidan) of shrimp was reduced by 50.81 to 68.06% over the control group. Immanuel et al. (2010) also reported the effect of hot water extracts of *S. wightii* and *S. duplicatum* on the reduction in mortality in *P. monodon* PL against WSSV. They pointed out that the lower concentration (250 mg l^{-1}) of both seaweed extracts showed lower (16.12 and 39.35%) inhibitory activity against WSSV, although at the highest tested concentration (750 mg l^{-1}), both seaweed extracts showed higher (47.92 and 65.83%) inhibitory activity against WSSV. Enhanced resilience against WSSV has also been reported for *P. monodon* PL and juveniles fed on diets containing β-1,3-glucan (Chang et al. 1999, 2003) and for

P. monodon fed diets containing either fucoidan extracted from the brown algae *S. polycystum* (Chotigeat et al. 2004) or a 2% extract of *Cynodon dactylon*, a medicinal herb (Balasubramanian et al. 2007, 2008).

Nested PCR analysis showed that WSSV loads in *Penaeus monodon* PL fed *Artemia* nauplii enriched with sodium alginate from *Sargassum wightii* decreased progressively as alginate concentrations fed to the *Artemia* were increased between 100 and 400 mg l^{-1}. Such decreases have been noted similarly in *P. monodon* PL fed *Artemia* enriched with hot water extracts of *S. wightii* and *S. duplicatum* (Immanuel et al. 2010). However, in comparison with Immanuel et al.'s (2010) study, decreases in WSSV loads detected in PL fed on the sodium alginate extracted from *S. wightii* were far more uniform in the present study, suggesting that the effects of the more highly purified extract were more potent. Amongst *P. monodon* fed extracts of *Cynodon dactylon* before a WSSV challenge, decreased infection loads were detected only amongst shrimp that were fed pellets coated with the undiluted 2% extract (Balasubramanian et al. 2008).

The V_3 loop motif of the WSSV gp120 envelope glycoprotein is essential for initial virus attachment to cell surface heparin sulfate before more specific binding occurs to the CD4 receptor of CD4+ cells (Witvrouw & De Clercq 1997). The mechanism by which WSSV replication is inhibited by sodium alginate extracted from seaweed may involve binding of negatively charged sulfate groups of the polysaccharide to positively charged amino acids in this V_3 loop region, thus interfering with its entry into cells. Sodium alginate enhances resistance of shrimp (Cheng et al. 2004, 2005, Liu et al. 2006) and fish (Fujiki et al. 1994, Fujiki & Yano 1997, Cheng et al. 2007) to various pathogens. Moreover, alginate derived from *Lessonia nigrescens* fed to *Litopenaeus vannamei* shrimp increases prophenol oxidase activity, phagocytic activity and viral clearance (Cheng et al. 2005), and sodium alginate also enhances both the immune and antioxidant defense systems in *Penaeus monodon* (Liu et al. 2006). Although the data obtained here with sodium alginate extracted from the brown seaweed *Sargassum wightii* is promising, additional studies are required to determine whether it can be exploited as a feed additive to help protect cultured shrimp against disease caused by WSSV.

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