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Short communication

Stimulating effects of polysaccharide from *Angelica sinensis* on the nonspecific immunity of white shrimps (*Litopenaeus vannamei*)

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ABSTRACT

Angelica sinensis polysaccharide (ASP) was prepared by hot water extraction. Then, high-performance liquid chromatography and ion chromatography analyses were conducted, and the results indicated that ASP is a heteropolysaccharide, has a molecular mass of 82,000 Da and consists of arabinose, galactose and glucose (molar ratio of 6:1:1). The effects of ASP on the nonspecific immunity of white shrimps (*Litopenaeus vannamei*) were investigated by feeding them with ASP-containing diets (0.5, 1 and 1.5 g/kg) during a 12-week breeding experiment. Oral ASP administration significantly improved the survival rate, phenoloxidase activity, superoxide dismutase activity, glutathione peroxidase level, disease resistance against *V. alginolyticus,* total haemocyte count and number of hyaline cells, semigranular cells and granular cells ($p < .05$). ASP exhibits immunostimulatory effects on Pacific white shrimps (*L. vannamei*) and may thus be used as a diet supplement for them.

1. Introduction

Litopenaeus vannamei is an important economic shrimp species; upon cultivation, the yield of *L. vannamei* is higher than those of *Penaeus monodon* and *Macrobrachium rosenbergii* [1]. *L. vannamei* possesses unique advantages, such as fast growth rate, low nutrient requirement, large amount of meat consumed and tolerance to water environment stress. However, the global expansion of shrimp farming and production has been limited by frequent outbreaks and spread of shrimp diseases. Farmers are compelled to excessively use antibiotics as a prophylactic agent in order to prevent such diseases. Nevertheless, long-term use of antibiotics may lead to adverse effects on the environment and the health of human consumers [2]. Long-term use of antibiotics may decrease their effectiveness owing to the emergence of resistant bacterial or viral strains in shrimp; furthermore, excessive levels of antibiotics can deteriorate the overall conditions of shrimp [3,4]. Thus, suitable alternatives to antibiotics must be developed.

Angelica sinensis (AS) is one of the most popular plants used in Chinese herbal medicine, and its roots have been used to treat various diseases for thousands of years [5]. Dried AS roots have a sweet-acrid taste and warm property, exhibit medicinal functions and can be applied to the heart, liver and spleen channels [6]. They are also used as antioxidative, tonic, anti-inflammatory, neuroprotective or haematopoietic ingredients for the treatment of anaemia, constipation, cardiovascular diseases and hepatic fibrosis [7] and are found to contain abundant polysaccharides exerting pharmacological effects, such as radioprotection, antioxidation, antitumour effects, haematopoiesis, gastrointestinal protection and immunomodulation [8–11]. Nevertheless, data regarding the effects of AS polysaccharides (ASPs) on the nonspecific immunity of white shrimps (*L. vannamei*) are limited.

In this study, ASP was prepared by hot water extraction. The effects of ASP on the nonspecific immunity of white shrimps (*L. vannamei*) were investigated.

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethics committee of Huahai Institute of Technology, China. All procedures were carried out in compliance with relevant laws and institutional guidelines.

2.2. Materials

Dried *A. sinensis* rhizomes were purchased from a local pharmaceutical company (Haizhou, China). Three standard monosaccharides

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(arabinose, galactose and glucose) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.3. ASP preparation

Dried *A. sinensis* rhizomes were pulverised and sifted through a 100-mesh sieve. Then, we immersed the fine powder in distilled water to yield a suspension with a concentration of ~1% (w/v). The resulting suspension was incubated in a thermostatic water bath at 85 °C for 6 h and centrifuged at 6000 × *g* for 10 min. The resulting supernatant was protein-separated through the Sevag method, precipitated with absolute ethanol (three volumes), filtered and freeze-dried. The product was redissolved in distilled water, loaded on a DEAE-52 cellulose anion-exchange chromatography column (30 cm × 2.6 cm, GE Healthcare, UK) and eluted successively with 0.05 mol/L phosphate-buffered saline and gradient solution of 0.05–1.0 mol/L NaCl at a flow rate of 1.0 mL/min. The eluted polysaccharide fraction was collected for subsequent experiments. ASP recovery rate was 20.79%.

2.4. ASP characterisation

The proximate components, i.e., total sugar, protein and ash, of the ASP products were assayed through the phenol–sulphuric acid colorimetric method, Kjeldahl method and method described by the Association of Official Analytical Chemists procedures [12], respectively. The Fourier transform infrared (FTIR) spectra of the representative ASP product samples were obtained from KBr pellets by using a Nicolet Nexus FTIR 470 spectrophotometer over a wavelength range of 400–4000 cm^{-1}. UV spectra were recorded on a UV spectrometer (Spectra Test, Germany). The molecular weight (MW) of the ASP was determined through high-performance gel filtration chromatography (HPLC; LC-10A, Shimadzu, Japan) by using an ultrahydrogel size exclusion column (LKB-Prodokter, AB, Bromma, Switzerland) and high-sensitive refractive index detector (Model ERC-7515 A, ERC Inc., Japan). The ASP was eluted with 0.1 N $NaNO_3$ at a flow rate of 0.9 mL/min. Standard pullulan samples (P20–P800; JM Science, Inc., NY, USA) were used as MW standard. The ASPs were hydrolysed through the methods described by Sheng et al. [13]. The monosaccharide compositions of the obtained adenosine monophosphate (AMP) were assayed by ion chromatography (IC; ICS-5000, Dionex, USA) using a carbohydrate column (CarboPac PA20, Dionex, USA) and pulse ampere detector (Dionex, USA). The AMP monosaccharides were eluted with mobile phase at a flow rate of 0.5 mL/min. The composition and conditions of the mobile phase were as follows: 0–21.1 min (97.4% of water and 2.6% of 250 mM NaOH); 21.1–30 min (92.4% of water, 2.6% of 250 mM NaOH and 5.0% of NaAc) and 30–50 min (20% of water and 80% of 250 mM NaOH).

2.5. Diet preparation

The experimental diet formulations are shown in Table 1. The crude ASP was added to the experimental diets at levels of 0.5, 1 and 1.5 g/kg diet with a corresponding decrease in the cellulose content. The ingredients were ground in a Hammer mill and passed through a 60 mesh screen. Experimental diets were prepared according to the methods described by Chang et al. [14].

2.6. Shrimp culture

Shrimps were purchased from a local hatchery in Lianyungang, China, reared in a semi-intensive culture pond, acclimated to the experimental conditions and fed with commercial diet for 2 weeks before the feeding trial experiments. Water was continuously aerated with two air stones. The laboratory conditions during the acclimation period are similar to those at the beginning of the experiments.

Table 1
Composition of the shrimp diets (g/kg) supplemented with *Angelica sinensis* polysaccharides (ASP).

Ingredient	ASP diets (g/kg)			
	Control	50	150	250
Fish meal	430	430	430	430
Soybean meal	65	65	65	65
Yeast meal	25	25	25	25
Shrimp shell meal	70	70	70	70
Wheat flour	345	345	345	345
Cellulose	6	5.5	5	4.5
ASP	0	0.5	1	1.5
Gluten	25	25	25	25
Fish oil	8	8	8	8
Mineral mixture	20	20	20	20
Vitamin mixture	6	6	6	6

The mineral and vitamin mixes were prepared according to the formula of Cheng and Hardy [9].

Before the experiments, 400 shrimps, with an average body weight of 0.63 ± 0.03 g, were randomly divided into four groups and individually fed with the control diet (without ASP addition) or ASP-containing diets. In each group, 50 shrimps were subjected to susceptibility test, and the remaining 50 shrimps were subjected to immune response assay.

Shrimps were fed four times daily at a ratio of 10% of their body weight at 07:00, 12:00, 16:00 and 20:00, wherein the amounts of diets fed are 30%, 20%, 20% and 30% of the total amount, respectively. The health status and death of the shrimps were observed and recorded throughout the experiment period. The shrimps were weighed once every 3 weeks, and the amount of diet fed was adjusted according to the shrimp body weight. During the experiments, the following conditions were maintained: water temperature, of 28 ± 1 °C; pH, 7.8–8.0 and salinity, 3.2%. The experiments were performed in triplicate.

2.7. Growth and survival rate

At the end of the 12-week feeding trial, the shrimps from each aquarium were weighed. Growth was measured according to the body weight gain of the surviving shrimp in each aquarium and was calculated as (final body weight − initial body weight). Feed efficiency (FE) was calculated as [(final body wt − initial body wt)/feed intake].

2.8. Nonspecific immune response assays

Each shrimp was blotted dry after the final weighing. The haemolymph was withdrawn from the ventral sinus of each shrimp with a 1 mL syringe fitted with a 25-gauge needle and then rinsed with 50 μL of precooled (4 °C) 10% sodium citrate as anticoagulant. The immunological parameters used for the evaluation of the stimulating effect of the strains were as follows: phenoloxidase (PO) activity, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) level, total haemocyte count (THC), hyaline cells (HC), semigranular cells (SGC) and granular cells (GC).

PO activity was measured according to the method described by Huang et al. [15]. Anticoagulant (200 μL, 4 °C) and shrimp haemolymph (200 μL) were added to a 2 mL syringe with a 2.5 G needle and then mixed. The mixture was centrifuged at 800 × *g* and at 4 °C for 10 min. The resulting upper layer was used as plasma. Plasma (20 mL) was strewed in spectrophotometer cuvette as an unknown sample, and anticoagulant (20 mL) was strewed in another cuvette as control. After 1 min, 880 mL of $_L$-DOPA solutions were added to the two cuvettes. Absorption was recorded at 490 nm every 10 and 120 s. One unit of enzyme activity was defined as a linear increased in absorption of 0.001 per min per mL haemolymph.