doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia https://veterinariamexico.fmvz.unam.mx/

Immunochemical response and gene expression in juvenile shrimp (*Litopenaeus vannamei*) exposed to microorganisms isolated from marine sediment

Ángel I. Campa-Córdova[1]
0000-0003-0099-3324
Gabriel Aguirre-Guzman[2*]
0000-0002-7374-2369
Yuniel Méndez-Martínez[3]
0000-0002-5365-5794
Diana Medina-Félix[4]
0000-0002-0904-4099
Carlos E. Ceseña[1]
0000-0002-8412-9172
Jocelyne García-Armenta[1]
Jesús A. Valenzuela-Chávez[1]

[1] Centro de Investigaciones Biológicas del Noroeste. La Paz, BCS, Mexico.

[2] Universidad Autónoma de Tamaulipas. Facultad de Medicina Veterinaria y Zootecnia. Cd Victoria, Tamps, México.

[3] Universidad Técnica Estatal de Quevedo. Facultad de Ciencias Pecuarias y Biológicas. Laboratorio de Acuicultura Experimental, Los Ríos, Ecuador.

[4] Universidad Estatal de Sonora. Licenciatura en Ecología. Hermosillo, Sonora. México.

***Corresponding author**
Email address:
gabaguirre@docentes.uat.edu.mx

Submitted: 2023-04-24
Accepted: 2023-10-02
Published: 2024-03-21

Additional information and declarations can be found on page 12

Abstract

The use of microorganisms isolated from the natural environment can improve the culture conditions in aquaculture systems and the immune and antioxidant response capacity of cultured organisms. In this study, the immunochemical response and gene expression of *Litopenaeus vannamei* juveniles were analyzed following exposure to microorganisms isolated from marine sediments after *in vitro* characterization of their probiotic properties. Two yeasts and one bacterium strain were used by immersion method (48 to 216 h) at a concentration of 1×10^6 CFU/mL with the following treatments: (1) Control, without probiotics; (2) Positive Control, commercial immunostimulant (Laminarin; β-1,3, glucan); (3) *Candida maris*, strain A1; (4) *Geotrichum candidum*, strain A3; and (5) *Curtobacterium* sp., strain S13. Shrimp treated with microorganisms and commercial immunostimulants showed a significant increase ($P<0.0500$) in the soluble protein content in hemocytes compared with the control group. At 72 h post-exposure (hpe), *G. candidum*, *Curtobacterium* sp., and laminarin treatments showed a significant increase ($P<0.0500$) in superoxide desmutase (SOD) activity compared with the control group. SOD enzyme gene expression showed a significant increase ($P<0.0500$) in all shrimp treated with microorganisms from 48 hpe. The results obtained showed that the use of three microorganisms isolated from a marine environment can stimulate the protein content in circulating hemocytes, antioxidant activity, and gene expression in *juvenile L. vannamei*.

Keywords: Hemocytes; Immunostimulants; Lipopolysaccharides; Superoxide dismutase; Yeasts.

Cite this as:

Campa-Córdova ÁI, Aguirre-Guzman G, Méndez-Martínez Y, Medina-Félix D, Ceseña CE, García-Armenta J, Valenzuela-Chávez JA. Immunochemical response and gene expression in juvenile shrimp (*Litopenaeus vannamei*) exposed to microorganisms isolated from marine sediment. Veterinaria Mexico OA. 2024;11. doi: 10.22201/fmvz.24486760e.2024.1207.

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Study contribution

Shrimp farming is important in generating products of high nutritional value and economic value in different countries. This industry is constantly growing in different topics such as physiology, nutrition, adaptability, immune system, stress, and resistance to pathogens, etc. However, information regarding the immunological changes that shrimp undergo when exposed to immunostimulants is poorly understood. This study focuses on the use of *C. maris*, G. *candidum*, and *Curtobacterium* sp. as activators of the immune system of *L. vannamei* and evaluates their immune response (hemocytes, total proteins, and expression of genes) to this stimulus. The results showed that marine microorganisms can stimulate protein content in circulating hemocytes, antioxidant activity, and gene expression. This information can help shrimp farmers have a better control of pathogens and diseases of diseases that affect production.

Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is one of the most cultivated crustacean species worldwide, with a global production of 1 237 016 t[1] in 2022 and of 270 807 t in 2021 in Mexico. Furthermore, it is an important source of income.[2] Due to the continuous demand for this product, shrimp farming has become an economically important activity, which is not immune among others, to nutritional, stress, environment, health, problems which are strongly associated with different factors that affect shrimp growth.[3, 4] Such problems in shrimp aquaculture favor the development of strategies to improve the health and growth of the organisms, the health through an early detection of diseases to reduce losses in production, and the reduction of environmental impact.[1, 5]

The problems generated by pathogenic agents in shrimp (bacteria and viruses) have increased research on the immune system of these organisms using studies at cellular, protein, proteomic, and gene expression levels among others.[6-8] An alternative for disease control is to activate or strengthen the shrimp immune system through the use of microorganisms, by-products, or cellular fractions thereof that improve resistance to pathogens.[5] Some marine microorganisms contribute to the prevention of health problems that affect shrimp by having an excellent nutritional level, in addition to functional properties such as probiotics and/or immunostimulants, adhesion to the intestinal mucosa, antagonism against pathogens, and removal of organic matter.[9-11]

Different marine microorganisms are sources of β-glucans, lipopolysaccharides, and nucleotides that are used as immunostimulants in *L. vannamei* and provide resistance against bacterial and viral pathogens.[9, 12] They also generate the activation of genes of different immune factors such as antimicrobial peptide, anti-lipopolysaccharide factor, penaeidin, prophenoloxidase (ProPO), and SOD.[13] Strains of microorganisms such as *Candida maris*, *Geotrichum candidum*, and *Curtobacterium* sp. have been used as a prophylactic measure against shrimp pathogens.[14-16]

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Ochoa[17] and Ochoa et al.[18] reported an important response in cultured *L. vannamei* hemocytes exposed to *G. candidum.* This microorganism has been used as a probiotic agent in fish, where an increase in the immunological and productive response was found.[19, 20] This research aimed to evaluate the cellular immune response, protein content, and gene expression in juvenile Pacific shrimp (*L. vannamei*) exposed to *C. maris*, *G. candidum*, and *Curtobacterium* sp. isolated from the marine environment.

Materials and methods

Ethical statement

All animal experimental protocols were carefully revised and approved by an internal council at Centro de Investigaciones Biológicas del Noroeste or CIBNOR (Ethics Committee: CICUAL-CIBNOR, Spanish acronym, Institutional approval number CIBNOR-CEI-2023-04) and complied with the guidelines established by the European Union Council (2010/63/EU) and the Mexican Government (NOM-062-ZOO- 1999) for the cultivation, care, and use of experimental animals.

Microorganisms strain

The experimental strains used were *Candida maris* (A1), *Geotrichum candidum* (A3), and *Curtobacterium* sp. (S13), isolated from the sediment in the mangrove area of La Paz Bay, Baja California Sur, Mexico (24°14′59.80″ N, 110°18′47.12″ W) and characterized *in vitro* and *in vivo* at the Humid Laboratory for Crustacean Culture and Marine Immunogenomics Laboratory at the CIBNOR, La Paz, Baja California Sur, Mexico.[21] The experimental strains were cultivated in YPD broth (Peptone-Dextrose for yeast) + 2.5 % NaCl with 50 µg/mL of chloramphenicol at 30 °C for 24 h. The non-hemolytic capacity of the strains (gamma hemolysis) was previously determined by the hemolysis test on blood agar plates + 2.5 % NaCl + 10 % rabbit plasma, using the puncture procedure (6 mm approx.) and incubation at 35 °C for 24 h.[22] The controlled dose was generated by culturing the strains in YPD agar + 2.5 % NaCl at 30 °C for 24 h. The obtained colonies were suspended in a sterile 2.5 % NaCl solution until they reached an absorbance of 1 at 540 nm (1×10^6 CFU/mL of final dose).[23]

Bioassay

The bioassay was performed using juvenile Pacific white shrimp, *Litopenaeus vannamei* (14.25 ± 1.5 g), in the CIBNOR laboratory for acclimatization and culture of aquatic organisms. The bioassay was performed in fiberglass tanks with 30 L of filtered seawater (1 µL) at 28 ± 1 °C, 37 ± 2 ups, and continuous aeration (dissolved oxygen of 5.02 ± 0.5 mg/mL). Shrimp were randomized in triplicate at a density of 15 organisms/tank and exposed to five treatments for 216 h: (1) shrimp fed a commercial shrimp diet (PIASA, 35 % protein) as a negative control (TCN); (2) shrimp exposed to laminarin (β-1,3 glucan; SIGMA) as a positive control (TCP); (3) shrimp exposed to *Candida maris* (TCM); (4) shrimp exposed to *Geotrichum*

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

candidum (TGC); and (5) shrimp exposed to *Curtobacterium* sp. (TCS). The replicates of each treatment were accommodated in three blocks of five tanks, where each block had a treatment that was also randomly distributed.

The microorganisms were applied to the corresponding treatments (TCM, TGC, TCS) by immersion at a dose of 1×10^6 CFU/mL to the respective experimental tanks.[23] The TCP dose was based on the manuscript of Campa-Córdova et al.[24] with the use of laminarin (Laminaria digitata, Sigma, L-9634) at a final concentration of 0.5 mg/mL. The TCN treatment contained only commercial feed. The dose of each treatment was applied at 0, 48, 96, 144, and 192 h. During the bioassay, daily maintenance was performed early in the morning and included the removal of molts and dead shrimp and siphoning of residual food and fecal material from the tanks. In addition, a daily exchange of 15 % of the total volume of seawater was conducted. The shrimp were fed *ad libitum* with the commercial diet (PIASA, 35 % protein) twice a day (09:00 and 15:00 h) at 1.9 % of the total biomass.

Sampling

Hemolymph from three rando (mL) and selected shrimp, one from each replicate, was collected at 24, 48, 72, and 216 h for evaluation of total hemocyte count (THC), total protein (TP), and gene expression analysis. Selected shrimps were euthanized by placing them in filtered seawater at 8 °C for 30 min, and 2 g of muscle was dissected from each shrimp. These tissues were stored at -80 °C to determine the superoxide dismutase (SOD) enzyme activity.

For hemolymph sampling, an isotonic shrimp solution with EDTA, an anticoagulant, SIC-EDTA (450 mM NaCl, 10 mM KCl, 10 mM EDTA-Na_2, 10 mM HEPES, pH 7.3, and 850 mOsm/kg) was used to obtain the hemolymph sample.[24] Hemolymph was obtained from the ventral hemolymphatic sac located at the base of the pleopod of the first abdominal segment near the genital pore, with a 5 (mL) syringe (22 × 32, 23 G) + 500 µL of SIC-EDTA at 4 °C (depending on the volume of hemolymph collected, the dilution factor was calculated).

The extracted hemolymph (1 mL approx.) was deposited in 1.5 mL microcentrifuge tubes (removing the needle so as not to break the cells) and cooled on an ice bed.[24, 25] 50 µL of collected hemolymph was used for total hemocyte count and protein analysis. The rest of the collected sample was used for gene expression and centrifuged at 3000 *g* for 3 min at 4 °C. The supernatant was removed and 500 µL of an ARNlater solution (Invitrogen™, catalog number AM7021) was applied to the cell package[6] for preservation at 4 °C for 24 h and subsequent storage at -80 °C, according to the manufacturer's instructions.

Total hemocyte count

Total hemocyte count (THC) was performed as described by Campa-Córdova et al.[24] and Pacheco et al.[25], where 200 mL of 10 % formaldehyde was added to 50 µL of hemolymph. The hemolymph sample was evaluated in a Neubauer chamber to determine the number of hemocytes using a 10× binocular optical microscope (CX-21). The number of hemocytes was evaluated in cells per milliliter according to the following formula:

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

$$\text{Number of hemocytes} = \left(\frac{\text{number of cells}}{\text{Number of quadrants read}}\right)(10000)\left(\text{Dilution factor with fixative solution}\right)\left(\text{Dilution factor with anticuagulant solution}\right)$$

Protein quantification

The evaluation of TP (mg/mL) in the hemocytes and muscle was performed using a Bradford modification,[26] analyzing the sample in a microplate at 595 nm. A final homogeneous hemocyte concentration was calculated for protein determination (1×10^5 cell/mL) and bovine serum albumin (BSA) was used as a standard by serial dilutions to establish a standard curve.[13, 25]

Evaluation of the activity of superoxide dismutase in muscle

The collected muscle samples were thawed and individually analyzed for SOD, where 100 mg of tissue from each sample was manually macerated and homogenized with a sterile plastic pestle in 0.5 (mL) of phosphate buffer (50 mM, pH 7.8) and subsequently centrifuged at 14 000 rpm for 10 min at 4 °C.[24, 27] The supernatant was placed in 1.5 mL microcentrifuge tubes chilled on a bed of ice for analysis. The SOD activity was evaluated according to Suzuki[28] using the xanthine/xanthine oxidase system as a constant generator of superoxide radicals, which reduce nitroblue tetrazolium to formazan, and was evaluated every 30 s for 5 min at an absorbance of 560 nm. SOD activity was defined as the amount of the enzyme required to inhibit 50 % dismutation of the superoxide radical. The results of SOD activity were expressed as U/mg of protein in the muscle.

Evaluation of the expression of immune response genes in hemocytes (RNA extraction and complementary DNA synthesis)

To determine gene expression, 500 µL of RNAlater stabilization solution (Ambion) was added to each hemocyte sample. Total RNA was obtained using TRIzol Reagent (Sigma-Aldrich®) according to the manufacturer's instructions. RNA was quantified by spectrophotometry between 260/280 nm.[8, 29] The synthesis of the complementary strand (cDNA) was obtained using the Improm II protocol (Promega®), adjusting the samples to a concentration of 10 µg of RNA and storing the obtained samples (30 µL) at -20 °C.

The expression of the superoxide dismutase gene (MnSOD) was analyzed by real-time polymerase chain reaction (qPCR) using the CFX96 qPCR System (Bio-Rad), as well as the EvaGreen Sso-fast super mix reagent (Bio-Rad) and primers for specific genes.[30,31] The PCR program was carried out as follows: denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s, where fluorescence was evaluated. A denaturation curve was obtained at the end of the PCR reaction to observe the specificity of the fragments and the absence of artifact formation. The primers used for gene amplification are listed in Table 1. After stability analysis, the ubiquitin and RpL8 genes were selected to normalize the data obtained using cMnSOD. The relative expression (RE) of the transcripts of the cMnSOD gene in hemocytes of *L. vannamei* was calculated starting from the relation between the relative quantity (QR) of each sample with the

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Table 1. Primers used for the expression of the MnSOD gene by qPCR after the challenges[31, 32]

Gen	Primer name	Sequence 5´– 3´	Efficiency %	Fragment size (bp)
Ribosomal protein S12	S12-F S12-R	GTGGAAGGAGACGTTGGTGT AGAGCCTTGACCGCTTCAT	2.00	150 pb
Ubiquitin	UBI-F UBI-R	GGGAAGACCATCACCCTTG TCAGACAGAGTGCGACCATC	1.98	146 pb
Ribosomal protein L8	L8-F L8-F	GCCTAAGGTGCGTGGTGT ATTCTGCCTTGGGTCCTTCT	2.00	181 pb
Manganese superoxide dismutase (MnSOD)	MnSOD-F MnSOD-R	ATTGGGTGAGGAACGAGGTG GGTGATGCTTTGTGTGGTGG	2.10	113 pb

following equation, where *t* is the target gene (reference gene) and *nf* is the factor of normalization obtained from the geometric mean calculated from the QRs of the most stable reference genes (S12 and RPL8).[32, 33]

$$\text{Relative quantity (RE)} = \left(\frac{\text{target of reference gene}}{\text{factor of normalization}}\right)$$

Statistical analysis

The homoscedasticity and homogeneity of the data were determined using the Kolmogorov– Smirnov and Bartlett tests. A one-way analysis of variance (ANOVA) was performed using the F test to analyze the differences between the treatment and control groups. Values of $P<0.05$ were considered significantly different, and Tukey's test (HSD) was applied to identify the nature of these differences ($P<0.05$). All data were evaluated using STATISTICA software (software version 2.0.).

Results

There was no significant increase in the THC content in any treatment. However, the highest average THC value was detected at 72 h post-exposure in the treatments with *C. maris* and *laminarina* compared with the control group and other treatments (Figure 1a). The laminarin treatment showed high mean THC values compared with any other treatment in almost all the samples except for 48 h post-exposure (Figure 1a). *Candida maris* and *G. candidum* showed low THC activity only at 24 h post-exposure, and their average values increased from 100 to 300 %, with twelve *C. maris* exhibiting these values (Figure 1a).

The total protein (TP) content in the hemocytes is shown in Figure 1b. At 24 h post-exposure a significant increase ($P=0.0136$) was observed in shrimp treated with *Curtobacterium* sp. and at 48 h post-exposure in juveniles treated with laminarin ($P=0.0418$) and *G. candidum* ($P=0.0375$) compared with the control group. At 72 h post-exposure, all treatments had higher average values of TP

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

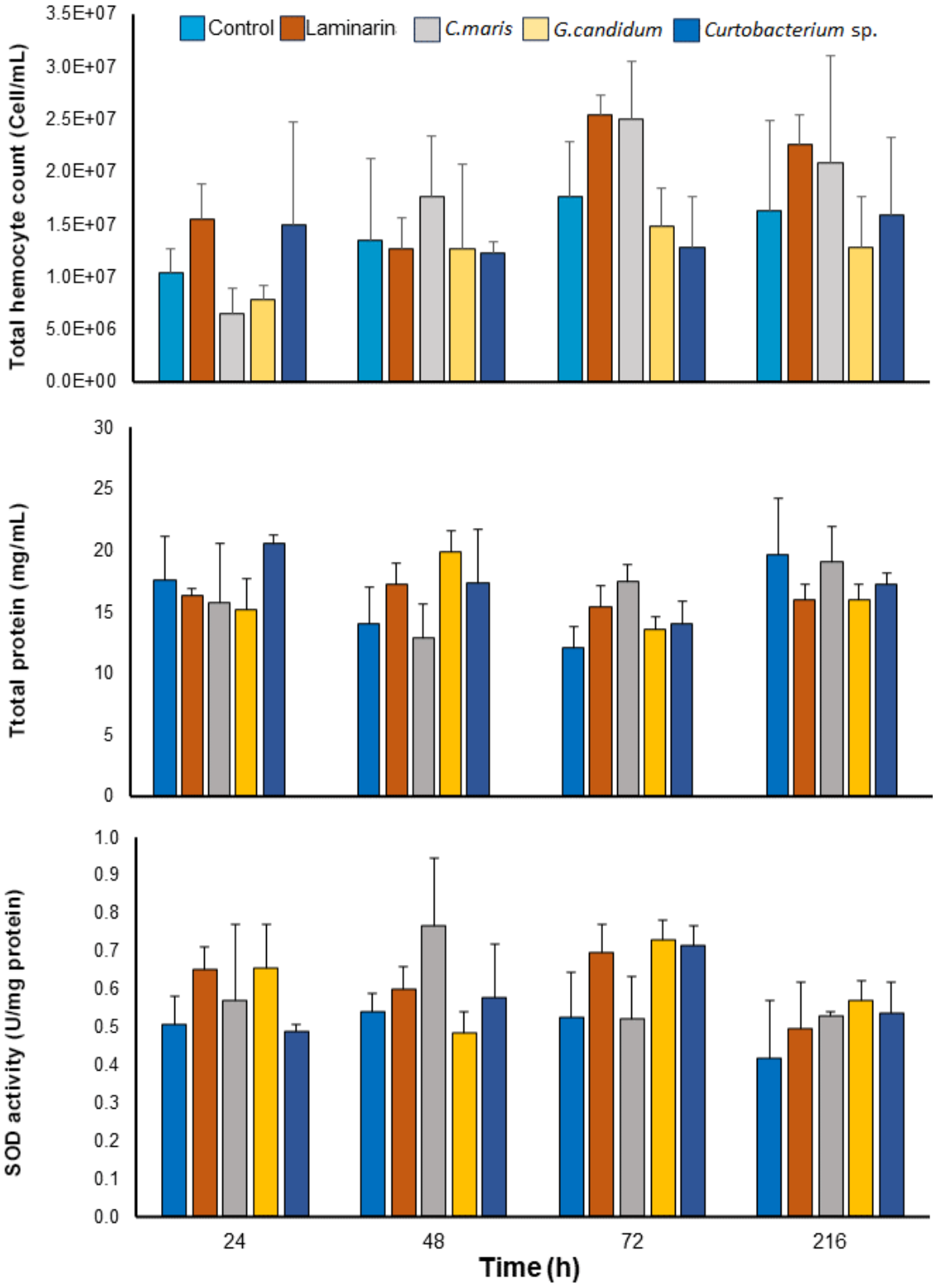

Figure 1. Hemocyte count and total protein in hemocytes, in addition to superoxide dismutase (SOD) activity in muscle in juvenile white shrimp *L. vannamei* exposed to the control group treatments (no treatment), laminarin (0.5 mg/mL), *Candida maris*, *Geotrichum candidum*, and *Curtobacterium* sp. at 1×10^6 CFU/mL each. *Significance compared with the control group at each time point.

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

than the control group (Figure 1b). However, at 216 h postexposure, all treatments showed values similar to the control (untreated shrimp, Figure 1b).

Shrimp treated with laminarin showed a significant increase in the mean SOD activity of the muscle at 24 ($P = 0.0435$) and 72 h post-exposure ($P = 0.0463$) compared with the control group (Figure 1c). *Candida maris* generated higher average values of SOD activity at 24, 48, and 216 h post-exposure compared with the control group, showing the greatest increase at 48 h compared with the control group and other treatments. At 72 h, *G. candidum* and *Curtobacterium* sp. also registered a significant increase ($P > 0.0500$) in the average values of SOD activity compared to the control group (Figure 1c). At 216 h post-exposure, all treatments showed values similar to the control group (Figure 1b).

Figure 2 presents the gene expression of MnSOD in shrimp (*L. vannamei*) hemocytes, where a significant decrease was observed in the expression at 24 h post-exposure in all treatments compared with the control group. At 48 h post-exposure, a significant increase was observed in the gene expression of the shrimp treated with laminarin ($P = 0.0273$), *C. maris* ($P = 0.0187$), and *Curtobacterium* sp. ($P = 0.0331$), being at least twice the expression compared with the control group. At 72 h post-exposure, this significant increase was observed ($P = 0.0357$) in shrimp exposed to *G. candidum* and at 216 h post-exposure in shrimp exposed to *C. maris* ($P = 0.0431$). In both cases (72 and 216 h post-exposure), the gene expression of the hemocytes obtained from the shrimp exposed to these two treatments was at least twice than that of the control group. MnSOD gene expression in the hemocytes of shrimp exposed to *G. candidum* showed a tendency to increase up to 72 h post-exposure, decreasing subsequently.

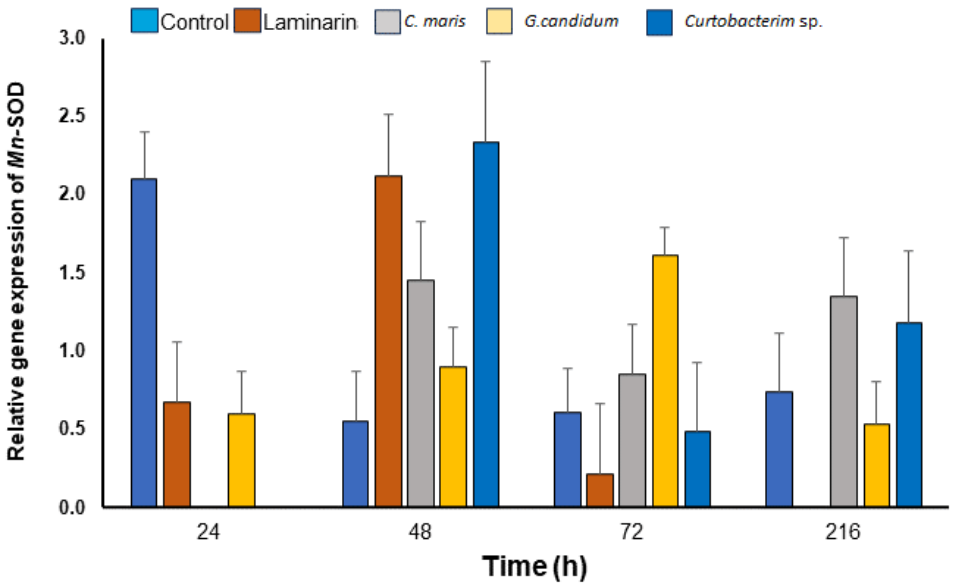

Figure 2. Relative gene expression of MnSOD in hemocytes of juvenile white shrimp *L. vannamei* exposed to the control group treatments (no treatment), laminarin (0.5 mg/mL), *Candida maris*, *Geotrichum candidum*, and *Curtobacterium* sp. at $1x10^6$ CFU/mL each. *Significance compared with the control group at each time point (h).

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Discussion

Different molecules act naturally to stimulate the defense mechanisms of organisms (immunostimulants), preventing the entry, invasion, and colonization of pathogens.[8, 11, 34] These immunostimulant molecules come from different sources (viruses, bacteria, fungi, etc.) and are regularly supplied to cultured shrimp by different routes depending on the volume of water, density or age of cultured organisms: by direct immersion in culture water (postlarvae), by inclusion in the diet (juveniles) or by intramuscular injection (adults), favoring disease resistance against potential pathogens (without causing the microbial resistance that antibiotics generate), enhancing the survival of organisms in the face of environmental and nutritional stress.[35, 36]

In this study, Pacific juvenile shrimp exposed by immersion at concentrations of 1×10^6 CFU/mL with microbial immunostimulants isolated from marine soil and a commercial immunostimulant (0.5 mg/mL), showed that microbial treatments increased antioxidant system and protein content in hemocytes. Ceseña et al.[31] reported increased immune and antioxidant responses and resistance against experimental infections with *V. parahaemolyticus* in *L. vannamei* juveniles after exposure of shrimp to immunostimulant microorganisms included in shrimp diet and by immersion.

Adequate use of immunostimulants in terms of concentration (dose), frequency, and route of administration are relevant to obtain favorable results on the activation of the shrimp immune system. This is to avoid potential immunosuppression caused by an excessive energy loss (oxidative stress) in the organism or by null effects generated for not use an adequate specific treatment in concentration, administration method, or administration frequency of the immunostimulants. [24, 36, 37]

The information obtained in the present study showed that the use of *C. maris*, *G. candidum*, and *Curtobacterium* sp. generated significant results at different periods throughout the bioassay, both in terms of hemocyte protein content and muscle SOD activity. A high level of hemocyte content was also detected at 72 and 216 h compared with the other post exposure times (24 and 48 h) used in the present study. Immunostimulants activate the immune responses of shrimp from the postlarval stage and develop the innate immunity of these crustaceans, creating a hostile environment for detected potential pathogens and promoting their timely elimination.[11, 34, 38, 39]

Apines-Amar & Amar[40] showed that when immunostimulants are detected by hemocytes, they initiate a chain of physiological events that prepare and strengthen the immune system in organisms against pathogens. This chain of shrimp physiological events generates binding with antibacterial peptides, phagocytosis by hemocytes, encapsulation of the detected molecule, melanization, and activation of the ProPO system.[16, 34, 37] This produces and releases different proteins from the hemocytes to the hemolymph by exocytoses, such as prophenoloxidase, serine proteinase, peroxynectin, proteinase inhibitor, and lysozyme, in response to stimuli produced by the invasion of pathogens,[41] where the destroyed materials and molecules are expelled. through the excretory system in branchial tissue, antennal gland, etc.

Hemocytes are a reliable indicator for determining and preventing diseases, as well as a sign of the physiological state of the animal. These are responsible for coagulation, release of transglutaminases, and thrombospondins, and phagocytosis

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

and removal of foreign materials that enter the hemolymph and shrimp tissues. The detection of a foreign molecule activates these cells, stimulating the production of different extra- and intracellular products associated with the immune response and generating hemocyte proliferation and phagocytosis. This also releases proteins such as agglutinins, ProPO, antimicrobial peptides, and lysozyme that increase the total protein content in hemocytes and hemolymph.[42] Some studies suggest that an increase in immunoproteins in hemocytes is induced by exposure to immunostimulants.[42–44]

The three experimental strains used (*C. maris*, *G. candidum*, or *Curtobacterium* sp.) showed immunostimulant activity in shrimp (*L. vannamei*), modifying the protein concentration in hemocytes at different post-challenge periods (Figure 1) and inducing significant increases in protein at 48 and 72 h postexposure compared with the control groups. Several authors[13, 38, 45] observed similar changes and increases in the level of hemocytes and proteins when exposing *L. vannamei* to immunostimulants. The results show that the experimental strains used could improve the immune response and resistance to diseases of white shrimp (*L. vannamei*).

An important immune response of hemocytes is phagocytosis, which eliminates foreign agents; however, during this process, reactive oxygen molecules (ROS) are produced that must be neutralized.[46] This neutralization is carried out by antioxidant enzymes, mainly by the SOD enzyme, which converts ROS into oxygen and hydrogen peroxide molecules. This last molecule is converted into water and oxygen by the catalase enzyme, thus neutralizing ROS and regulating cell homeostasis. The results show a significant increase in SOD when using laminarin *G. candidum* and *Curtobacterium* sp. at 72 h compared with the control group.

The SOD enzyme uses a metallic cofactor for catalysis, where manganese is one of the metals involved, thus generating MnSOD.[46] García-Triana et al.[47] indicated that MnSOD is an antioxidant enzyme that transforms or dismutates toxic superoxide into hydrogen peroxide and oxygen, protecting cells from the damage generated by this molecule. The MnSOD transcription level in hemocytes decreased at 24 h postexposure, with the products used, compared with the control group, followed by an increase at 48, 72, and 216 h post-exposure (Figure 2). This decrease in antioxidant expression is similar to other studies related to the use of immunostimulants, which is associated with cellular oxidative stress caused by activation of the host's immune system at the beginning after exposure to treatments and with a posterior increase of cellular response.[43, 48]

In addition, a decrease in shrimp immune and antioxidant response has been reported after exposure to external stressors such as pollutants, microbial infections, and hypoxia.[36, 48–50] Neves et al.[36] reported a significant decrease in SOD enzyme activity when shrimp (*Palaemonetes argentinus*) was infected with *Probopyrus ringueleti*. Campa-Córdova et al.[24] obtained a significant increase in SOD activity in *L. vannamei* hemocytes at 6 h post-exposure (immersion) with β-1,6 glucan (0.5 mg/mL) and a subsequent significant decrease at 72 h postexposure compared with the control group. Licona-Jain et al.[35] reported increases in SOD activity in hemocytes of juvenile shrimp (*L. vannamei*) after the second week post-exposure to marine yeasts (1.1 % in the diet), improving shrimp resistance after exposure to a pathogenic bacterium (*Vibrio parahaemolyticus*).

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Conclusions

Based on the results observed for the three microorganisms obtained from the marine environment, these showed that juvenile Pacific shrimp (*L. vannamei*) exposed to a concentration of 1×10^6 CFU/mL for 48 h present an increase in the protein content in circulating hemocytes, in addition to an increase in the enzymatic activity and gene expression of superoxide dismutase. This study shows the importance of increasing studies to clarify the issue.

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

Data availability

All relevant data are included within the manuscript, and its supporting information files are presented as supplementary material on the journal's website.

Acknowledgments

The authors thank the CIBNOR technical staff, Sandra de la Paz, Pablo Monsalvo, Martín Ramírez, and Norma Ochoa for their technical support in carrying out this study.

Funding statement

This research was funded by Mexico's National Council of Science and Technology (https://conahcyt.mx), grant SEP-CONACYT 243532, awarded to A. Campa. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest related to this manuscript.

Author contributions

Conceptualization and Data curation: AICC, GAG.
Methodology: AICC, YGA, JAVC.
Formal analysis and software: AICC, YMM, DMF, JAVC.
Funding acquisition, Project administration, and supervision: AICC.
Visualization: AICC, GAG
Writing-original draft: AICC, GAG, YMM, DMF, CES, YGA, JAVC.
Writing-review and editing: AICC, GAG, YMM DMF, CES.

References

1. FAO. The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome; 2022. doi: 10.4060/cc0461en.
2. CONAPESCA. Anuario estadístico de pesca. CDMX, México: SAGARPA; 2021. https://nube.conapesca.gob.mx/sites/cona/dgppe/2021/ANUARIO_ESTADISTICO_D E_ACUACULTURA_Y_PESCA_2021.pdf
3. Arreguín-Sánchez F, Arcos-Huitrón E. Fishing in Mexico: state of exploitation and use of ecosystems. Hidrobiológica. 2011;21(3):431-462.
4. Pérez-Castañeda R, Sánchez-Martinez JG, Aguirre-Guzman G, Rabago-Castro JL, Vazquez-Sauceda (ML). Interaction of fisheries and aquaculture in the production of marine resources: advances and perspectives in Mexico. In: CW Finkl, C Makowski. editors. Switzerland: Environmental Management and Governance–Advances in Coastal and Marine Resources, Coastal Research Library 8, Springer International Publishing. 2014;111-140. doi: 10.1007/978-3-319-06305-8_5.
5. Morales-Cristóbal Y, Cortés-Jacinto E, Saucedo PE, Cadena-Roa M, Campa-Córdova AI. Dietary enrichment with crude protein content and feed additives (*Bacillus* spp. and yeast strains) improves growth performance, survival and circulating hemocytes in juvenile white shrimp, *Litopenaeus vannamei*. Revista de Biologia Marina y Oceanografia. 2022;57(1):45–56. doi: 10.22370/rbmo.2022.57.1.3361.

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

6. Burge E, Madigan DJ, Burnett LE, Burnett KG. Lysozyme gene expression by hemocytes of Pacific white shrimp, *Litopenaeus vannamei*, after injection with Vibrio. Fish & Shellfish Immunology. 2007;22(4):327-39. doi: 10.1016/j.fsi.2006.06.004.
7. Robalino J, Almeida JS, McKillen D, Colglazier J, Trent HF, Chen YA, Peck MET, Browdy CL, Chapman RW, Warr GW, Gross PS. Insights into the immune transcriptome of the shrimp *Litopenaeus vannamei*: tissue-specific expression profiles and transcriptomic responses to immune challenge. Physiology Genomics. 2007;29:44–56. doi: 10.1152/physiolgenomics.00165.2006.
8. Luna-González A, Moreno-Herrera JT, Campa-Córdova AI, González-Ocampo HA, Fierro-Coronado JA, Álvarez-Ruíz P, Bueno-Ibarra MA. Respuesta inmune y expresión de genes en el camarón blanco (*Litopenaeus vannamei*) inducida por inmunoestimulantes microbianos. Latin American Journal of Aquatic Research. 2013;41(5):898-907. doi: 103856/vol41-issue5-fulltext-10.
9. Ceseña CE, Vega-Villasante F, Aguirre-Guzman G, Luna-González A, Campa-Córdova AI. Update on the use of yeast in shrimp aquaculture: a minireview. International Aquatic research. 2021b;13:1-16. doi: 10.22034/IAR.2021.1904524.1066.
10. Méndez-Martínez Y, Torres-Navarrete YG, Cortés-Jacinto E, García-Guerrero MU, Hernández-Hernández LH, Verdecía DM. Biological, nutritional, and hematoimmune response in juvenile *Cherax quadricarinatus* (Decapoda:Parastacidae) fed with probiotic mixture. Revista MVZ Córdoba. 2022;27(3). doi: 10.21897/rmvz.2578.
11. Kumar S, Verma AK, Singh SP, Awasthi A. Immunostimulants for shrimp aquaculture: paving pathway towards shrimp sustainability. Environmental Science and Pollution Research. 2022(Jan);13. doi: 10.1007/s11356-021-18433-y.
12. Türker M. Yeast biotechnology: diversity and applications. Advances in science and industrial productions of baker's yeast. 27th VH Yeast Conference, April 14th–15th. Istanbul, Turkey: 2014; 1-26.
13. Campa-Córdova AI, Hernández-Saavedra NY, Aguirre-Guzmán G, Ascencio F. Immunomodulatory response of superoxide dismutase in juvenile American white shrimp (*Litopenaeus vannamei*) exposed to immunostimulants. Ciencias Marinas. 2005;31(4):661-669. doi: 10.7773/cm.v31i4.32.
14. Samuel P, Prince L, Prabakaran P. Antibacterial activity of marine derived fungi collected from South East Coast of Tamilnadu, India. Journal of Microbiology and Biotechnology Research. 2011;1(4):86-94.
15. Fell JW. Yeast in marine environments. In: EB Gareth, KL Pang, editors. Marine fungi and fungal-like organisms. De Gruyter: University of Malaya; 2012. pp: 92-103.
16. Campa-Córdova AI, Valenzuela-Chávez JA, García-Armenta J, Medina D, Licona-Jain AB, Angulo-Valadez CE, Aguirre-Guzmán G, Mejía-Ruíz CH. Uso profiláctico de aditivos inmunoestimulantes en el cultivo del camarón blanco, *Litopenaeus vannamei*. In: LE Cruz Suárez, D Ricque D, M Tapia M, MG Nieto, DA Villarreal, J Gamboa, L Mercedes L, MA Galaviz editors. Nuevo León, México: Universidad Autónoma de San Nicolás de los Garza, Nuevo León, México: 2017. pp. 541-559.
17. Ochoa N. Evaluación de factores de virulencia de hongos infecciosos en camarón blanco (*Litopenaeus vannamei*) [tesis de maestría]. La Paz, Baja Cal-

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

ifornia Sur, México: Instituto Politécnico Nacional, Centro Interdisciplinario en Ciencias Marinas; 2004.

18. Ochoa JL, Ochoa-Alvarez N, Guzmán-Murillo MA, Hernandez S, Ascencio F. Isolation and risk assessment of *Geotrichum* spp. in the white shrimp (*Litopenaeus vannamei* Boone, 1931) from culture ponds. Latin American Journal of Aquatic Research. 2015;43(4):755-765. doi: 10.3856/vol43-issue4-fulltext-14.
19. Ibrar M, Zuberi A, Amir I, Imran M, Noor Z. Effect of probiotic *Geotrichum candidum* on early rearing of *Labeo rohita* (Hamilton, 1822). Turkish Journal of Fisheries and Aquatic Sciences. 2017;17:1263-1270. doi: 10.4194/1303-2712-v17_6_19.
20. Amir I, Zuberi A, Kamran M, Imran M, Ul M, Murtaz H. Evaluation of commercial application of dietary encapsulated probiotic (*Geotrichum candidum* QA-UGC01): effect on growth and immunological indices of rohu (*Labeo rohita*, Hamilton 1822) in semi-intensive culture system. Fish & Shellfish Immunology. 2019;95:464-472. doi: 10.1016/j.fsi.2019.11.011.
21. Ibarra-Serrano, AC. Evaluación de microorganismos aislados de sistema de mangle con capacidades biorremediadoras e inmunoestimulantes en el cultivo de camarón blanco del Pacífico *Litopenaeus vannamei* (Boone, 1931) [tesis de maestría]. La Paz, Baja California Sur, México: Centro de Investigaciones Biológicas del Noroeste; 2018. pp. 62.
22. Cowan ST, Steel LJ. Manual for the identification of medical bacteria. Cambridge, UK: University of Cambridge; 1993.
23. Abasolo-Pacheco F, Campa- Córdova AI, Mazón-Suástegui JM, Tovar-Ramírez D, Araya R, Saucedo PE. Enhancing growth and resistance to *Vibrio alginolyticus* disease in catarina scallop (*Argopecten ventricosus*) with *Bacillus* and *Lactobacillus* probiotic strains during early development. Aquaculture Research. 2017;48:4597-4607. doi: 10.1111/are.13283.
24. Campa-Córdova AI, Hernández-Saavedra NY, de Philippis R, Ascencio F. Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to β-glucan and sulphated polysaccharide. Fish and Shellfish Immunology. 2002;12:353-366. doi: 10.1006/fsim.2001.0377.
25. Pacheco M, Campa A, Aguirre G, Luna A, Guzmán M, Ascencio F. Effect of *Debaryomyces hansenii* on the antioxidant response of juvenile white shrimp *Litopenaeus vannamei*. Revista MVZ. Córdoba. 2012;17(1):2820-2826.
26. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72:248-54.
27. Wang Z, Zhu F. MicroARN-100 is involved in shrimp immune response to white spot syndrome virus (WSSV) and *Vibrio alginolyticus* infection. Scientific Reports. 2017;7:42334. doi: 10.1038/srep42334.
28. Suzuki K. Measurements of Mn-SOD and Cu, Zn-SOD. In: N Taniguchi and J Gutteridge, editors. Experimental protocols for reactive oxygen and nitrogen species. Oxford, UK: Oxford University Press. 2000;91-95.
29. Gutiérrez-Dagnino A, Luna-González A, Fierro-Coronado JA, Álvarez-Ruíz P, Flores-Miranda MC, Miranda-Saucedo S, Medina-Beltrán V, Escamilla-Montes R. Efecto de la inulina y del ácido fúlvico en la supervivencia, crecimiento, sistema inmune y prevalencia de WSSV en *Litopenaeus vannamei*. Latin

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

American Journal of Aquatic Research. 2015;43(5):912-921. doi: 10.3856/vol43-issue5-fulltext-11.

30. Fernandes TJR, Costa J, Oliveira MBPP, Mafra I. COI barcode-HRM as a novel approach for the discrimination of hake species. Fisheries Research. 2018;197:50-59. doi: 10.1016/j.fishres.2017.09.014.
31. Ceseña CE, Cortés-Jacinto E, Luna-González A, Vega-Villasante F, Ochoa-Álvarez NA, Morelos-Castro RI, Escamilla-Montes R, Tovar-Ramírez D, Sánchez-Ortiz AC, Campa-Córdova AI. Dietary supplementation of *Debaryomyces hansenii* enhanced survival, antioxidant and immune response in juvenile shrimp *Penaeus vannamei* challenged with *Vibrio parahaemolyticus*. Tropical and Subtropical Agroecosystems. 2021;24(2):1-13. doi: 10.56369/tsaes.3616.
32. Ventura-Lopez C, Gomez-Anduro G, Arcos FG, Llera-Herrera R, Racotta IS, Ibarra AM. A novel CHH gene from the Pacific white shrimp *Litopenaeus vannamei* was characterized and found highly expressed in gut and less in eyestalk and other extra- eyestalk tissue. Gene. 2016;582(2):148-60. doi: 10.1016/j.gene.2016.02.011.
33. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biology. 2007;8(2):R19.
34. Zhang Z, Aweya JJ, Yao D, Zheng Z, Tran NT, Li S, Zhang T. Ubiquitination as an important host-immune response strategy in penaeid shrimp: inferences from other species. Frontiers in Immunology. 2021;12:697397. doi: 10.3389/fimmu.2021.697397.
35. Licona-Jain A, Racotta I, Angulo C, Luna-González A, Escamilla-Montes R, Cortés-Jacinto E, Morelos-Castro RM, Campa-Córdova AI. Combined administration routes of marine yeasts enhanced immune-related genes and protection of white shrimp (*Penaeus vannamei*) against *Vibrio parahaemolyticus*. Fish & Shellfish Immunology. 2022;124:192-200. doi: 10.1016/j.fsi.2022.04.004.
36. Neves CA, Santos EA, Bainy ACD. Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Paleminedae), infected by *Probopyrus ringueleti* (Isopoda, Bopyridae). Diseases of Aquatic Organisms. 2000;39:155-158.
37. Aguirre-Guzmán G, Sánchez-Martínez JG, Campa-Córdova AI, Luna-González A, Ascencio F. Penaeid shrimp immune system: a minireview. Thai Journal of Veterinary Medicine. 2009;39(3):205-215.
38. Ji PF, Yao CL, Wang ZY. Immune response and gene expression in shrimp (*Litopenaeus vannamei*) hemocytes and hepatopancreas against some pathogen-associated molecular patterns. Fish & Shellfish Immunology. 2009;27:563-570. doi: 10.1016/j.fsi.2009.08.001.
39. Lin YC, Chen JH, Man SNC, Morni WZW, Suhaili ASNA, Cheng SY, Hsu CH. Modulation of innate immunity and gene expressions in white shrimp *Litopenaeus vannamei* following long-term starvation and re-feeding. Results in Immunology. 2012;2:148-156. doi: 10.1016/j.rinim.2012.07.001.
40. Apines-Amar MJS, Amar EC. Use of immunostimulants in shrimp culture: an update. In: CMA Caipang, MBI Bacano-Maningas, FF Fagutao, editors. Biotechnological Advances in Shrimp Health Management in the Philippines, Research Signepost. 2015;45-71.
41. Lin YC, Tayag CM, Huang CL, Tsui WC, Chen JC. White shrimp *Litopenaeus vannamei* that had received the hot-water extract of *Spirulina platensis* showed

doi: 10.22201/fmvz.24486760e.2024.1207
Vol. 11 | 2024

earlier recovery in immunity and up-regulation of gene expressions after pH stress. Fish & Shellfish Immunology. 2010;29:1092-1098.

42. Jennissen HP. Ubiquitin and the enigma of intracellular protein degradation. European Journal of Biochemistry. 1995;231:1-30.
43. Dugger D. Bio-modulation of the non-specific immune response in marine shrimp with beta-glucan. Aquaculture Magazine. 1999;1:81-89.
44. Karunasagar I, Otta SK, Devaraj TN, Shubha G, Iddya K. Immunostimulation of *Penaeus monodon* through the oral route. In: Workshop: Shrimp Immunity and Disease Control. Thailand; 1999. http://www.ifremer.fr/incode/htm
45. Hien TTT, Tao CT, Hoa TTT, Huynh TG, Tu TLC, Hai TN, Nguyen DH, Kim SH, Song JW, Nhan HT, Duc PM. Effects of dietary supplementation with Pro-A on growth performance, feed utilization, immune responses, and intestinal microbiota of whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture Reports. 2022;24. Article: 101125. doi: 10.1016/j.aqrep.2022.101125.
46. Zhang Q, Li F, Wang B, Zhang J, Liu Y, Zhou Q, Xiang J. The mitochondrial manganese superoxide dismutase gene in Chinese shrimp *Fenneropenaeus chinensis*: Cloning, distribution and expression. Developmental and Comparative Immunology. 2007;31:429-440. doi: 10.1016/j.dci.2006.08.005.
47. García-Triana A, Zenteno-Savín T, Peregrino-Uriarte AB, Yepiz-Plascencia G. Hypoxia, reoxygenation and cytosolic manganese superoxide dismutase (cMn-SOD) silencing in *Litopenaeus vannamei*: Effects on cMnSOD transcripts, superoxide dismutase activity and superoxide anion production capacity. Developmental and Comparative Immunology. 2010;34:1230–1235. doi: 10.1016/j.dci.2010.06.018.
48. Le Moullac G, Soyez C, Saulnier D, Ansquer D, Avarre JC, Levy P. Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. Fish & Shellfish Immunology. 1998;8:621-629.
49. Downs C, Fauth JE, Woodley CM. Assessing the health of grass shrimp (*Palaemonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. Marine Biotechnology. 2001;3:380-397.
50. Truscott R, White KN. The influence of metal and temperature stress on the immune system of crabs. Functional Ecology. 1990;4:455-461.