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Short Communication

Individual and synergistic effect of highly diluted bioactive compounds and probiotic actinomycetes on the growth and survival of juvenile shrimp *Penaeus vannamei*

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ABSTRACT. The objective of this study was to evaluate two *Streptomyces* spp. probiotic strains (RL8 and N7) and four highly-diluted bioactive compounds (HDBC) in the growth, survival, and modulation of the microbial content in culture water and hepatopancreas of juvenile white shrimp *Penaeus vannamei*. An experimental design was applied with five treatments in triplicate, including T1: (RL8+N7); T2: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C)]; T3: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C) + (N7)]; T4: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C) + (RL8)] and T5 as the control without actinobacteria or HDBC. T2 showed significant differences ($P < 0.5$) in height increase compared to the other treated and control groups. The organisms in T2, T3, and T4 recorded higher weight gain compared to T1 and T5. A survival rate from 94.13 ± 2.12 to 99.01 ± 2.45% was obtained with a significant difference ($P < 0.05$) between T1, T2, and T3, concerning the untreated control (T5). A significant decrease in *Vibrio* spp. the content was observed in water and shrimp in T1 and T2 concerning the control. The heterotrophic bacterial count in hepatopancreas increased with all treatments compared to T5. Our results suggest a positive synergy between the HDBC mixture [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C)] and N7 *Streptomyces* sp. strain, whose probiotic action has been previously demonstrated by improving culture conditions for *P. vannamei* juveniles in terms of growth, survival, and reduction of potentially pathogenic bacteria.

Keywords: *Penaeus vannamei*; *Streptomyces*; crustaceans; probiotic; bacteria; homeopathy; synergistic treatments; water quality; growth, survival

Shellfish production is increasingly important globally to provide a food source for human populations. Shrimp are the main cultured crustaceans, with white leg (Pacific white) shrimp *Penaeus vannamei* constituting the largest 4.97 million t yielded in 2018 (FAO 2020).

Probiotics are the most effective and safe alternative to reduce the use of antibiotics in white shrimp *P. vannamei* aquaculture (Lakshmi et al. 2013). The gradual and progressive resistance that various farm animals have to antibiotics represents a critical and serious global threat to human health due to the use and

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abuse of these chemotherapeutic agents in producing aquatic food (Hasan et al. 2020). Probiotics offer several biological benefits; they reduce the proliferation of pathogenic bacteria through competitive exclusion (Zorriehzahra et al. 2016) and improve survival (Peñalosa-Martinell et al. 2021). Probiotic bacteria are supplied live and can colonize the digestive tract, modify intestinal microbiota, and improve the immune response (Hoseinifar et al. 2018). Several gram-positive and gram-negative bacterial candidates, such as *Bacillus* species (Chai et al. 2016, Chen et al. 2017), *Vibrio* species (El-Sersy et al. 2006, Thompson et al. 2010), lactic acid bacteria (Wang & Gu 2010), and some other bacterial species (Das et al. 2010, García-Bernal et al. 2017, 2018) have been used as probiotics in shrimp aquaculture in recent times.

Microorganisms with probiotic capacities include actinobacteria that produce a wide variety of exoenzymes such as cellulose, amylase, protease, and lipase that can improve feed digestibility and utilization in various aquaculture species (Sarkar & Suthindhiran 2022). Actinomycetes are also excellent producers of secondary metabolites that can inhibit the growth of pathogens of interest in aquaculture (Das et al. 2010).

Other eco-friendly alternatives to make aquaculture systems more efficient are highly-diluted bioactive compounds (HDBC). Including in this category, homeopathic medicines for human use authorized for this purpose by official government entities, such as the Health Ministry (Secretaría de Salud 2015) in Mexico, whose positive effects have been demonstrated in aquaculture (Mazón-Suástegui et al. 2018a,b, Nagai et al. 2022) and agriculture (Mazón-Suástegui et al. 2019a).

The application of these highly diluted treatments has been related to increases in growth rate, digestive enzymatic activity, condition index, energetic reserves, oocyte quality, and gut microbiota modulation (Mazón-Suástegui et al. 2018b, 2020, 2021). The HDBC improved growth rate in shrimp *P. vannamei* juveniles (Mazón-Suástegui et al. 2018a) as well in marine bivalves, such as *Argopecten ventricosus* juveniles (López-Carvallo et al. 2019) and mussel *Modiolus capax* adults (García-Corona 2018).

Therefore, the objective of this research is to study the independent and synergistic action of *Streptomyces* genus probiotic strains (RL8 and N7) of actinomycetes, and HDBC on juvenile white shrimp *P. vannamei* growth, survival, and modulation of the microbial content in culture water and hepatopancreas.

White shrimp *P. vannamei* juveniles measuring an initial weight and size of 0.6 ± 0.14 g and 4.13 ± 0.52 cm (mean ± standard deviation) were provided by Acuacultura Mahr Company, a commercial shrimp hatchery located at La Paz, MX. The juveniles were acclimatized for seven days in 1500 L fiberglass tanks in the Laboratorio Experimental de Homeopatía Acuícola y Semillas Marinas of Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR) with seawater filtered at 1 μm and sterilized with ultraviolet (UV) light, salinity 37, continuous aeration and temperature of 29 ± 1°C. The shrimp were fed to satiety thrice a day with a balanced commercial diet (Purina® 35% protein; Ciudad Obregón, MX).

Two *Streptomyces* spp. strains (RL8 and N7) with proven probiotic action (García-Bernal et al. 2017, 2018) were isolated and characterized in Cuba (Centro de Biactivos Químicos, Universidad Central de Las Villas) and Mexico (CIBNOR). Experimental HDBC for aquaculture use and officially licensed HDBC for humans were also evaluated.

Commercial HDBC [(*Phosphoricum acid* Similia® (PhA-6C) and *Silicea terra* Similia® (SiT-6C)] have official registration and authorization from the Health Ministry of Mexico as homeopathic medicines for human use, and manufactured under procedures of the "Farmacopea Homeopática de Los Estados Unidos Mexicanos" (Secretaría de Salud, 2015). PhA and SiT were purchased in sixth centesimal dilution (1:99) in 87°GL ethanol in an authorized pharmacy (Farmacia Homeopática Nacional, FHN®, CDMX, MX).

Shrimp were randomly distributed in 120-L fiberglass tanks (50 shrimp $tank^{-1}$) containing seawater filtered at 5 μm and sterilized with UV radiation at 29 ± 1°C and constant aeration. Five experimental groups with three replicates each were used in the bioassay, including T1: (RL8+N7); T2: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C)]; T3: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C) + (N7)]; T4: [(ViP-7C+ViA-7C) + (PhA-7C+SiT-7C) + (RL8)]; and T5 a control group without actinobacteria or HDBC.

The probiotic suspensions and treatments formulated with HDBC were added by spraying them to the commercial pelletized balanced feed (Purina® 35% protein; Ciudad Obregón, MX), which was dried in the shade at a temperature of 25 ± 0.5°C and stored at 4°C. Only the amount to be used in a maximum time of one week was prepared. The probiotic treatments (RL8, N7) were sprayed at a final concentration of 1×10^8 CFU $g^{-1,}$ according to García-Bernal et al. (2017, 2018). The HDBC in the sixth centesimal alcoholic vehicle (6C) was diluted again in distilled water to obtain a seventh centesimal dilution (7C) and thus avoid the potential side effects of ethanol (Mazón-

Suástegui et al. 2019a). The shrimp were fed to satiety thrice a day with the balanced feed described above, added with probiotics and/or HDBC (García-Bernal et al. 2017, 2018, Mazón-Suástegui et al. 2018a).

At the end of the experiment (30 days), the following response variables were evaluated: height increase (HI), weight gain (WG), and survival percentage (S) of the different experimental groups, applying the method described by Venkat et al. (2004).

HI = final height (cm) - initial height (cm)

WG = final weight (g) - initial weight (g)

S = (number of living organisms / number of total organisms) ×100

For the count of total heterotrophic bacteria and *Vibrio* spp., samples of the culture water and shrimp hepatopancreas were taken from the three replicates of all the experimental groups (treated and control). After disinfecting each shrimp with 70% ethanol, the hepatopancreas was removed with a sterile scalpel and homogenized in a physiological sterile saline solution. For all the experimental groups and their replicates, decimal dilutions of the corresponding homogenate were made, and 100 µL were spread over the culture media (marine agar medium 2216 Difco®) was used for heterotrophic bacteria. Thiosulfate citrate bile sucrose agar (TCBS, Difco®) was used for counting *Vibrio* spp. Plates were incubated at 35°C for 24-48 h. The same procedure was carried out to evaluate the bacteriological quality of the culture water of each treatment and its replicates.

The normality of data was initially analyzed with the Kolmogorov-Smirnov test and then confirmed with Levene's test for homogeneity of variance (Sokal & Rohlf 2020). After that, one-way ANOVA was used to assess the experimental groups' differences in growth, survival, and bacterial counts in the culture water and hepatopancreas. The percentage data (S) were arcsine-transformed before the ANOVA. Tukey's multiple range test was applied to detect significant differences in growth, S, and count values of microorganisms depending on the treatments provided and the control groups. Differences were considered significant at $P < 0.05$. The analysis was performed using the SPSS Statistics package version 21 for Windows (SPSS Inc., Chicago, IL).

The recorded S ranged between 94.13 ± 2.12 to 99.01 ± 2.45%, with significant differences ($P < 0.05$) between the treated groups. Different responses in shrimp growth parameters (HI, WG, and S) were attained after the administration of the treatment (Actinobacteria and HDBC) incorporated in the feed for 30 days (Fig. 1).

The best results were in the shrimp treated with T1, T2, and T3 concerning the untreated control group T5 (Fig. 1c). A greater increase (the organisms reached $P < 0.05$) in size in T2 compared to the other treated groups, including the control group. Significant differences ($P < 0.05$) were observed in groups T2, T3, and T4 regarding WG compared to T1 and T5.

Vibrio spp. count in the culture water was significantly lower ($P < 0.05$) in the experimental groups that received T1 and T2, compared to T5 (Table 1). Regarding the count of total marine heterotrophic bacteria in the culture water, a significant increase ($P > 0.05$) was observed only in the T3 group compared to T5. *Vibrio* spp. bacterial count in hepatopancreas was significantly lower ($P < 0.05$) in groups T1, T2, and T4 with respect to T5. In the count of total heterotrophic bacteria in hepatopancreas, all the groups' treatment showed significant differences compared to T5 (Table 1).

The probiotics application in aquaculture is related to increased water quality and biological control of pathogens and their associated infectious diseases. It is also related to an improvement in growth (Farsani et al. 2020, Nargesi et al. 2020), survival and enzymatic activity (Makled et al. 2020), increase in the immune response (Park et al. 2020), and greater resistance to biotic and abiotic stress (Rengpipat et al. 2000).

In this study, we have shown that actinobacteria and HDBC treatments could improve the growth, microbiological, and survival parameters of *P. vannamei*. Das et al. (2010) used marine strains of *Streptomyces* (CLS-28, CLS-39) in *Artemia* culture and concluded that this probiotic significantly increased the resistance of nauplii and adults against *Vibrio harveyi* and *V. proteolyticus*. These authors supplemented the black tiger shrimp postlarval diet with 1% *Streptomyces* for 15 days. They observed greater growth in probiotic-fed shrimp and more resistance against *V. harveyi* than the control group. In this study, the use of the mixture of two *Streptomyces* spp. (RL8 and N7) strain at a concentration of 1×10^8 CFU mL^{-1} significantly increased the survival of white shrimp juveniles and decreased *Vibrio* microbial density in culture water and shrimp hepatopancreas.

The HDBC can strengthen the immunomodulatory response and promote growth in marine organisms (Nagai et al. 2022). These HDBCs are primarily used as prophylactic treatments to improve growth, survival, and stimulation of the immune system in fish, mollusks,

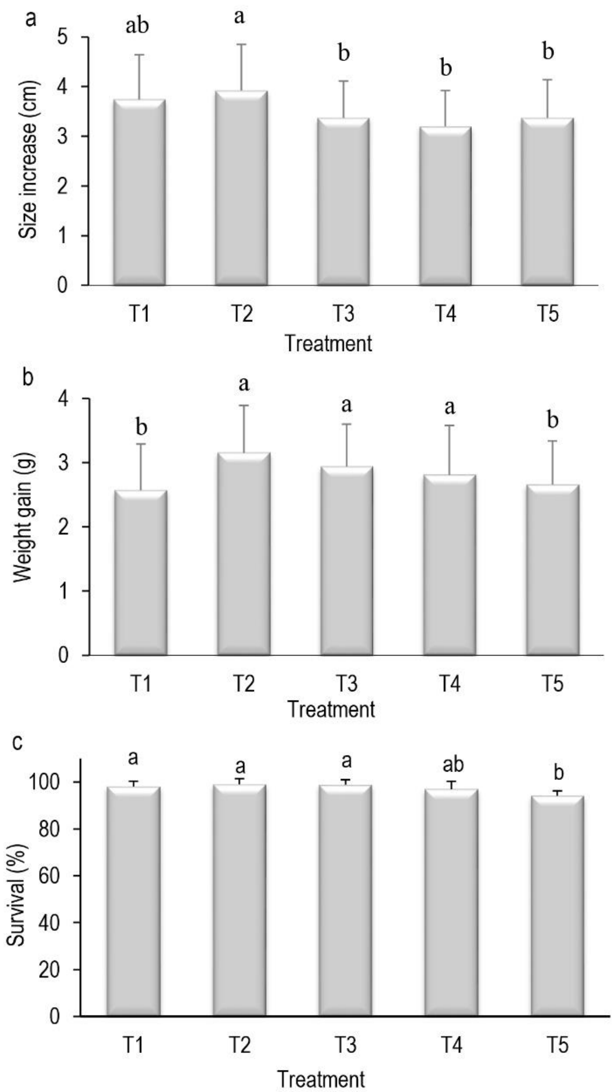

Figure 1. Synergistic effect of highly-diluted bioactive compounds (ViP, ViA, PhA, SiT) and probiotic actionmycetes (RL8, N7) in productive indicators of Pacific white shrimp *Penaeus vannamei* aquaculture. a) Size increase; b) weight gain; c) survival rate. Data are expressed as mean ± standard deviation. Bars with different letters differed significantly ($P < 0.05$).

and crustaceans (Mazón-Suástegui et al. 2017, 2018a, 2020). They are considered eco-friendly treatments because of their high dilution and innocuousness, useful for the prevention of high mortality in hatcheries of freshwater fishes such as *Oreochromis niloticus*, *Piaractus mesopotamicus*, *Colossoma macropomun* (Feitosa et al. 2013, Merlini et al. 2014, Pinheiro et al. 2015) and marine species such as *P. vannamei*, *Seriola rivoliana*, *A. ventricosus* (Mazón-Suástegui et al. 2017, 2018a, 2019b, López-Carvallo et al. 2020, 2022). Due to their high dilution and safety, these treatments have been reported as a potential and sustainable alternative for aquaculture (Ortiz-Cornejo et al. 2017, Mazón-Suástegui et al. 2018a, 2019a, López-Carvallo et al. 2019, 2020, García-Bernal et al. 2020).

Regarding the growth and survival parameters, the shrimp groups that received T2 and T3 recorded the highest values concerning the untreated control group (T5); T2 is a mixture of medicines authorized for human use and homeopathic products formulated at CIBNOR. The application of these HBDCs has made it possible to obtain increases in growth rate, digestive enzyme activity, condition index, energy reserves, oocyte quality, and modulation of the intestinal microbiota in marine organisms such as *S. rivoliana*, *A. ventricosus*, and *P. vannamei* (Mazón-Suástegui et al. 2018, 2021, López-Carvallo et al. 2019, 2020, 2022).

Through the application of these and other HDBCs, the immune response and survival rate of *P. vannamei* has increased when challenged against the pathogenic bacteria *V. parahaemolyticus* (Mazón-Suástegui et al. 2018a). A study by Mazón-Suástegui et al. (2020) showed that using ViP-7C and ViA-7C increased chymotrypsin activity -the digestive enzyme in juveniles of *S. rivoliana*- suggesting an improvement in food assimilation that allows organisms to increase their digestion rate. In another investigation, Mazón-Suástegui et al. (2017) applied PhA-7C+SiT-7C in the culture water of the Catarina scallop *A. ventricosus*, managing to improve growth, survival, and the immune response in these organisms during a challenge against the pathogen *V. alginolyticus*. In the case of T3, it proved that synergy exists between this HDBC mixture and the actinobacterial strain *Streptomyces* sp. N7, whose previous application has shown a probiotic action (García-Bernal et al. 2017).

Using beneficial bacteria (probiotics) to control or inhibit pathogenic bacteria through competitive processes is a very efficient disease control strategy suggested by researchers (Jamal et al. 2019, Li et al. 2022, Thompson et al. 2022).

The success of shrimp farming depends on water quality maintenance and the dynamic balance between beneficial and pathogenic bacteria (Iber & Kasan 2021). This investigation revealed that applying probiotic actinomycetes and HDBC mixtures decreased *Vibrio* spp. concentration, both in culture water and shrimp hepatopancreas thus treated. The results showed crop benefits, particularly with T1 and T2 concerning *Vibrio* spp. count in the culture water, and with T1, T2 and T4 in relation to *Vibrio* spp. count in shrimp hepatopancreas.

Table 1. Total count of heterotrophs and *Vibrio* spp. in water (CFU mL^{-1}) and hepatopancreas Hp (CFU g^{-1}) during the application of probiotic actinomycetes and highly-diluted bioactive compounds in juvenile Pacific white shrimp *Penaeus vannamei*. V: vibrios, Ht: heterotrophs; A: culture water; Hp: hepatopancreas; CFU: colony forming units. Values expressed as mean ± standard deviation; means with different letters in the same column differ significantly ($P < 0.05$).

Total count	Treatment				
	T1	T2	T3	T4	T5
Log V in water (CFU mL^{-1})	2.36 ± 0.11^{a}	2.43 ± 0.11^{a}	2.90 ± 0.20^{ac}	3.30 ± 0.17^{bc}	3.00 ± 0.26^{bc}
Log Ht in water (CFU mL^{-1})	6.33 ± 0.11^{ab}	6.36 ± 0.11^{ab}	6.76 ± 0.57^{c}	6.46 ± 0.21^{abc}	6.20 ± 0.00^{a}
Log V in Hp (CFU g^{-1})	1.85 ± 0.12^{a}	1.81 ± 0.29^{a}	2.42 ± 0.35^{ab}	2.29 ± 0.04^{a}	3.26 ± 0.47^{b}
Log Ht in Hp (CFU g^{-1})	6.06 ± 0.16^{a}	5.82 ± 0.18^{a}	6.08 ± 0.08^{a}	5.99 ± 0.13^{a}	3.73 ± 0.23^{b}

Regarding the count of heterotrophic bacteria in culture water, the T3 group showed significant differences from the T5. Regarding bacterial count in the hepatopancreas, all the treated groups had greater total bacteria than the T5. Similar results were obtained by García-Bernal et al. (2020) with probiotic actionmycetes and HDBC application, which decreased the concentration of *Vibrio* spp., both in culture water and in shrimp hepatopancreas.

In aquatic organisms, gut bacterial microbiota mainly consists of Gram-negative bacteria, predominantly *Vibrio*, *Pseudomonas*, and *Aeromonas* (Vine et al. 2006). Nevertheless, several studies have demonstrated that such bacterial microbiota can be modified by the addition of Gram-positive bacteria in the diet (Ziaei-Nejad et al. 2006, Vieira et al. 2008) or in the culture water (Rengpipat et al. 2000).

The evidence found in this study demonstrates that both actinomycete strains and HDBC can be used in shrimp farming to improve pond water quality and increase the growth and survival parameters of these aquatic organisms.

The synergistic effect of probiotic actinomycetes and HDBC improved culture water quality by increasing the total count of heterotrophic marine bacteria in the water of the experimental units and in the hepatopancreas of the shrimp studied, which also reduced the count of potentially pathogenic *Vibrio* spp. strains in hepatopancreas and culture water. Therefore, considering that the treatments T2 and T3 increased *P. vannamei* growth and survival. These HDBC and N7 *Streptomyces* sp. strains may be used alone or in synergy to increase biological productivity during the culture and rearing of the white shrimp *P. vannamei*.

The results suggest that both treatments, alone or combined, have potential applicability as a natural and technically viable alternative to increase the production of white shrimp *P. vannamei* in an eco-friendly manner, thus contributing to reducing the use and abuse of antibiotics in the shrimp industry.

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REFERENCES

Chai, P.C., Song, X.L., Chen, G.F., Xu, H. & Huang, J. 2016. Dietary supplementation of probiotic *Bacillus* PC465 isolated from the gut of *Fenneropenaeus chinensis* improves the health status and resistance of *Litopenaeus vannamei* against the white spot syndrome virus. Fish and Shellfish Immunology, 54: 602-611. doi: 10.1016/j.fsi.2016.05.011

Chen, H., Ullah, J. & Jia, J. 2017. Progress in *Bacillus subtilis* spore surface display technology towards environment, vaccine development, and biocatalysis. Journal of Molecular Microbiology and Biotechnol, 27: 159-167. doi: 10.1159/000475177

Das, S., Ward, L.R. & Burke, C. 2010. Screening of marine *Streptomyces* spp. for potential use as probiotics in aquaculture. Aquaculture, 305: 32-41. doi: 10.1016/j.aquaculture.2010.04.001

El-Sersy, N.A., AbdelRazek, F.A. & Taha, S.M. 2006. Evaluation of various probiotic bacteria for the survival of *Penaeus japonicus* larvae. Fresenius Environmental Bulletin, 15: 1506-1511.

Farsani, M.N, Bahrami-Gorji, S., Hoseinifar, S.H., Rashidian, G. & Van Doan, H. 2020. Combined and singular effects of dietary primaLac® and potassium diformate (kdf) on growth performance and some physiological parameters of rainbow trout (*Oncorhynchus mykiss*). Probiotics and Antimicrobial Proteins, 12: 236-245. doi: 10.1007/s12602-019-9523-2

Feitosa, O.C., Aparecido-Povhayme, J. & Sampaio de Abreu, J. 2013. Physiological responses of pacu (*Piaractus mesopotamicus*) treated with homeopathic product and submitted to transport stress. Homeopathy, 102: 268-73. doi: 10.1016/j.homp.2013.07.004

Food and Agriculture Organization (FAO). El estado mundial de la pesca y la acuicultura. 2020. FAO, Rome.

García-Bernal, M., Medina-Marrero, R., Campa-Córdova, Á.I. & Mazón-Suástegui, J.M. 2017. Probiotic effect of *Streptomyces* strains alone or in combination with *Bacillus* and *Lactobacillus* in juveniles of the white shrimp *Litopenaeus vannamei*. Aquaculture International, 25: 927-939. doi: 10.1007/s10499-016-0085-y

García-Bernal, M., Medina-Marrero, M., Campa-Córdova, Á.I., Tovar-Ramírez, D., Barajas, D., Ormart-Castro, P. & Mazón-Suástegui, J.M. 2020. Growth and survival of the shrimp *Penaeus vannamei* with application of probiotic actinomycetes and homeopathy. AquaTechnica, 2: 76-85. doi: 10.33936/at.v2i2.2654

García-Bernal, M., Medina-Marrero, R., Rodríguez-Jaramillo, C., Marrero-Chang, O., Campa-Córdova, Á.I., Medina-García, R. & Mazón-Suástegui, J.M. 2018. Probiotic effect of *Streptomyces* spp. on shrimp (*Litopenaeus vannamei*) postlarvae challenged with *Vibrio parahaemolyticus*. Aquaculture Nutrition, 24: 865-871. doi: 0.1111/anu.12622

García-Corona, J.L. 2018. Análisis fisiológico y transcriptómico del efecto de medicamentos homeopáticos en la maduración gonádica del mejillón café *Modiolus capax* (Conrad, 1837). Master's Thesis. Centro de Investigaciones Biológicas del Noroeste, La Paz.

Hasan, J., Rahman, M.H., Ullah, M.R. & Mredul, M.M.H. 2020. Availability of aqua drugs and their uses in semi-intensive culture farms at Patuakhali district in Bangladesh. Archives of Agriculture and Environmental Science, 5: 368-376. doi: 10.26832/24566632.2020.0503019

Hoseinifar, S.H., Sun, Y., Wang, A. & Zhou, Z. 2018. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Frontiers in Microbiology, 9: 2429. doi: 10.3389/fmicb.2018.02429

Iber, B.T. & Kasan, N.A. 2021. Recent advances in shrimp aquaculture wastewater management. Heliyon, 7: e08283. doi: 10.1016/j.heliyon.2021.e08283

Jamal, M.T., Abdulrahman, I.A., Harb, M.A. & Chithambaran, S. 2019. Probiotics as alternative control measures in shrimp aquaculture: a review. Journal of Applied Biology & Biotechnology, 7: 69-77. doi: 10.7324/JABB.2019.70313

Lakshmi, B., Viswanath, B. & Sai-Gopal, D.V.R. 2013. Probiotics as antiviral agents in shrimp aquaculture. Journal of Pathogens, 2013: 1-13. doi: 10.1016/j.aquaculture.2015.10.011

Li, X., Wang, Q., Hu, X. & Liu, W. 2022. Current status of probiotics as supplements in the prevention and treatment of infectious diseases. Frontiers in Cellular and Infection Microbiology, 14: 789063. doi: 10.3389/fcimb.2022.789063

López-Carvallo, J.A., Arcos-Ortega, G.F., Tovar-Ramírez, D., Hernández-Oñate, M.A., Abasolo-Pacheco, F., García-Corona, J.L. & Mazón-Suástegui, J.M. 2019. Effect of immunomodulatory medication over the general response of juvenile Catarina scallop (*Argopecten ventricosus* Sowerby II, 1842). Latin American Journal of Aquatic Research, 47: 65-77. doi: 10.3856/vol47-issue1-fulltext-8

López-Carvallo, J.A., Mazón-Suástegui, J.M., Arcos-Ortega, G.F., Hernández-Oñate, M.A., Tovar-Ramírez, D., Abasolo-Pacheco, F. & García-Bernal, M. 2022. Highly diluted bioactive compounds in marine aquaculture: a potential alternative for sustainable production. Reviews in Aquaculture, 14: 1-24. doi: 10.1111/raq.12644

López-Carvallo, J.A., Mazón-Suástegui, J.M., Hernández-Oñate, M.A., Tovar-Ramírez, D., Abasolo-Pacheco, F., Morelos-Castro, R.M. & Arcos-Ortega, G.F. 2020. Transcriptome analysis of Catarina scallop (*Argopecten ventricosus*) juveniles treated with highly-diluted immunomodulatory compounds reveals activation of non-self-recognition system. Plos One, 15: e0233064. doi: 10.1371/journal.pone.0233064

Makled, S.O., Hamdan, A.M. & El-Sayed, A.F.M. 2020. Growth promotion and immune stimulation in Nile tilapia, *Oreochromis niloticus*, fingerlings following dietary administration of a novel marine probiotic, *Psychrobacter maritimus* S. Probiotics and Antimicrobial Proteins, 12: 365-374. doi: 10.1007/s12602-019-09575-0

Mazón-Suástegui, J.M., Salas-Leiva, J., Teles, A. & Tovar-Ramírez, D. 2019b. Immune and antioxidant enzyme response of longfin yellowtail (*Seriola rivoliana*) juveniles to ultra-diluted substances derived from phosphorus, silica and pathogenic *Vibrio*. Homeopathy, 108: 43-53. doi: 10.1055/s-0038-167 2197

Mazón-Suástegui, J.M., Salas-Leiva, J., Teles, A. & Tovar-Ramírez, D. 2020. Evaluation of homeopathic phosphoric acid, silica and pathogenic *Vibrio* on digestive enzyme activity of longfin yellowtail fish (*Seriola rivoliana*). Homeopathy, 109: 3-13. doi: 10.1055/s-0039-1692998

Mazón-Suástegui, J.M., García-Bernal, M., Saucedo P.E., Campa-Córdova, A. & Abasolo-Pacheco, F. 2017. Homeopathy outperforms antibiotics treatments in juvenile scallop *Argopecten ventricosus*: effects on growth, survival, and immune response. Homeopathy, 106: 18-26. doi: 10.1016/j.homp.2016.12.002

Mazón-Suástegui, J.M., García-Bernal, M., Avilés-Quevedo, A., Campa-Córdova, A.I., Salas-Leiva, J. & Abasolo-Pacheco, F. 2018a. Assessment of homeopathic medicines on survival and antioxidant response in white shrimp *Litopenaeus vannamei*. Revista MVZ Córdoba, 23: 6850-6859. doi: 10.21897/rmvz.1373

Mazón-Suástegui, J.M., López-Carvallo, J.A., Rodríguez-Jaramillo, C., García-Corona, J.L., Arcos-Ortega, G.F. & Abasolo-Pacheco, F. 2021. Ultra-diluted bioactive compounds enhance energy storage and oocyte quality during gonad conditioning of Pacific calico scallop *Argopecten ventricosus* (Sowerby II, 1842). Aquaculture Research, 52: 1490-1500. doi: 10.1111/are. 15002

Mazón-Suástegui, J.M., Ojeda-Silvera, C.M., García-Bernal, M., Avilés-Quevedo, M.A., Abasolo-Pacheco, F., Batista-Sánchez, D., et al. 2019a. Agricultural homoeopathy: a new insight into organics. In: Moudry, J. (Ed.). Multifunctionality and impacts of organic and conventional agriculture. IntechOpen Books, London, pp. 1-18.

Mazón-Suástegui, J.M., Tovar-Ramírez, D., Salas-Leiva, J.S., Arcos-Ortega, G.F., García-Bernal, M., Avilés-Quevedo, M.A., et al. 2018b. Aquacultural homoeopathy: a focus on marine species. In: Diarte-Plata, G. & Escamilla, R. (Eds.). Aquaculture: plants and invertebrates. IntechOpen Books, London, pp. 67-91.

Merlini, L.S., Vargas, L., Piau, R., Ribeiro, P.R. & Merlini, N.B. 2014. Effects of a homeopathic complex on the performance and cortisol levels in Nile tilapia (*Oreochromis niloticus*). Homeopathy, 103: 139-142. doi: 10.1016/j.homp.2013.08.005

Nagai, M.Y.O., Von Ancken, A.C.B. & Bonamin, L.V. 2022. Effects of highly diluted substances on aquatic animals: a review. Water Special Edition: High Dilutions. A Partnership with IPWS - International Panel on Water Structure for the 9th World Water Forum, Dakar, Senegal, March, 2022. doi: 10.14294/ WATER.2022.S11

Nargesi, E.A., Falahatkar, B. & Sajjadi, M.M. 2020. Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition, 26: 98-108. doi: 10.1111/anu.12970

Ortiz-Cornejo, N.L., Tovar-Ramírez, D., Abasolo-Pacheco, F. & Mazón-Suástegui, J.M. 2017. Homeopatía, una alternativa para la acuicultura. Revista Médica de Homeopatía, 10: 28-34. doi: 10.1016/j. homeo.2017.04.006

Park, Y., Kim, H., Won, S., Hamidoghli, A., Hasan, M.T., Kong, I. & Bai, S.C. 2020. Effects of two dietary probiotics (*Bacillus subtilis* or *licheniformis*) with two prebiotics (mannan or fructo oligosaccharide) in Japanese eel, *Anguilla japonica*. Aquaculture Nutrition, 26: 316-327. doi: 10.1111/anu.12993

Peñalosa-Martinell, D., Araneda-Padilla, M., Dumas, S., Martinez-Díaz, S. & Vela-Magaña, M. 2021. The use of probiotics in larval whiteleg prawn (*Litopenaeus vannamei*) production: a marginal analysis of bioeconomic feasibility. Aquaculture Research, 52: 943-951. doi: 10.1111/are.14949

Pinheiro, D.A., Cavero, B.A.S., Vargas, L., Braccini, G.L., Yoshioka, E.T., Oliveira, M.S. & Tabares-Dias, M. 2015. Performance, parasitic infections, hematology and hepatic histology of *Colossoma macropomum* (tambaqui) fed on homeopathic product. African Journal of Pharmacy and Pharmacology, 9: 82-90. doi: 10.5897/-AJPP2014

Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. & Menasaveta, P. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquaculture, 191: 271-288.

Sarkar, G. & Suthindhiran, K. 2022. Diversity and biotechnological potential of marine actinomycetes from India. Indian Journal of Microbiology, 62: 475-493. doi: 10.1007/s12088-022-01024-x

Secretaría de Salud. 2015. Farmacopea homeopática de los Estados Unidos Mexicanos. Secretaría de Salud, México D.F.

Sokal, R.R. & Rohlf, F.J. 2000. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., New York.

Thompson, J., Gregory, S., Plummer, S., Shields, R.J. & Rowley, A.F. 2010. An *in vitro* and *in vivo* assessment of the potential of *Vibrio* spp. as probiotics for the Pacific white shrimp, *Litopenaeus vannamei*. Journal of Applied Microbiology, 109: 1177-1187. doi: 10.1111/j.1365-2672.2010.04743.x

Thompson, J., Weaver, M.A., Lupatsch, I., Shields, R.J., Plummer, S., Coates, C.J. & Rowley, A.F. 2022. Antagonistic activity of lactic acid bacteria against pathogenic *Vibrios* and their potential use as probiotics in shrimp (*Penaeus vannamei*) culture. Frontiers in Marine Science, 9: 807989. doi: 10.3389/fmars.2022.807989

Venkat, H.K., Sahu, N.P. & Jain, K.K. 2004. Effect of feeding Lactobacillus-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). Aquaculture Research, 35: 501-507. doi: 10.1111/j.1365-2109.2004.01045.x

Vieira, F.N., Buglione, C.C., Mouriño, J.L.P., Jatobá, A., Ramirez, C., Martins, M.L., et al. 2008. Time-related action of *Lactobacillus plantarum* in the bacterial microbiota of shrimp digestive tract and its action as immunostimulant. Pesquisa Agropecuaria Brasileira, 43: 763-769.

Vine, N.G., Leukes, W.D. & Kaiser, H. 2006. Probiotics in marine larviculture. FEMS Microbiology Reviews, 30: 404-427. doi: 10.1111/j.1574-6976.2006.00017.x

Wang, Y. & Gu, Q. 2010. Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response, Marine Biology Research, 6: 327-332. doi: 10.1080/17451000903300893

Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R. & Shakour, M. 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, 252: 516-524. doi: 10.1016/j.aquaculture.2005.07.021

Zorriehzahra, M.J., Delshad, S.T., Adel, M., Tiwari, R., Karthik, K., Dhama, K. & Lazado, C.C. 2016. An update on their multiple modes of action: probiotics as beneficial microbes in aquaculture: a review. Veterinary Quarterly, 36: 228-241. doi: 10.1080/01652176.2016.1172132

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