Latin American Journal of Aquatic Research, 53(1): 79-88, 2025
DOI: 10.3856/vol53-issue1-fulltext-3278

*Research Article*

# Effective doses of probiotics to prevent infection of *Crassostrea gigas* larvae by *Vibrio parahaemolyticus*

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**ABSTRACT.** Most bacterial diseases in aquaculture production systems are caused by Gram-negative bacteria that belong to the Vibrionaceae family. The administration of probiotics is a promising area of research for the biocontrol and prevention of diseases in aquaculture. In this work, the objective was to investigate three probiotic strains of the genus *Lactobacillus*. Two trials were performed to determine the mean effective dose of the *Lactobacillus* strains and the mean lethal dose of the pathogenic *Vibrio parahaemolyticus* strain. The LD50 for *V. parahaemolyticus* was 5.5 log CFU $mL^{-1}$. The three probiotic strains, *L. plantarum* 69Cr with $10^4$ CFU $mL^{-1}$, *L. fermentum* 101Cc with $10^4$ CFU $mL^{-1}$, and *L. casei* 43Cg with $10^3$ CFU $mL^{-1}$ conferred greater survival to the larvae than the control. The bacteria *L. plantarum* 69Cr and *L. fermentum* 101Cc conferred protection against the pathogen and significantly improved larval survival with an optimal dose of $10^4$ CFU $mL^{-1}$, whereas *L. casei* 43Cg did so with an optimal dose of $10^3$ CFU $mL^{-1}$. The best survival percentage was achieved when the probiotic *L. plantarum* 69Cr was supplied. These results indicate that probiotic bacteria associated with, or originating from, *Crassostrea gigas* can protect their host from a pathogen such as *V. parahaemolyticus*.

**Keywords:** *Crassostrea gigas*; larvae; *Lactobacillus*; probiotic; *Vibrio*; dose; aquaculture

## INTRODUCTION

Aquaculture is growing rapidly and is now considered a major contributor to global food production (Naylor et al. 2021, Garlock et al. 2022). According to the Food and Agriculture Organization (FAO), the growth of the aquaculture sector is higher than that of any other type of food production system (FAO 2022). However, the growth of the aquaculture industry is hampered by sudden mortalities, regularly caused by pathogenic microorganisms (Prado et al. 2014, 2015, Richards et al. 2015, Rojas et al. 2015, Dubert et al. 2016, 2017, Le Roux et al. 2016, King et al. 2018, Zhao et al. 2020). Bacterial diseases have been attributed to the bottlenecks of organic production in intensive aquaculture, which is why antibiotics are currently the most used method in aquaculture (Mohamad et al. 2019). The application of antibiotics was an effective strategy at the beginning (Ng et al. 2024). Still, indiscriminate use led to the emergence of antibiotic-resistant bacteria in aquaculture environments (Santos & Ramos 2018). The selection of probiotics is important to avoid undesirable effects on the host (Pandiyan et al. 2013). When selecting probiotic microorganisms, different criteria must be met, one of the most important being the ability to adhere to intestinal surfaces (Nikoskelainen et al. 2001). Among the genera *Bacillus* spp. and *Lactobacillus* spp., probiotics are prominent for aquaculture through modulating the host immune system, promoting attachment, producing digestive en-

Associate Editor: Carlos Alvarez

zymes and nutrition to promote animal growth, competing for adhesion receptors on the intestinal cells and exerting significant antimicrobial activity against pathogenic microorganisms. They can help alleviate stress, especially during transport and changes in ecological conditions, and reduce the release of excess nutrients into the aquatic environment, to the induction of host genes involved in innate immunity, contributing to better water quality and minimizing environmental pollution (Abasolo-Pacheco et al. 2017, Hoseinifar et al. 2018, 2019, Dawood, et al. 2019b, Stavropoulou & Bezirtzoglou. 2020, Van Zyl et al. 2020, Cristofori et al. 2021, Savin-Amador et al. 2021, Rohani et al. 2022, Amenyogbe 2023, Muthu et al. 2024). Infectious diseases affect production and can cause high economic losses. Some pathogens, including the most prolific species of the *Vibrio* genus, affect all stages of the life of their hosts (Rojas et al. 2015, Dubert et al. 2016, 2017, Todorov et al. 2024) due to the production of toxic extracellular products (Labreuche et al. 2010, Rojas et al. 2015). In general, the dosage and duration of the feeding period also play an important role in achieving the desired results. In aquaculture, overdosing and prolonged administration of probiotics is common, possibly resulting in immunosuppression from ongoing responses of non-specific immune systems (Sharifuzzaman & Austin 2010, Hasan & Banerjee. 2020). Muthu et al. (2024) mention promising applications of probiotics in terms of their effective dose, duration, and frequency of administration. This study investigated the effective dose of probiotic bacteria previously isolated from ostreids and their role in attacking the pathogenic bacteria *Vibrio parahaemolyticus* to *Crassostrea gigas* larvae.

## MATERIALS AND METHODS

### Bacterial strains

The probiotic strains used were *Lactobacillus fermentum* 101Cc isolated from *Crassostrea corteziensis*, *Lactobacillus plantarum* 69Cr isolated from *C. rhizophorae*, *Lactobacillus casei* 43Cg isolated from *C. gigas* in the state of Baja California Sur, Mexico. All of them are characterized by their adhesion to the mucosa of the digestive gland, their antagonism with bacteria of the genus *Vibrio*, and their effect on the survival and resistance of larvae of oysters to attack by *V. parahaemolyticus* (Savín-Amador et al. 2021). These strains, stored at -85°C in the Laboratory of Food Science and Technology (LABCyTA, by its Spanish acronym) of the Autonomous University of Baja California Sur (UABCS, by its Spanish acronym), were reactivated on MRS agar (DIFCO) and incubated in an anaerobiosis glass jar at a temperature of 30°C for 48 h. Subsequently, a colony of each strain was inoculated in MRS broth (DIFCO) and incubated at 30°C for 12 to 18 h before use. The pathogenic strain *V. parahaemolyticus*, also stored at -85°C, was reactivated on thiosulfate citrate bile sucrose agar (TCBS agar, DIFCO) and incubated at 30°C for 48 h. Subsequently, all strains were inoculated in trypticasein and soy broth (TSB) before use and incubated at 30°C for 12 to 18 h. The optical density at 600 nm of each strain was adjusted to get the colony-forming unit per milliliter (CFU $mL^{-1}$) required according to the standard curve previously performed.

### Obtaining and cultivating larvae

Adult oysters were acclimatized for four days at the Pichilingue Research Unit of the UABCS, and larvae were obtained using standard protocols for the culture of this species (Helm et al. 2006). Seawater with a salinity of 37 ± 0.5 was used, previously filtered at 1 µm, UV-sterilized at 25 ± 1°C and with constant aeration. The larvae were placed in seawater until they reached stage D (early veliger) for approximately 24 to 36 h and then were fed daily with a mixture of the microalgae *Isochrysis galbana* and *Chaetoceros calcitran*s (1:1) at a concentration of $3\times10^4$ cells $d^{-1}$ $mL^{-1}$.

### Calculation of the LD50 for the pathogenic bacteria *Vibrio parahaemolyticus*

The two days-old oyster larvae were challenged with five individual doses of the pathogen *V. parahaemolyticus* ($10^6$, $10^5$, $10^4$, $10^3$ and $10^2$ CFU $mL^{-1}$ and control without bacteria, n = 6 per treatment), with 12 replicates per treatment and an exposure period of 48 h. The experiment was conducted in 96-well microplates (Estes et al. 2004, modified) at 25°C. Each well contained 100 µL of sterile filtered seawater, 10 two-day-old larvae, and the indicated pathogen dose. Larvae were observed using a phase contrast microscope (Nikon Eclipse E-600) every 6 h until 48 h of incubation to determine survival. The average lethal dose, LD50, for larvae was calculated using the following equation (Reed & Muench 1938):

$$\text{Log LD50} = \frac{(\text{logDn}+50)-\%\text{ of death in Dn}}{(\%\text{ of death in Dv}-\%\text{ of death in Dn})\times \log(\text{dilutionfactor})}$$

where Dn is the dilution when the percentage of death is immediately less than 50%, and Dv is the dilution when the percentage of death is immediately greater than 50%. The dilution factor is the log of 10 = 1, based on serial dilution 10 times pathogenic doses. Furthermore, this value was confirmed using the reported

Spearman-Karber method (Hamilton et al. 1977) to estimate the mean lethal dose.

**An effective dose of probiotics on a laboratory scale**

The effective dose of the three probiotic strains was determined through a 21-day bioassay with two days-old oyster larvae, in which each treatment began with 10 larvae $mL^{-1}$, one of the three doses of the probiotic strains ($10^3$, $10^4$, $10^5$ CFU $mL^{-1}$) and a dose of the pathogen *V. parahaemolyticus* ($3.14\times10^5$ CFU $mL^{-1}$). In addition, three control treatments were incorporated: without bacteria, only probiotic ($10^3$ CFU $mL^{-1}$), and only pathogen ($10^5$ CFU $mL^{-1}$ *V. parahaemolyticus*), as shown in Table 1. The larvae were fed daily with a mixture of *I. galbana* and *C. calcitrans* (1:1) at a concentration of $3\times10^4$ cells $d^{-1}$ $mL^{-1}$. The experiment was conducted in 2.5-L containers with 2 L of sterile seawater maintained at 25°C per treatment. Water exchange and bacterial dosing were performed every three days during the challenge.

**Confirmation of the effective dose of the probiotic on a pilot scale**

In this bioassay, there were four treatments in triplicate as follows: 1) control treatment, without bacteria, 2) with the probiotic 69Cr (effective dose), 3) with the pathogen (*V. parahaemolyticus*), and 4) with the probiotic 69Cr plus the *V. parahaemolyticus* pathogen, added until the third day. The bioassay was carried out in 20 L containers with 18 L of filtered seawater at a concentration of approximately 8 larvae $mL^{-1}$. The concentrations used were $1\times10^4$ of the probiotic and $3.14\times10^5$ of the pathogen, according to the previous bioassays; the bacteria were added every two days, along with water exchange. The bioassay lasted for 16 days at 25°C until the pediveliger stage (16 days) in a temperature-controlled room, and the larvae were fed daily with a mixture of *I. galbana* and *C. calcitrans* (1:1) at a concentration of $3\times10^4$ cells $d^{-1}$ $mL^{-1}$. Survival was determined by observing the internal structure of the larvae using a compound microscope (Nikon Eclipse E-600) with a 10x objective. Deterioration or retraction of internal organ structures or lack of food was taken as indicative of dead or dying larvae. The survival rate was calculated using the survival formula:

Survival rate (%) = 100 × (% number of live larvae) / (% total number of larvae).

**Statistical analysis**

The results were subjected to Bartlett's homoscedasticity test and the D'Agostino-Pearson normality test with an $\alpha = 0.05$, followed by a one-way analysis of variance (ANOVA) to compare the survival status among the treatments with the addition of probiotic bacteria, the pathogenic agent and the respective controls. Determination of factors that may contribute to significant differences was performed using the LSD multiple comparison test (Sokal & Rohlf 1980). Data collected as a percentage were arcsine-transformed before analysis.

**Table 1.** Doses of the probiotics and the pathogen *Vibrio parahaemolyticus* were used as treatments in the bioassays to determine the optimal dose of probiotics that significantly increases larval survival.

| Treatments | Probiotics | Dose (CFU $mL^{-1}$) |
|---|---|---|
| | | Pathogen |
| 101Cc + *V. parahaemolyticus* | 105 | $3.14\times10^5$ |
| 101Cc + *V. parahaemolyticus* | 104 | $3.14\times10^5$ |
| 101Cc + *V. parahaemolyticus* | 103 | $3.14\times10^5$ |
| 101Cc Control | 103 | 0 |
| 69Cr + *V. parahaemolyticus* | 105 | $3.14\times10^5$ |
| 69Cr + *V. parahaemolyticus* | 104 | $3.14\times10^5$ |
| 69Cr + *V. parahaemolyticus* | 103 | $3.14\times10^5$ |
| 69Cr Control | 103 | 0 |
| 43Cg + *V. parahaemolyticus* | 105 | $3.14\times10^5$ |
| 43Cg + *V. parahaemolyticus* | 104 | $3.14\times10^5$ |
| 43Cg + *V. parahaemolyticus* | 103 | $3.14\times10^5$ |
| 43Cg Control | 103 | 0 |
| *V. parahaemolyticus* Control | 0 | $3.14\times10^5$ |
| Control | 0 | 0 |

## RESULTS

**LD50 for the pathogenic bacterium *V. parahaemolyticus***

According to the equation of Reed & Muench (1938), the LD50 for *V. parahaemolyticus* resulted in infected *C. gigas* larvae dying at two days of age at $3.14\times10^5$ CFU $mL^{-1}$. This result was confirmed using the reported Spearman-Karber method (Hamilton et al. 1977) to estimate the mean lethal concentration.

**The effective dosage of probiotics**

**Laboratory scale**

After three days in cultures, the *C. gigas* larvae with *L. plantarum* 69Cr, $10^4$ CFU $mL^{-1}$ treatment were alive, and significative differences were observed with *V. parahaemolyticus* control treatment. There were no significant differences in the other treatments. On day 9 of the larvae cultures, the 69Cr, $10^4$ CFU $mL^{-1}$ treatment presented 83.88% survival, the highest percentage observed. Significant differences were

found in the 69Cr dose $10^3$ CFU $mL^{-1}$ treatments without *V. parahaemolyticus* and the 43Cg dose $10^4$ CFU $mL^{-1}$ with 75% survival compared with the rest of the treatments. On day 16 of larvae cultures, the 69Cr treatment at a dose of $10^4$ CFU $mL^{-1}$ presented the highest survival (58.66%), followed by treatments 69Cr control without pathogen (54%), 43Cg dose $10^3$ CFU $mL^{-1}$ (46%) and control treatment without bacteria (46%), all of them with significant differences with the rest of the treatments (Fig. 1).

**Pilot scale test**

In this pilot-scale bioassay, the oyster larvae were exposed to the probiotic *L. plantarum* 69Cr. The probiotic was added by immersion and ingested by the larvae, and a day three challenge with the pathogen *V. parahaemolyticus* was performed. On day 3, after larval sampling, water change, feeding, and dosing with the 69Cr probiotic and before exposure to the pathogen, no significant differences were observed between control larvae and larvae supplemented with the 69Cr probiotic (Fig. 2), indicating that there were no negative effects of the probiotic on larvae survival. The effects of the pathogen were observed on day 5 (Fig. 2) when mortalities were observed for both the pathogen treatment and the combined pathogen and probiotic treatment. The survival rates of the larvae in the 69Cr probiotic treatment were 83.33% on day 9, 79.16% on day 12, and 75% on day 16. In the pathogen *V. parahaemolyticus* treatment, the survival rate on day 9 was 45.83%; on day 12, it was 33.33%; and on day 16, it was 8.33%. Survival in the treatment group inoculated with the effective dose of the probiotic 69Cr and subsequently with the pathogen *V. parahaemolyticus* was 66.66% on day 9, 54.16% on day 12, and 41.66% on day 16. These results indicate that the survival of the larvae challenged with the pathogen *V. parahaemolyticus* was significantly improved by the presence of the probiotic 69Cr on days 9 and 12 ($P$ = 0.0314). Also, on day 16, survival improved significantly ($P$ = 0.0074), but only in comparison to the larvae challenged with the pathogen.

## DISCUSSION

Using species-specific probiotic bacteria as biological control agents is considered an environmentally friendly method for disease prevention in bivalve larvae and seed production laboratories. In this research, an evaluation of the bacteria isolated from ostreids was carried out. They were selected for their probiotic potential and molecularly identified to apply them safely and efficiently. The probiotic bacteria isolated from ostreids, selected from and used in the same genus of organisms, improved the survival rate in the larval stage. Such was the case of *C. gigas* larvae in laboratory and pilot trials. In previous studies, these probiotic bacteria were evaluated for their safe use in the culture of oyster larvae. They positively interacted with the microalgae *I. galbana* and *C. calcitrans* (Kesarcodi-Watson et al. 2008). These results also indicate that the most effective dose of the probiotic *L. plantarum* 69Cr was $10^4$ CFU $mL^{-1}$ (Table 2). Furthermore, the LD50 for the pathogenic strain *V. parahaemolyticus* was $3.14\times10^5$ CFU $mL^{-1}$, established as the stable pathogenic dose for the bioassays. Rojas et al. (2019) demonstrated that the LD50 of the bivalvicid *Vibrio* strain in the scallop *Argopecten purpuratus* larval cultures was $1.3\times10^4$ CFU $mL^{-1}$.

Bioassays are generally performed *in vivo* to determine the efficiency of probiotics. These bioassays with living organisms allow the direct effects of probiotic bacteria on the animal host to be observed and quantified through any mode of action (Kesarcodi-Watson et al. 2008). The effective dose of the probiotic bacteria and the stable pathogenic dose of *V. parahaemolyticus* for oyster larvae were confirmed in the pilot-level larva-probiotic-pathogen bioassay, which suggested the amount of probiotic to be used and the monitoring of *Vibrio* at commercial scale.

In this work, we obtained results close to those reported by Lim et al. (2011) for small-scale bioassays. However, Riquelme et al. (2000) found that a pre-exposure time of 6 h was required for scallop larvae to ingest probiotic strains at doses of $10^6$ CFU $mL^{-1}$ to achieve competitive exclusion of the pathogen by the probiotic. In contrast, Lim et al. (2011) used a pre-exposure period of three days for the probiotic to produce a positive effect before the pathogen was introduced, allowing *C. virginica* larvae to ingest the probiotic and establish themselves in the culture system. Likewise, in the case of the two bioassays performed in this study, there was a 3-day exposure period with the probiotic bacteria, leading to positive results; the larvae treated with the probiotic *L. plantarum* 69Cr had higher survival rates. In the final bioassay, the effective dose of $10^4$ CFU $mL^{-1}$ of the probiotic *L. plantarum* 69Cr significantly improved larval survival by 34% when the larvae were challenged with *V. parahaemolyticus* and by 83% when they were treated only with the probiotic.

The species of lactic acid bacteria from oysters that were used in this study have recently been used in aquaculture, namely *L. plantarum* (Correa et al. 2018, Gao et al. 2018, Li et al. 2018, Pacheco-Vega et al.

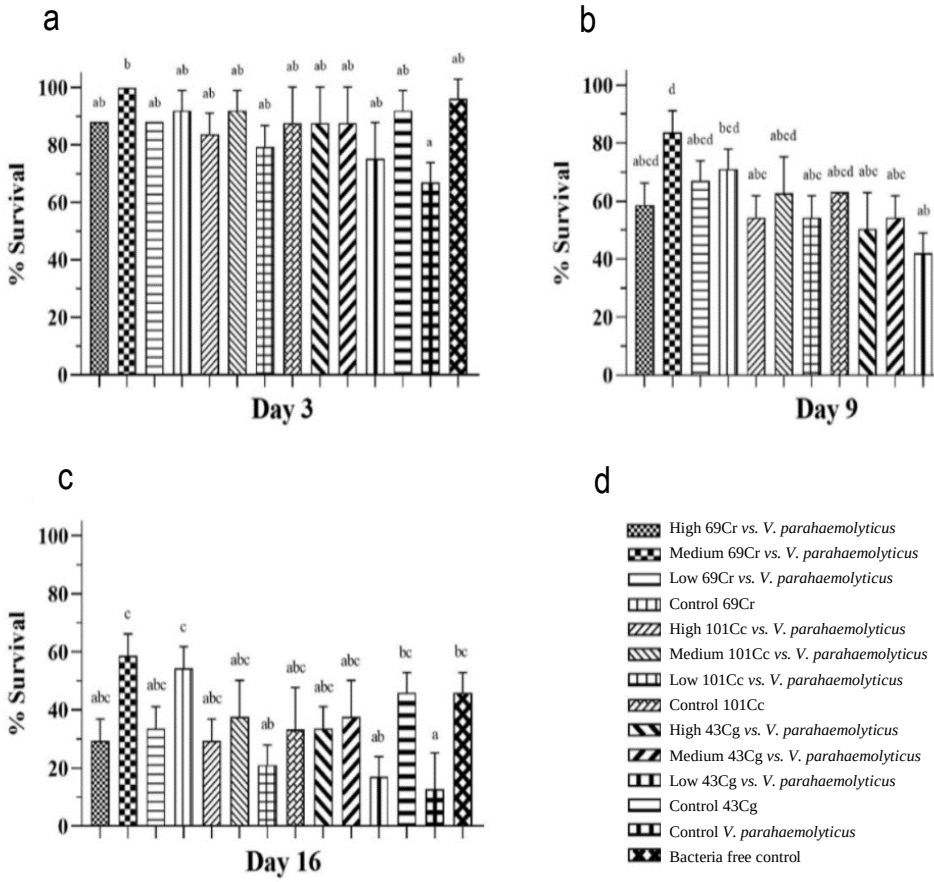


**Figure 1.** Optimal doses of the probiotics *Lactobacillus plantarum* 69 Cr, *L. fermentum* 101Cc, and *L. casei* 43Cg. a) Day 3, b) day 9, c) day 16, and d) treatments: high 69Cr *vs. V. parahaemolyticus*, medium 69Cr *vs. V. parahaemolyticus*, low 69Cr *vs*. *V. parahaemolyticus*, control 69Cr, high 101Cc *vs*. *V. parahaemolyticus*, medium 101Cc *vs. V. parahaemolyticus*, low 101Cc *vs. V. parahaemolyticus*, control 101Cc, high 43Cg *vs. V. parahaemolyticus*, medium 43Cg *vs. V. parahaemolyticus*, low 43Cg *vs. V. parahaemolyticus*, control 43Cg, control *V. parahaemolyticus* and bacteria free control. Treatments with different letters were significantly different (ANOVA, $P < 0.05$).

2018, Zheng et al. 2018, Dawood et al. 2019a, Valipour et al. 2019), *L. fermentum* (Mohammadían et al. 2019a, Vazirzadeh et al. 2019, Wang et al. 2019), and *L. casei* (Mirghaed et al. 2018, Mohammadían et al. 2018, 2019b, Savin-Amador et al. 2021).

Results like those obtained with $1\times10^4$ CFU mL$^{-1}$ of *L. plantarum* were reported by Venkat et al. (2004) with *Macrobrachium rosenbergii* larvae and Mazón-Suástegui et al. (2009) with *C. corteziensis* larvae cultured with *Lactobacillus* sp. and a mixture of strains of the genus *Bacillus*. Campa-Córdova et al. (2011) suggested that the greatest benefits associated with strengthening the immune system of the bacteria used were the significant increases in the growth and survival of *C. corteziensis* larvae produced in the laboratory. Kesarcodi et al. (2012) administered probiotics to *C. gigas* larvae and reported that using *Neptunomonas* sp. 0536 increased larval mortality compared to non-inoculated controls. This same author also administered *A. macleodii* 0444, *P. gallaeciensis*, or *Pseudoalteromonas* sp. D41, but they did not influence the survival of *C. gigas* larvae compared to that of the larvae of the non-inoculated control. Takyi et al. (2023) demonstrated that *Phaeobacter* inhibitans S4 formulation ($10^4$ CFU mL$^{-1}$) is safe, easy to handle, and stable for use in oyster farms. This formulation may help control the impact of *Vibriosis* when used prophylactically in oyster farms. However, it may not offer protection against other causes of larval mortality that are not yet fully characterized. Recently, Čanak et al. (2023) used juvenile queen scallop (*Aequipecten opercularis*) fed with the addition of *Lactiplantibacillus plantarum* (also called *L. plantarum*) at a concentration of $10^5$-$10^7$ CFU mL$^{-1}$, exhibiting in creased

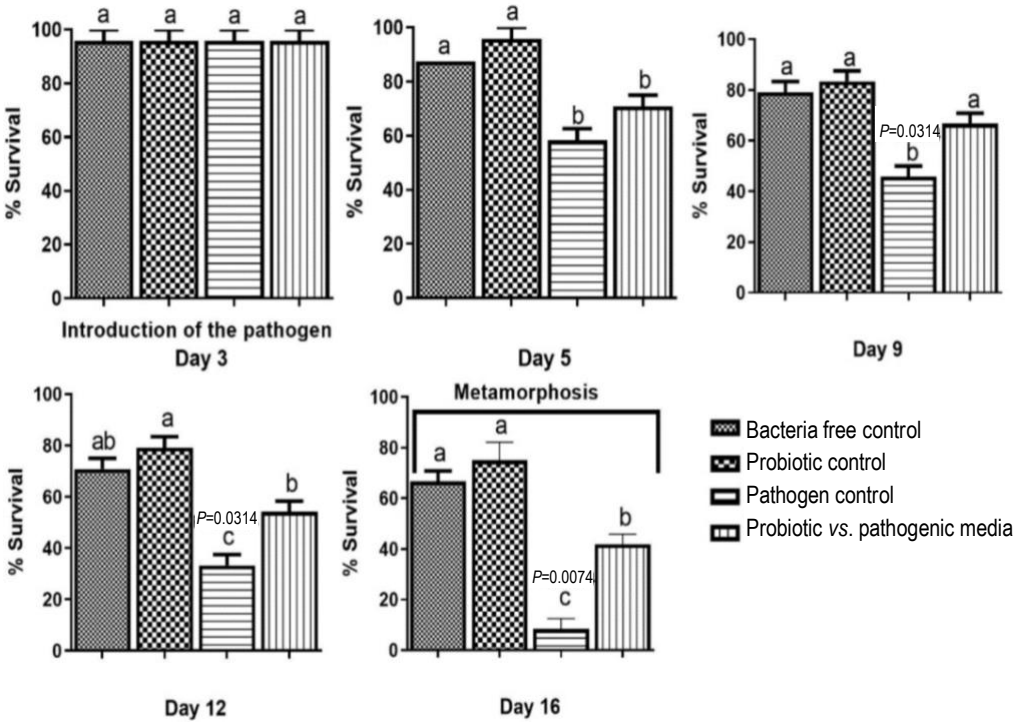


**Figure 2.** Survival percentage of oyster larvae fed with *Isochrysis galbana* and *Chaetoceros calcitrans* microalgae and stimulated with a dose of $3.14\times10^5$ CFU mL$^{-1}$ of the pathogen *Vibrio parahaemolyticus* and with a dose of $10^4$ CFU mL$^{-1}$ of the probiotic *Lactobacillus plantarum* 69Cr, as controls the larvae were stimulated only with probiotic, with pathogen or without bacteria.

**Table 2.** Summary of the highly significant difference multiple comparison test (GraphPad Software Inc. 2015) of the larva-probiotic-pathogen assay to determine the optimal doses of the probiotics *L. plantarum* 69Cr, *L. fermentum* 101Cc and *L. casei* 43 Cg. See Table 1 for low, medium, and high concentrations of microbial agent. *Highly significant *P*-value.

| Day | Significant comparisons by treatment | *P*-value |
|---|---|---|
| 9 | Medium dose ($10^4$ CFU mL$^{-1}$) probiotic 69Cr *L. plantarum* + *V. parahaemolyticus vs*. low dose ($10^4$ CFU mL$^{-1}$) probiotic 43Cg *L. casei* + *V. parahaemolyticus*. | <0.0001* |
| 9 | Medium dose ($10^4$ CFU mL$^{-1}$) probiotic 69Cr *L. plantarum* + *V. parahaemolyticus vs*. pathogen control ($3.14\times10$^5 CFU mL$^{-1}$) *V. parahaemolyticus.* | <0.0001* |
| 16 | Medium dose ($10^4$ CFU mL$^{-1}$) probiotic 69Cr *L. plantarum* + *V. parahaemolyticus vs*. low dose ($10^3$ CFU mL$^{-1}$) probiotic 43Cg *L. casei* + *V. parahaemolyticus.* | 0.0005* |
| 16 | Medium dose ($10^4$ CFU mL$^{-1}$) probiotic 69Cr *L. plantarum* + *V. parahaemolyticus vs*. pathogen control ($3.14\times10^5$ CFU mL$^{-1}$) + *V. parahaemolyticus.* | 0.0001* |
| 16 | Dose control ($10^4$ CFU mL$^{-1}$) probiotic 69Cr *L. plantarum vs*. pathogen control ($3.14\times10^5$ CFU mL$^{-1}$) + *V. parahaemolyticus.* | 0.0005* |

weight and length compared to those in control tanks. Supplementation of *Barbus gryprus* with various concentrations of *L. casei*, $5\times10^7$ CFU g$^{-1}$ for 30 days and $5\times10^8$ CFU g$^{-1}$ for 60 days, improved the growth and enzymatic activity of *B. gryprus* (Vand et al. 2014). In this research, $10^3$ and $10^5$ CFU mL$^{-1}$ of *L. casei* were used to improve the survival of *C. gigas* larvae, obtaining a survival rate of 46%.

In bivalve seed production laboratories, the continuous flow of water and other factors induce the adaptation of the environment's microbial community and the organisms. For organisms that have been exposed to a limited range of microorganisms during their development, it is not guaranteed that a single dose of the probiotic will result in long-term colonization (Verschuere et al. 2000). In this study, it was confirmed that the best probiotic was *L. plantarum* 69Cr, with an effective dose ($10^4$ CFU $mL^{-1}$). These results were observed in survival with and without the presence of the pathogen *V. parahaemolyticus*, thus demonstrating the reliability of the probiotic even with the microbiota of the larvae and the microalgae *I. galbana* and *C. calcitrans*.

## CONCLUSIONS

The *Lactobacillus* strains tested in this research showed an important beneficial interaction with the larvae of the Japanese oyster *C. gigas* and an extra benefit as protectors against the pathogenic agent *V. parahaemolyticus*. The biotechnological use of these probiotic bacteria to improve oyster seed production is a sustainable and reliable option to reduce losses in seed production laboratories. Furthermore, a valuable strategy for selecting strains with probiotic potential is through the effective dose, which, for the probiotic *L. plantarum* 69Cr, $10^4$ CFU $mL^{-1}$ was sufficient to protect the larvae against the pathogenic agent, thus avoiding the excessive use of antibiotics.

These bacteria are notable allies for the development of oyster farming in the study region because they were isolated from oysters and selected for their ability to generate a beneficial interaction with their hosts. In this study, we established the foundations for more in-depth studies with molecular and genetic tools, with a broader perspective on the probiotic bacteria-host and probiotic/pathogen-host interaction mechanisms, emphasizing interactions with the host's native microbiota.

### Credit author contribution

M. Savin-Amador: conceptualization, validation, methodology, formal analysis, writing-original draft; M. Rojas-Contreras: funding acquisition, project administration, supervision, review, and editing; R. Vázquez-Juárez: methodology, validation, supervision, review, and editing. All authors have read and accepted the published version of the manuscript.

### Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

## ACKNOWLEDGEMENTS

The authors thank CONAHCYT for financial assistance (Graduate scholarship 593764).

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*Received: May 22, 2024; Accepted: August 30, 2024*