Article

N-Acyl Homoserine Lactone-Degrading *Bacillus* sp. Improves the Survival of *Penaeus vannamei* Larvae Challenged with *Vibrio harveyi*

Reinaldo González [1], Héctor Cabrera [2], Yufeng Niu [3], Alessandra Roncarati [4,*], Adrian Toledo [1], Yulaine Corrales [1], Yanelis Quevedo [1], Livio Galosi [4], Peter Bossier [3] and Amilcar Arenal [5,*]

[1] Microbiology Laboratory, Department of Morphophysiology, University of Camagüey, Km 5 1/2, Camagüey 74650, Cuba; reinaldo.gonzalez@reduc.edu.cu (R.G.); adrian.toledo@redu.udu.cu (A.T.); yulainecorralesbarrios@gmail.com (Y.C.); yanelis.quevedo@reduc.edu.cu (Y.Q.)
[2] Yaguacam Shrimp Hatchery, Yaguanabo, Cumanayagua, Cienfuegos 57600, Cuba; hectorherreraloe@gmail.com
[3] Laboratory of Aquaculture & Artemia Reference Center, Faculty of Bioscience Engineering Blok F, Ghent University, Coupure Links 653, 9000 Gent, Belgium; yufeng.niu@ugent.be (Y.N.); peter.bossier@ugent.be (P.B.)
[4] School of Biosciences and Veterinary Medicine, University of Camerino, 62024 Matelica, Italy; livio.galosi@unicam.it
[5] Department of Infectious Diseases and Pathology, School of Veterinary Medicine, St. Nicholas University, Morne Daniel, Roseau 00109-8000, Dominica
* Correspondence: alessandra.roncarati@unicam.it (A.R.); amilcar.arenal@gmail.com (A.A.)

Abstract: This study aimed to isolate AHL-degrading bacteria from the intestine of *Penaeus vannamei* and evaluate their ability to control pathogenic *Vibrio harveyi* in *P. vannamei* larvae. Twenty-seven isolates were obtained from the digestive tract of healthy Pacific white shrimp juveniles (*P. vannamei*) after six cycles of pasteurization at 70 °C, but only three isolates (E_1LP_2, E_2LP_1, and E_2LP_2) could degrade AHL. The 16S sequence results gave a high identity (>95%) with *Bacillus* sp. The isolates exhibited quorum-quenching abilities by degrading AHLs, thereby disrupting Vibrio quorum sensing and virulence. In Zoea and Mysis, the challenged larvae plus the administration of E_1LP_2 resulted in the lowest survival compared to the other groups. Isolates degrading N-acyl homoserine lactone improved the survival of shrimp Zoea and Mysis larvae when challenged with pathogenic *V. harveyi*. This is the first report on the use of quorum-sensing disrupter bacteria in *P. vannamei* larval shrimp culture. Our findings suggest that these *Bacillus* spp. strains have potential as biocontrol agents for sustainable shrimp aquaculture, reducing the reliance on antibiotics while mitigating vibriosis outbreaks.

Keywords: probiotic; *Bacillus*; quorum sensing; diseases; aquaculture

Academic Editor: Beniamino T. Cenci-Goga

Received: 8 January 2025
Revised: 12 February 2025
Accepted: 17 February 2025
Published: 28 February 2025

Citation: González, R.; Cabrera, H.; Niu, Y.; Roncarati, A.; Toledo, A.; Corrales, Y.; Quevedo, Y.; Galosi, L.; Bossier, P.; Arenal, A. N-Acyl Homoserine Lactone-Degrading *Bacillus* sp. Improves the Survival of *Penaeus vannamei* Larvae Challenged with *Vibrio harveyi*. *Microbiol. Res.* **2025**, *16*, 56. https://doi.org/10.3390/microbiolres16030056

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Penaeid shrimp culture plays a crucial role in the economies of Asia and Latin America [1]. However, intensive farming practices often lead to stress in shrimp, making them more susceptible to viral and bacterial infections [2,3]. Vibriosis, caused by bacteria of the genus *Vibrio*, is the most prevalent bacterial disease and can cause mortality up to 100% in cultures [4–6]. The *Vibrio* species with the highest impact are *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio anguillarum* [7,8].

Traditionally, the only method for the management of undesirable bacterial populations was the application of chemotherapeutics. However, the improper use of these

chemicals causes bacterial resistance, in turn producing loss of control over certain infectious diseases. Additionally, the residual effects of these chemicals pose significant environmental risks [9].

Probiotics serve as an alternative approach to managing bacterial diseases in shrimp farming. Their ability to reduce mortality rates and increase yields has been well documented, highlighting their potential as a viable strategy for improving shrimp health and productivity [10–12].

Bacillus species are particularly promising probiotics because they produce spores, compete for nutrients, and inhibit the growth of pathogens [13]. In microbial systems, metabolic processes are regulated not only by enzymatic activity and gene expression but also by interactions with other microorganisms. Additionally, these interactions are often mediated by quorum sensing (QS), a bacterial communication mechanism that involves the detection of extracellular signaling molecules, termed autoinducers, enabling bacteria to monitor and respond to population density in their vicinity [14].

The strain *Cobetia* sp. W1B, displaying quorum quenching (QQ) activity, was iso-lated and characterized for its ability to degrade N-acylhomoserine lactones (AHLs), a group of QS signaling molecules [15]. Additionally, prior researches have demonstrated that strain W1B effectively reduces mortality rates in juvenile shrimp infected with *Vibrio harveyi* [16].

Some species of bacteria from the genus *Bacillus* produce lactonase enzymes, to defend themselves and survive in the environment [17]. These enzymes break down acyl homoserine lactones (AHLs), which are chemical signals that regulate the activation of virulence genes via quorum sensing in Gram-negative bacteria like *Vibrio* sp. [18]. Moreover, some probiotics were able to modulated the expression profiles of immune-related genes in shrimp infected with *Vibrio harveyi*. Additionally, it significantly downregulated the relative expression of apoptosis-associated genes in *V. harveyi* infected shrimp [19]. The impact of this strategy on the saprophyte or harmless microbiota is low and rarely triggers bacterial resistance mechanisms [20]. The degradation of AHLs plays a critical role in protecting aquatic organisms from pathogenic infections, positioning AHL-degrading Bacillus species as promising biocontrol candidates for sustainable aquaculture practices [21].

Recent studies have identified bioactive substances derived from animals, plants, and microbes that can inhibit quorum sensing, key mechanism in the pathogenesis of vibriosis. However, probiotics secreting quorum sensing-inhibiting compounds remain underexplored in Penaeus species. The present study aimed to isolate AHL-degrading bacteria from the intestine of *P. vannamei* and evaluate their control of pathogenic *Vibrio harveyi* in *P. vannamei* larval cultures.

2. Materials and Methods

2.1. Bacterial Strains

A mini-Tn5 mutant of *Chromobacterium violaceum* strain CV026, derived from *C. violaceum* strain ATCC31532 [22], was used as a reporter for the AHL. This strain lacks the ability to produce AHL but synthesizes violacein, a purple pigment. This enables it to detect and respond to a range of AHL molecules with acyl side chains. For the degradation assays, the soil bacterium *P. fluorescens* transformant strains P3/pME6000 and P3/pME6863 served as the positive and negative controls, respectively. The *aiiA* gene, carried on plasmid pME6863, originates from *Bacillus* sp. A24 encodes lactonase, an enzyme that degrades AHL molecules [23]. *Vibrio harveyi* strain ATCC 14126 was donated by the Center for Genetic Engineering and Biotechnology, Camaguey, Cuba.

2.2. Preparation of Microbial Communities

At the Yaguanabo shrimp hatchery, healthy juvenile Pacific white shrimp (*P. vannamei*) were cultured on formulated feeds, and their digestive tracts were sampled for microbial communities (MCs). After dissection, the digestive systems of the shrimp were extracted and homogenized using a stomacher blender (Seward Ltd, Worthing, West Sussex, UK). The resulting suspensions were centrifuged at 1600× *g* for 5 min to ensure thorough homogenization, and the supernatant was preserved at −80 °C in a solution containing 20% glycerol. These microbial communities (MCs) provided the seed material for isolating AHL-degrading strains. A minimal culture medium consisting of 9 g/L NaCl and 5 mg/L of commercially available N-hexanoyl-DL-homoserine lactone was used for the isolation process (Fluka, Buchs, Switzerland), with 200 microliters of the MC suspension. The cultures were incubated at 28 °C with shaking at 120 rpm.

The isolation process was conducted over six consecutive 48 h cycles. After each cycle, 200 µL of the sample was transferred into a new flask containing 20 mL of fresh medium. Cell densities were measured at the beginning and end of each cycle using OD_{550} nm readings and by plating the samples onto Marine Agar (Becton Dickinson and Co., Le Pont De Claix, France). Isolates derived from three different shrimp specimens were obtained after the sixth cycle and preserved at −80 °C in 20% glycerol for subsequent characterization.

2.3. Culture Media

Marine Broth (Difco, Detroit, MI, USA) was utilized for cultivating the unknown bacterial isolates as well as *Vibrio harveyi* strains ATCC 14126. Strain P3/pME6863 was cultured in Luria–Bertani (LB) medium, consisting of 1% (*w/v*) tryptone (Becton Dickinson and Co., Le Pont De Claix, France), 0.5% (*w/v*) yeast extract (Sigma-Aldrich, Saint Louis, MO, USA), and 0.4% (*w/v*) NaCl. Strain CV026 was maintained in LB medium supplemented with 20 mg L^{-1} kanamycin to preserve the plasmid responsible for violacein production.

2.4. Isolation of Bacillus Strains from the Cultures

Enrichment cultures for AHL degradation were inoculated into nine-salt solution (NSS) supplemented with 5 mg/L *N*-hexanoyl-*L*-homoserine lactone (HHL) as the sole carbon and nitrogen source, pH 6.5 [22]. The suspensions were incubated at 28 °C for 48 h at 120 rpm. After incubation, the cultures were pasteurized at 70 °C for 30 min. This cycle was repeated six times, after which the pasteurized cultures were plated on Luria–Bertani (LB) agar containing 20 g/L synthetic sea salt (LB 20). Individual colonies were then isolated and assessed for AHL degradation activity.

2.5. 16S rRNA Gene Sequencing

Polymerase chain reaction (PCR) amplification targeting a 1500 base pair fragment of the 16S ribosomal RNA (rRNA) gene of the isolates was performed as described by Boon et al. [24], using the GM3f and GM4r primer pair (Biolegio, Nijmegen, The Netherlands). Amplification was carried out under the following cycling conditions: an initial denaturation at 95 °C for 5 min, followed by 32 cycles of denaturation at 94 °C for 1 min, annealing at 42 °C for 1 min, extension at 72 °C for 3 min, and a final extension at 72 °C for 10 min. The PCR products were subsequently sequenced at IIT Biotech (Bielefeld, Germany). Sequence homology analysis was performed using the BLASTn algorithm provided by the National Center for Biotechnology Information (NCBI). Additional sequences were retrieved from the NCBI GenBank database (National Center for Biotechnology Information, USA) using the Clustal W program [23] within the MEGA12 software (Version 12.0) package [24]. Evolutionary distances were calculated using the Kimura two-parameter model [25].

Phylogenetic trees were constructed using three methods implemented in the MEGA 12 package: Neighbor-Joining [26]. The robustness of the trees was assessed through bootstrap resampling analysis, based on 1000 random replicates.

2.6. Detection of N-Hexanoyl-L-Homoserine Lactone (HHL)

A plate diffusion assay was employed for HHL detection, utilizing *Chromobacterium violaceum* CV026 as the reporter strain [27]. This strain does not synthesize AHLs but produces the purple pigment violacein when exposed to exogenous AHLs [19]. CV026 was cultured in Luria–Bertani (LB, pH 6.5) medium containing 20 mg/L kanamycin, and grown to an optical density of approximately two at 550 nm before being spread onto LB plates. Subsequently, 10 µL of the HHL (1000 mg/L) was applied to the plate center and then incubated at 28 °C for 48 h.

2.7. Penaeus vannamei Larval Challenge Tests

The experiments were carried out at the Yaguacam Hatchery, Cienfuegos, Cuba. Larvae derived from a single female breeder were employed for each trial. Twenty-four hours post-hatch, the larvae were randomly sampled and acclimated to the experimental tanks at 50 larvae per liter. Two challenge tests were performed, one at Protozoea-I (PZ-1) stage and the other at Mysis-I (M-I). Each treatment was performed in five tanks.

The experiments were conducted in cylinder-conical rearing tanks, each containing 1 L of seawater with a salinity of 30 ‰, following a completely randomized design with five tanks per treatment. A light intensity of approximately 900–1000 lux was maintained at the water surface for 12 h per day. The rearing tanks were gently aerated to maintain dissolved oxygen levels above 5 mg L^{-1} in the rearing water.

The larvae were treated with the isolated bacteria (10^7 cells mL^{-1}) for 8 h. Afterward, *Vibrio harveyi* was introduced to the tubes (10^7 cells mL^{-1}), except for the control, where no pathogen was added. Larval survival was evaluated 48 h post-pathogen exposure.

The larval diet consisted of diatoms [*Chaetoceros gracilis*, Protozoea I-II (PZ-I to PZ-II): 60,000 cells mL^{-1}; Protozoea III (PZ-III) to Postlarvae 2 (PL-2): 50,000 cells mL^{-1}], flagellates [*Tetraselmis suecica*, PZ-I to PZ-II: 2000 cells mL^{-1}; PZ-III to Mysis III (M-III)-PL 7: 3000 cells mL^{-1}], and fresh *Artemia* nauplii (Aquatic Eco-Systems Inc., Apopka, FL, USA) [PZ-III: 0.5 nauplii mL^{-1}; M-I to M-II: 1 nauplii mL^{-1}; M-III: 1.5 nauplii mL^{-1} (four times daily)]. The rearing conditions were maintained at 30 ± 2 °C, a salinity of 30 g/L, and pH 8.5 ± 0.5, with continuous aeration.

2.8. Statistical Analysis

Survival data were arcsine-transformed to meet the assumptions of normality and homoscedasticity. The transformed data were subsequently analyzed using a one-way analysis of variance, followed by Tukey's multiple-range test for post hoc comparisons. When normality was not met, a non-parametric Kruskal–Wallis test followed by Dunn's post hoc range test was used. Statistical analysis was conducted using GraphPad Prism 7.0 to assess significant differences between treatments, with a significance level set at $p < 0.05$.

3. Results

3.1. Isolation and Identification of Bacillus Strains

Twenty-seven colonies were obtained in the first pasteurization. The number of colonies decreased to eight after the six cycles of pasteurization. The isolates were Gram-positive bacilli, characterized by the formation of central spores without swelling of the sporangium, and were facultative anaerobes. Three distinct morphological colonies were observed. Figure 1 shows the phylogenetic tree based on the 16S rRNA gene sequence of

the isolates (E_1LP_2, E_2LP_1, or E_2LP_2), constructed using the Neighbor-Joining method. The 16S rDNA sequence analysis showed that the three isolates are localized in the same clade as *Bacillus cereus*. The relationships among the isolates exhibited bootstrap support values over the threshold of 70%.

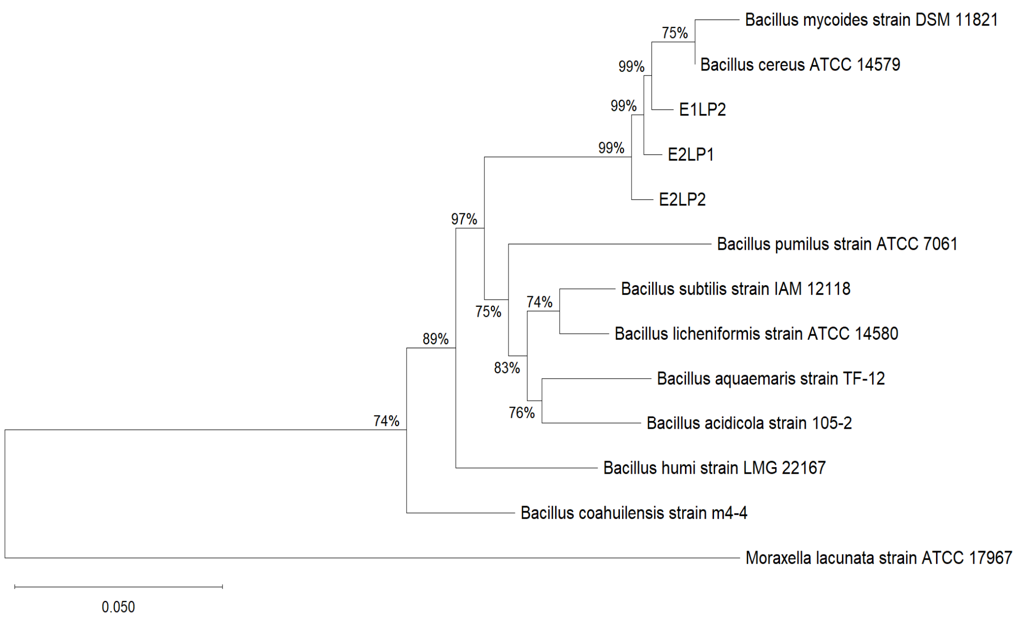

Figure 1. Phylogenetic tree of the isolates based on 16S rRNA gene sequences. The phylogenetic tree was constructed using the Neighbor-Joining method. The strain *Moraxella lacunata* ATCC 17967^T (NR_114416.1) was used as the outgroup. Branch nodes display bootstrap values (expressed as percentages from 1000 replicates) greater than 50%. The scale bar represents five substitutions per 100 nucleotides.

3.2. Acyl Homoserine Lactone (AHL) Inactivation Assay

The isolates E_1LP_2, $E_2LP_{1,}$ and E_2LP_2 failed to induce pigment production in the reporter strain, suggesting the complete degradation of HHL (Figure 2). These isolates were able to degrade HHL to levels below 0.1 mg L^{-1} after 24 h, which correspond to the detection limit of the reporter strain CV026. In contrast, the other isolates exhibited a purple zone with a diameter comparable to that of the non-HHL-degrading control (36 $\pm$ 2 mm), suggesting minimal HHL inactivation.

3.3. Bacillus sp. Effect on the Survival of Zoea and Mysis Challenged with Vibrio harveyi

All the AHL degrader isolates significantly improved the survival of challenged Zoea and Mysis larval stages (Figures 3 and 4). In Zoea, the challenged larvae treated with E_1LP_2 resulted in the lowest of survival (70.2%) among all tested groups. Nevertheless, it was similar to the unchallenged larvae treated with the isolate (66.6%) and to the unchallenged control (77.4%). In Mysis, the challenged group plus the administration of E_1LP_2 culture again provided the lowest survival rate (70.0%) compared to the rest of the combinations. However, this survival rate was similar to the unchallenged larvae treated with E_2LP_1 (79.0%) and to the unchallenged control larvae (82.0%).

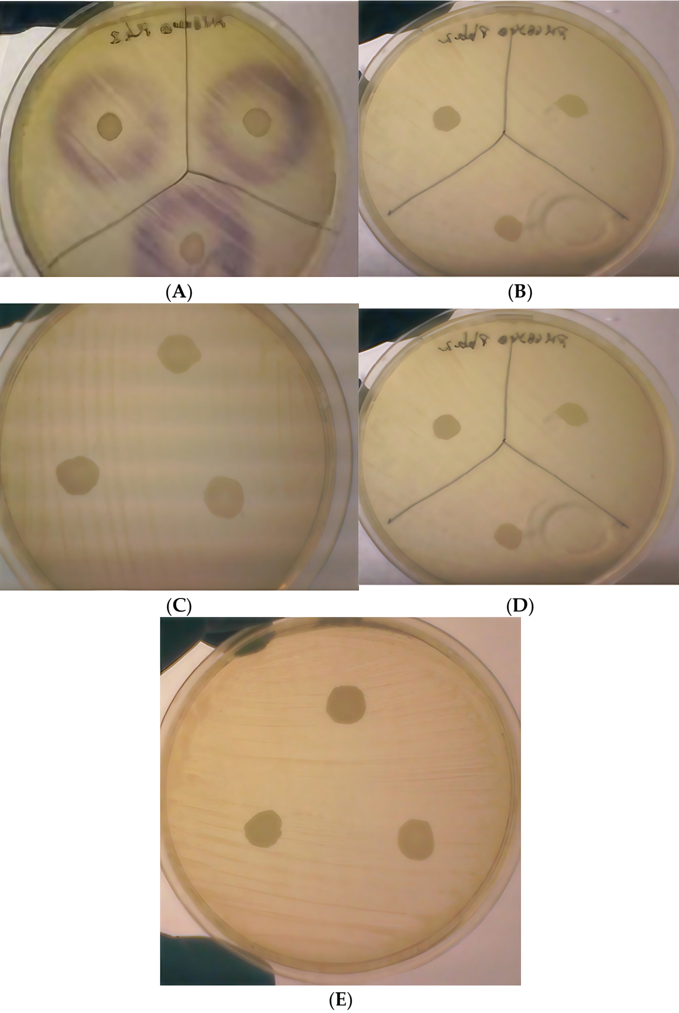

Figure 2. Inhibition of violacein production. The experiment was carried out on Luria–Bertani agar plates overlaid with a lawn of HSL-induced CV026, which was incubated overnight. (**A**) Positive control (P3/pME6863); (**B**) negative control (P3/pME6000); (**C**) E_1LP_2; (**D**) E_2LP_1; (**E**) E_2LP_2.

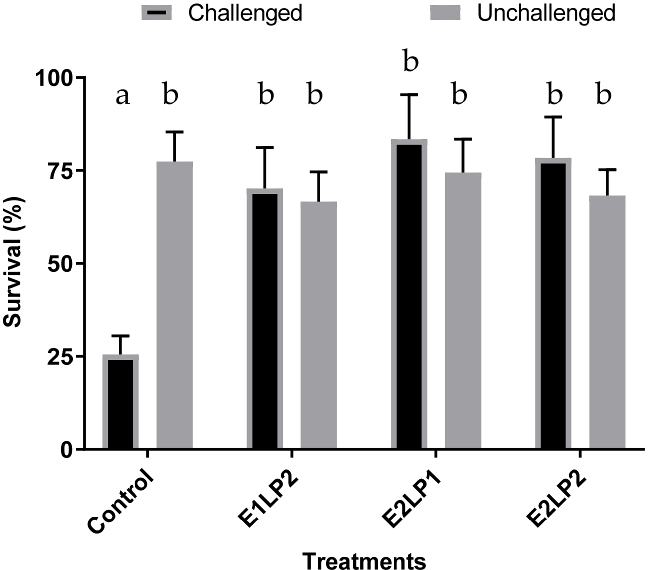

Figure 3. Survival of Zoea I larvae 36 h post-challenge with *V. harveyi*. The larvae were treated with either E_1LP_2, E_2LP_1, or E_2LP_2 at 10^7 cells ml^{-1} before the challenge (n = 5). Similar letters indicate no significant difference ($p > 0.01$).

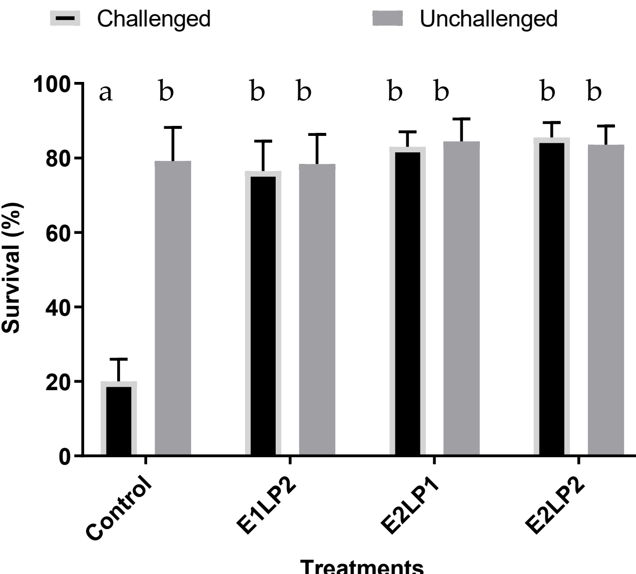

Figure 4. Survival of Mysis I larvae 36 h post-challenge with *V. harveyi*. The larvae were treated with either E_1LP_2, E_2LP_1, or E_2LP_2 at 10^7 cells ml^{-1} before the challenge (n = 5). Similar letters indicate no significant difference ($p < 0.01$).

4. Discussion

Pathogenic Vibrio species affecting aquaculture organisms possess quorum sensing systems. The link between quorum sensing and virulence factor expression has been demonstrated. Vibrio species use AHL as signal molecules in the regulation of quorum sensing [28]. AHL-degrading bacteria have been identified in a variety of aquatic habitats, including the open water, bottom sediments, seaweed surfaces, and the gastrointestinal systems of healthy aquatic animals [29]. These bacteria can be exploited as a luminescent vibrio control in aquaculture and serve as potential candidates for probiotic selections.

In the present work, three in vitro AHL degrading isolates (E_1LP_2, E_2LP_1, and E_2LP_2) were obtained from *P. vannamei* intestines. The intestinal microbiota of aquatic animals can harbor AHL-degrading bacteria, which may provide protection against bacterial infections in aquaculture [30]. The isolation of probiotics that inhabit the same ecosystem as the host is recommended [31]. Probiotics from the natural flora enhance host immunity and physiological functions [32].

The 16S sequences indicated that E_1LP_2, E_2LP_1, and E_2LP_2 are *Bacillus* spp. As a DNA-sequence-based identification method for Bacillus, the 16S rRNA gene is regarded as a suitable tool for the identification of marine Bacillus species [33]. However, the presence of highly conserved regions within the 16S rDNA gene hinders the discrimination between closely related or similar species, as observed in members of the Bacillus cereus group and certain members of the Bacillus subtilis group [34,35].

Several AHL-degrading bacteria reported for aquaculture are *Bacillus* sp. Lactonases are the enzymes that inactivate the AHLs by hydrolyzing the lactone ring. Lactonases from different *Bacillus* sp. have been cloned and purified, and their activities have been characterized [3]. Additionally, other bacterial genera have the ability to produce lactonase, and their probiotic applications in aquaculture systems have been proposed [36]. N-acyl homoserine lactone-degrading microbial enrichments from *P. vannamei* shrimp gut were isolated and assessed for their probiotic properties in *Brachionus plicatilis* cultures.

Herein, the three isolates E_1LP_2, E_2LP_1, and E_2LP_2 protected Zoea and Mysis from the *V. harveyi* challenge. The isolates exhibited the ability to completely degrade *N*-hexanoyl-L-homoserine lactone, this degradation capability is consistent with previous studies highlighting the role of AHL-lactonase activity in disrupting quorum sensing-mediated

virulence. Previous studies have identified three distinct quorum sensing systems in *Vibrio harveyi*, each regulated by specific autoinducer molecules: HAI-1, AI-2, and CAI-1. Of these, HAI-1 is classified as an *N*-acyl homoserine lactone (AHL), while AI-2 and CAI-1 are non-AHL signaling molecules [37].

Lactonase enzyme from *Vibrio anguillarum* effectively degraded *N*-acyl homoserine lactones, this degradation did not significantly impact the growth of the pathogen or the production of its virulence factors. This suggests that the tested strain may employ additional regulatory mechanisms independent of AHL-mediated quorum sensing to control virulence and growth. In contrast, it was demonstrated that AHL-lactonase produced by *Bacillus licheniformis* DAHB1 disrupts *Vibrio* biofilm formation in vitro. This disruption translates to reduced *Vibrio* colonization in the shrimp gut and a significant decrease in shrimp mortality [38]. The recombinant AiiA lactonase derived from *Bacillus thuringiensis* BF1 exhibits a strong inhibitory effect on the bioluminescence of *Vibrio harveyi* VIB391, resulting in an 85% reduction in intensity.

Quorum sensing disruption is a new approach that has been studied to inactivate the in vitro and in vivo virulence of *V. harveyi*. In vivo challenge tests revealed that cinnamaldehyde at 1 μM and thiophenone at 10 μM, when administered to the culture water, provided significant protection for giant freshwater prawn (*Macrobrachium rosenbergii*) larvae against *Vibrio harveyi* infection [39]. *Vibrio harveyi* luminescence and toxin production were both inhibited by an N-hydroxy butanoyl-l-homoserine lactone antagonist from the marine algae *Delisea pulchra* at concentrations that had no impact on *Vibrio* growth [40]. *B. subtilis* STC (1×10^6 cells/mL) treatment improved the survival rate of *V. harveyi*-infected *P. monodon* larvae to 65.56% [41]. Furthermore, the bacterial degradation of AHLs improves water quality in freshwater prawn rearing through direct addition to the rearing water or the enrichment of *Artemia* nauplii, significantly enhancing larval survival and quality.

Recent studies have shown that *Bacillus velezensis* D-18 possesses significant potential for preventing *Vibrio anguillarum* infections in aquaculture due to its quorum-quenching capabilities, which involve interfering with bacterial communication and inhibiting biofilm formation [42]. Bacteria capable of degrading quorum sensing molecules and accumulating poly-β-hydroxybutyrate demonstrated protective effects against *Vibrio harveyi* as evidenced by experiments using gnotobiotic *Artemia* [43].

Enrichment cultures of AHL-degrading bacteria were used to mitigate the proliferation of potential opportunistic and pathogenic bacteria linked to *Macrobrachium* larvae, whose virulence is potentially regulated by the AHL-dependent quorum sensing system [44]. These enrichment cultures were bio-encapsulated in *Artemia*, and subsequent gut analysis confirmed successful colonization [45]. These findings align with the current results, further confirming that *Bacillus* species have the ability to disrupt the virulence mechanisms of *Vibrio* species.

5. Conclusions

The present study showed that *Bacillus* isolates E_1LP_2, $E_2LP_{1,}$ and E_2LP_2, which are N-acyl homoserin lactone degraders, improved the survival of Zoea and Mysis shrimp larvae infected with pathogenic *V. harveyi*. E_1LP_2, $E_2LP_{1,}$ and E_2LP_2 isolates could be used for the control of luminescent vibriosis in *P. vannamei* larvae. Our strategy is an eco-friendly alternative to traditional antibiotics for managing bacterial diseases in aquaculture.

Author Contributions: Conceptualization and design of experiments, sample collection, sample processing, analysis of data, drafting of manuscript, and approval of final submitted version, R.G. and Y.N.; data collection, critical revision of manuscript, and approval of final submitted version, H.C. and Y.Q.; analysis of data, critical revision of manuscript, and approval of final submitted version, Y.C., A.R., A.T. and L.G.; conceptualization and design of experiments, analysis of data, drafting and

critical revision of manuscript, and approval of final submitted version, P.B. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: Project: Improving sustainably shrimp larviculture in Cuba. This research has been supported by the Belgian Development Cooperation through VLIR-UOS. VLIR-UOS supports partnerships between universities and university colleges in Flanders (Belgium) and the South looking for innovative responses to global and local challenges. Visit www.vliruos.be for more information.

Institutional Review Board Statement: All protocols were approved by the Animal Care and Ethics Committee of Camaguey University, Cuba (protocol No. 2013002). Following the recommendations of the Cuban Legislation No 180/07 (Gaceta Official 084, 19 December 2007), this field study on commercial farms did not require ethical approval.

Informed Consent Statement: This study did not involve humans.

Data Availability Statement: The nucleotide sequences of the isolates were deposited in the Genbank database (http://www.ncbi.nlm.nih.gov/Genbank, accessed on 15 February 2025) under the following accession numbers: E_2LP_2: PV093882; E_2LP_1: PV093883; and E_1LP_2: PV093884.

Acknowledgments: The authors would like to thank the researchers of the Center for Genetic Engineering and Biotechnology, Camaguey, Cuba, for the donation of the strains.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AHL	Acyl-homoserine lactone
MCs	Microbial communities
NSS	Nine-salt solution
HHL	Hexanoyl-L-homoserine lactone

References

1. Deachamag, P.; Intaraphad, U.; Phongdara, A.; Chotigeat, W. Expression of a phagocytosis activating protein (PAP) gene in immunized black tiger shrimp. *Aquaculture* **2006**, *255*, 165–172. [CrossRef]
2. Kurath, G. Biotechnology and DNA vaccines for aquatic animals. *Rev. Sci. Tech.-Off. Int. Épizooties* **2008**, *27*, 175. [CrossRef]
3. Zhang, G.; Zhang, F.; Ding, G.; Li, J.; Guo, X.; Zhu, J.; Zhou, L.; Cai, S.; Liu, X.; Luo, Y.; et al. Acyl homoserine lactone-based quorum sensing in a methanogenic archaeon. *ISME J.* **2012**, *6*, 1336–1344. [CrossRef] [PubMed]
4. Saulnier, D.; Haffner, P.; Goarant, C.; Levy, P.; Ansquer, D. Experimental infection models for shrimp vibriosis studies: A review. *Aquaculture* **2000**, *191*, 133–144. [CrossRef]
5. Pruzzo, C.; Gallo, G.; Canesi, L. Persistence of vibrios in marine bivalves: The role of interactions with haemolymph components. *Environ. Microbiol.* **2005**, *7*, 761–772. [CrossRef]
6. Beaz-Hidalgo, R.; Balboa, S.; Romalde, J.L.; Figueras, M.J. Diversity and pathogenecity of *Vibrio* species in cultured bivalve molluscs. *Environ. Microbiol. Rep.* **2010**, *2*, 34–43. [CrossRef]
7. Mohney, L.L.; Lightner, D.V.; Bell, T.A. An Epizootic of Vibriosis in Ecuadorian Pond-Reared *Penaeus vannamei* Boone (Crustacea: Decapoda). *J. World Aquac. Soc.* **1994**, *25*, 116–125. [CrossRef]
8. Tyagi, A.; Khushiramani, R.; Karunasagar, I.; Karunasagar, I. Antivibrio activity of recombinant lysozyme expressed from black tiger shrimp, *Penaeus monodon*. *Aquaculture* **2007**, *272*, 246–253. [CrossRef]
9. Nayak, S.K.; Mukherjee, S.C. Screening of gastrointestinal bacteria of Indian major carps for a candidate probiotic species for aquaculture practices. *Aquac. Res.* **2011**, *42*, 1034–1041. [CrossRef]
10. Gomez-Gil, B.; Roque, A.; Turnbull, J.F. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* **2000**, *191*, 259–270. [CrossRef]
11. Chythanya, R.; Karunasagar, I.; Karunasagar, I. Inhibition of shrimp pathogenic vibrios by a marine Pseudomonas I-2 strain. *Aquaculture* **2002**, *208*, 1–10. [CrossRef]
12. Wang, Y.B.; XU, Z.R.; Xia, M.S. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. *Fish. Sci.* **2005**, *71*, 1036–1041. [CrossRef]

13. Decamp, O.; Moriarty, D.J.; Lavens, P. Probiotics for shrimp larviculture: Review of field data from Asia and Latin America. *Aquac. Res.* **2008**, *39*, 334–338. [CrossRef]
14. Mihailović, D.T.; Budinčević, M.; Balaž, I.; Mihailović, A. Stability of intercellular exchange of biochemical substances affected by variability of environmental parameters. *Mod. Phys. Lett. B* **2011**, *25*, 2407–2417. [CrossRef]
15. Yu, P.; Ye, H.B.; Shan, H.W.; Ma, S.; Wang, T. Screening, safety evaluation and fermentation conditions optimization of quorum quenching bacteria from *Litopenaeus vannamei* culture system. *Progr. Fish. Sci.* **2020**, 1–11.
16. Yu, P.; Wang, T.; Ye, H.B.; Shan, H.W.; Ma, S. Isolation and identification of pathogenic Vibrio spp. retrieved from diseased *Litopenaeus vannamei* and beneficial role of some functional probiotic bacteria for control. *Aquac. Int.* **2020**, *6*, 1403–1420. [CrossRef]
17. Yu, P.; Ye, H.; Ma, S.; Shan, H. Study of quorum quenching bacteria *Cobetia* sp. reducing the mortality of *Penaeus vannamei* infected with *Vibrio harveyi*: In vitro and in vivo tests. *Aquaculture* **2021**, *534*, 736244. [CrossRef]
18. Natrah, F.M.I.; Kenmegne, M.M.; Wiyoto, W.; Sorgeloos, P.; Bossier, P.; Defoirdt, T. Effects of micro-algae commonly used in aquaculture on acyl-homoserine lactone quorum sensing. *Aquaculture* **2011**, *317*, 53–57. [CrossRef]
19. Fuqua, C.; Parsek, M.R.; Greenberg, E.P. Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* **2001**, *35*, 439–468. [CrossRef]
20. Yousefian, M.; Amiri, M.S. A review of the use of prebiotic in aquaculture for fish and shrimp. *Afr. J. Biotechnol.* **2009**, *8*, 7313–7318.
21. Defoirdt, T.; Thanh, L.D.; Van Delsen, B.; De Schryver, P.; Sorgeloos, P.; Boon, N.; Bossier, P. N-acylhomoserine lactone-degrading Bacillus strains isolated from aquaculture animals. *Aquaculture* **2011**, *311*, 258–260. [CrossRef]
22. McClean, K.H.; Winson, M.K.; Fish, L.; Taylor, A.; Chhabra, S.R.; Camara, M.; Daykin, M.; Lamb, J.H.; Swift, S.; Bycroft, B.W.; et al. Quorum sensing and *Chromobacterium violaceum*: Exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology* **1997**, *143*, 3703–3711. [CrossRef]
23. Molina, L.; Constantinescu, F.; Michel, L.; Reimmann, C.; Duffy, B.; Défago, G. Degradation of pathogen quorum-sensing molecules by soil bacteria: A preventive and curative biological control mechanism. *FEMS Microbiol. Ecol.* **2003**, *45*, 71–81. [CrossRef] [PubMed]
24. Boon, N.; De Windt, W.; Verstraete, W.; Top, E.M. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol. Ecol.* **2002**, *39*, 101–112. [CrossRef] [PubMed]
25. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef] [PubMed]
26. Kumar, S.; Stecher, G.; Suleski, M.; Sanderford, M.; Sharma, S.; Tamura, K. MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Mol. Biol. Evol.* **2024**, *41*, msae263. [CrossRef] [PubMed]
27. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef]
28. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406425.
29. Tinh, N.T.N.; Gunasekara, R.A.; Boon, N.; Dierckens, K.; Sorgeloos, P.; Bossier, P. N-acyl homoserine lactone-degrading microbial enrichment cultures isolated from *Penaeus vannamei* shrimp gut and their probiotic properties in *Brachionus plicatilis* cultures. *FEMS Microbiol. Ecol.* **2007**, *62*, 45–53. [CrossRef]
30. Tang, K.H.; Zhang, X.H. Quorum quenching agents: Resources for antivirulence therapy. *Mar. Drugs* **2014**, *12*, 3245–3282. [CrossRef]
31. Dang, W.; Hu, Y.H.M.; Zhang, L. Sun Identification and molecular analysis of a stress-inducible Hsp70 from *Sciaenops ocellatus*. *Fish Shellfish Immunol.* **2010**, *29*, 600–607. [CrossRef]
32. Gatesoupe, F.J. Probiotic and formaldehyde treatments of Artemia nauplii as food for larval pollack, *Pollachius pollachius*. *Aquaculture* **2002**, *212*, 347–360. [CrossRef]
33. Nikoskelainen, S.; Salminen, S.; Bylund, G.; Ouwehand, A.C. Characterization of the properties of human-and dairy-derived probiotics for prevention of infectious diseases in fish. *Appl. Environ. Microbiol.* **2001**, *67*, 2430–2435. [CrossRef] [PubMed]
34. Blackwood, K.S.; Turenne, C.Y.; Harmsen, D.; Kabani, A.M. Reassessment of sequence-based targets for identification of Bacillus species. *J. Clin. Microbiol.* **2004**, *42*, 1626–1630. [CrossRef] [PubMed]
35. Hakovirta, J.R.; Preziuso, S.; Hodge, D.; Pillai, S.P.; Weigel, L.M. Identification and analysis of informative single nucleotide polymorphisms in 16S rRNA gene sequences of the Bacillus cereus group. *J. Clin. Microbiol.* **2016**, *54*, 2749–2756. [CrossRef]
36. Liu, B. Uncertain Reliability Analysis. In *Uncertainty Theory. Studies in Computational Intelligence*; Springer: Berlin/Heidelberg, Germany; Volume 300.
37. Vinoj, G.; Vaseeharan, B.; Thomas, S.; Spiers, A.J.; Shanthi, S. Quorum-quenching activity of the AHL-lactonase from Bacillus licheniformis DAHB1 inhibits Vibrio biofilm formation in vitro and reduces shrimp intestinal colonisation and mortality. *Mar. Biotechnol.* **2014**, *16*, 707–715. [CrossRef]

38. Taga, M.E.; Bassler, B.L. Chemical communication among bacteria. *PNAS* **2003**, *100*, 14549–14554. [CrossRef]
39. Pande, G.S.J.; Scheie, A.A.; Benneche, T.; Wille, M.; Sorgeloos, P.; Bossier, P.; Defoirdt, T. Quorum sensing-disrupting compounds protect larvae of the giant freshwater prawn *Macrobrachium rosenbergii* from *Vibrio harveyi* infection. *Aquaculture* **2013**, *406*, 121–124. [CrossRef]
40. Manefield, M.; Harris, L.; Rice, S.A.; de Nys, R.; Kjelleberg, S. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Appl. Environ. Microbiol.* **2000**, *66*, 2079–2084. [CrossRef]
41. Yuniarti, A.; Maftuch, M.; Soemarno, S.; Aulanni'am, A. In vitro and in vivo study of acyl homoserine lactone degrading Bacillus against *Vibrio harveyi*. *Int. J. Biosci.* **2015**, *6*, 338–348.
42. Monzón-Atienza, L.; Bravo, J.; Torrecillas, S.; Gómez-Mercader, A.; Montero, D.; Ramos-Vivas, J.; Galindo-Villegas, J.; Acosta, F. An In-Depth Study on the Inhibition of Quorum Sensing by *Bacillus velezensis* D-18: Its Significant Impact on Vibrio Biofilm Formation in Aquaculture. *Microorganisms* **2024**, *12*, 890. [CrossRef] [PubMed]
43. Van Cam, D.T.; Van Hao, N.; Dierckens, K.; Defoirdt, T.; Boon, N.; Sorgeloos, P.; Bossier, P. Novel approach of using homoserine lactone-degrading and poly-β-hydroxybutyrate-accumulating bacteria to protect Artemia from the pathogenic effects of *Vibrio harveyi*. *Aquaculture* **2009**, *291*, 23–30. [CrossRef]
44. Juhas, M.; Eberl, L.; Tummler, B. Quorum sensing: The power of cooperation in the world of Pseudomonas. *Environ. Microbiol.* **2005**, *7*, 459–471. [CrossRef] [PubMed]
45. Van Cam, D.T.; Nhan, D.T.; Ceuppens, S.; Van Hao, N.; Dierckens, K.; Wille, M.; Sorgeloos, P.; Bossier, P. Effect of N-acyl homoserine lactone-degrading enrichment cultures on *Macrobrachium rosenbergii* larviculture. *Aquaculture* **2009**, *294*, 5–13.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.