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SHORT PAPER

Salinicoccus hispanicus (OL638305), a Potential Probiotic Isolated from Saline Tilapia Greenwater, Exhibits *Vibrio parahaemolyticus* Killing Activity and Prevents Acute Hepatopancreatic Necrosis Disease in *Penaeus vannamei*

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Abstract

The present study investigates the probiotic potential and inhibitory activity of *Salinicoccus hispanicus*, isolated from saline tilapia culture water, against *Vibrio parahaemolyticus*. Results showed that the isolate is not pathogenic to *Penaeus vannamei* when administered orally at a dose of 10^6 cfu g^{-1} diet. Dietary supplementation for 45 days did not affect growth, reduced the gut content of *V. parahaemolyticus* to about five-folds and protected the shrimp against *V. parahaemolyticus* infection following a pathogen challenge test. Supplementation of *S. hispanicus* at 10^6 cfu g^{-1} diet can inhibit *V. parahaemolyticus* gut colonization and protects *P. vannamei* against this pathogen.

Introduction

Since the first reported incidence of shrimp mass mortalities in Southern China in 2010, Early Mortality Syndrome (EMS) disease, technically known as Acute Hepatopancreatic Necrosis Disease, has been associated with serious economic losses in shrimp farming activities across the Asia-Pacific region particularly in China, Vietnam, Malaysia and Thailand (Zorriehzahra and Banaederakhshan, 2015). Until the present, this disease is considered a significant concern on the sustainability and economic viability of penaeid shrimp aquaculture (Shinn et al., 2018). The disease is caused by a novel strain of *Vibrio parahaemolyticus* that harbours insect neurotoxin genes and is highly pathogenic to both the *Penaeus vannamei* and the P*enaeus monodon*. The disease manifests between 20 to 30 days after stocking wherein shrimp becomes anorexic and lethargic leading to high mortality rates (Lightner et al., 2012). To address this issue, techniques, previously proven effective against the control of Luminous Vibriosis disease, such as the application of immunostimulants (Mameloco and Traifalgar, 2020) and probiotics (Barcenal et al., 2015; Mahdhi et al., 2011; Verschuere et al., 2000) have been tried to mitigate the disease outbreak and to improve the survival and production biomass of cultured shrimp. Also, the use of tilapia green water (TGW) culture system was tested as it has been previously shown to inhibit the growth and proliferation of pathogenic Vibrios including *V. harveyi, V. campbelli* and

V. parahaemolyticus (Sampollo et al., 2018; Cadiz et al., 2016; Corre et al., 2005).

In aquaculture, the tilapia green water (TGW) system is described as a culture system with salt tolerant tilapia reared at a salinity of 15-35 ppt. It has been observed that the culture of salt tolerant tilapia in seawater environment promotes the proliferation and growth of green microalgae comprised mainly of *Chlorella* spp. and *Nanochloropsis* spp. This culture system has been also documented and referred as a mature microbial ecosystem, known to support a diverse species of microbes that has been shown to prevent the growth of pathogenic *Vibrio* species in the culture water (Sampollo et al., 2018; Corre et al., 2005). The pathogenic *Vibrio* inhibitory activities of TGW have been linked to the low molecular weight organic metabolites secreted by bacteria, fungi, and algae growing in this culture system (Lio-po et al., 2005).

Though these earlier works suggest the effectivity of TGW in suppressing the growth of shrimp pathogenic Vibrios, but a detailed information on microbial components with probiotic potential and the involvement of these microbes in suppressing the growth of pathogenic *V. parahaemolyticus* have been lacking. For the TGW system to work, there is a need of a reservoir pond where tilapia is raised to generate the green water and another variation is that the tilapia is raised in a pen inside the shrimp rearing pond (Corre et al., 2005). Both systems need an additional area to rear the tilapia and therefore there is a risk that tilapia could be unintentionally transferred to the rearing system and prey or compete with the shrimp. It has been suggested that a better way is to find a potential probiotic bacteria isolated from TGW and utilize these to control *V. parahaemolyticus* in shrimp aquaculture. To date, there have been limited information regarding the isolation of bacterial probiotics from tilapia green water that are effective against the pathogenic *V. parahaemolyticus*, the causative agent of the early mortality syndrome disease in *P. vannamei*. The present work isolated, from tilapia green water, a bacteria species and elucidates its probiotic potential against the pathogenic *V. parahaemolyticus*, a potent pathogen of cultured *P. vannamei*. This study aims to isolate a probiotic bacteria from shrimp ponds employing saline tilapia greenwater, assess its pathogenicity to *Penaeus vannamei*, and evaluate its capacity to inhibit *V. parahaemolyticus* growth in the culture of *P. vannamei.*

Materials and Methods

Isolation and Identification of Potential Probiotic Bacteria

One ml of water samples from Tilapia Greenwater (TGW) culture were collected and serially diluted, 100ul of these dilutions were spread-plated on nutrient agar (Condalab, Spain). After 24 hours of incubation at 28°C, colonies were picked according their morphology and isolated for further evaluation. The individual bacterial isolates were cultured in 10 ml tryptic soy broth (TSB, Condalab, Spain) with 2% NaCl for 24 hours at 28°C. The bacterial culture was then centrifuged at 16, 000 x g for 3 minutes and the supernatant was collected and passed through a sterile 20 µm syringe filter (Whatman, USA). Eight (8) ml of the collected filtrate was added with equal volume of ethyl acetate (Sharlau, Australia) following a liquid to liquid solvent extraction scheme (Lei et al., 2020; Rajan, and Kannabiran, 2014). Prior to this test, ethyl acetate was previously selected and optimized as the best solvent in this extraction scheme (data not shown). The two liquid solutions were partitioned into an aqueous and organic layer and the organic ethyl acetate layer was collected and evaporated in a rotary evaporator at 3 psi and 40°C. After evaporating the solvent, the remaining organic compound was added and dissolved with 300 µL ethyl acetate. One hundred fifty microliters of the dissolve extract were collected and tested for its antimicrobial activity against *V. parahaemolyticus* in a standard disk diffusion assay (Gou et al., 2022). Isolates with positive inhibitory activity were selected and stored under 90% glycerol (Sigma-Aldrich, Singapore) in a -80°C freezer until used.

One active isolate (isolate code HbT) that exhibited inhibition zones on the lawn of *V. parahaemolyticus* was selected and evaluated for its probiotic potential in the present study. Identification of HbT isolate was done by sequence analysis of the 16s rDNA gene. Bacterial genomic DNA was extracted using Wizard® Plus SV Minipreps DNA Purification System (Promega, USA) according to manufacturer's protocol. The 16s rDNA gene was amplified by PCR using the forward primer 27F:5'-AGAGTTTGATCCTGGCTCAG (Lane, 1991) and 1492R:5'-TACCTTGTTACGACTT (Turner et al., 1999) as the reverse primer. The PCR reaction mix includes 1x Taq PCR Master Mix (Promega, USA), 2µL of template DNA and 0.2 µM of each primer at a final reaction volume of 50µL. The PCR reaction was run at 2 min initial denaturation at 94°C followed by 30 cycles at 25 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C and the reaction was ended by a final extension step of 7 minutes at 72°C (Turner et al., 1999). Amplified PCR product was purified and sequenced at the facilities of AIT Biotech PTE Ltd. (Singapore). The resulting nucleotide sequences were aligned to the bacterial 16s rDNA gene database of the National Center for Biotechnology Information using the Basic Local Alignment Search Tool (BLAST) program (Altschul et al., 1990).

Pathogenicity Test of HbT to *P. vannamei*

Pathogenicity test was done through immersion exposure of the shrimp to the potential probiotic isolate for 15 days. The test was run in a plastic rearing container filled with 3 L sterile 25 ppt seawater. The potential probiotic bacteria, *Salinicoccus hispanicus*

(OL638305), designated as Hbt that was used in this study was isolated from saline tilapia green water system maintained in the saline tilapia rearing facilities of the Institute of Aquaculture University of Philippines Visayas. The potential probiotic isolate was stored under 90% glycerol (Sigma-Aldrich, Singapore) in a -80°C freezer until used. Prior to the experimental test, the stored bacterial culture was activated in tryptic soy broth (CondaLab, Spain) added with 2% sodium chloride for 18–24 h at 28°C. Following the activation, the bacterial culture was centrifuged at 16, 000 x g for 3 minutes and the concentrated bacterial cells were collected and used to prepare a suspension in the rearing water at concentrations of 0, 10^3, 10^4, 10^6, 10^8 cfu ml^{-1} respectively. Bacterial concentration was estimated using McFarland Standards (Condalab, Spain). The experimental shrimp, with mean weight of 1.15±0.14 g, used in the study was obtained from the nursery rearing facilities of the Institute of Aquaculture, University of the Philippines Visayas. The pathogenicity test was conducted in a controlled Stress Physiology Laboratory facility of the Institute of Aquaculture University of Philippines Visayas. The pathogenicity test was carried out by immersing 75 test shrimp per treatment in the different probiotic bacterial concentrations for 1 hour (Temario et al, 2022). Following immersion, 25 shrimps were transferred into 12 clean 50 L tanks containing UV filtered seawater and mild aeration. This comprises the 4 treatment groups run in triplicate. Shrimp conditions and mortality were monitored and recorded every 6 hours daily for 15 days.

***Vibrio parahaemolyticus* Exclusion Test**

To test the efficacy of the potential probiotic to exclude *V. parahaemolyticus* in the shrimp gut, two experimental diets were prepared by supplementing a commercial shrimp feed (VF3, Oversea Feeds Corporation, Philippines) with the pathogenic *V. parahaemolyticus* and the test potential probiotic HbT. For the HbT-supplemented feed, bacterial suspension was prepared by inoculating an overnight plate culture of HbT in a prepared sterile Normal Saline Solution (Condalab, Spain) (NSS) (0.85% NaCl w/v) to reach a concentration of 10^9 cfu ml^{-1}, estimated using McFarland standards. This solution was sprayed on the commercial feeds to attain a concentration of 10^9 cfu g^{-1} feed and air dried under sterile conditions. The *V. parahaemolyticus* supplemented diet was prepared at a bacterial concentration of 10^8 cfu g^{-1} using the same procedure as before. Ninety shrimps weighing 2.0±0.13 g were randomly distributed into 6 tanks that were divided into the 2 treatment groups (3 tanks per treatment). During the first 3 days of the trial all shrimp in each treatment were fed with *V. parahaemolyticus* supplemented diets and on the 4th day sampling (6 shrimp per treatment group) were done to quantify *V. parahaemolyticus* that is present the in shrimp gut in each treatment groups. Also on the 4^{th} day of the trial, 1 treatment group was fed with the control diet (without the *S. hispanicus*) while the other group was fed with HbT supplemented diet until day 12. *Vibrio parahaemolyticus* in shrimp gut was quantified every 3 days using *V. parahaemolyticus* Chromogenic Agar (Condalab, Spain). Gut bacterial quantification was done following Thakur et al. (2004) with modifications. The shrimp external surface was disinfected by swabbing with 70% ethanol (Sharlau, Austarlia). The shrimp was aseptically dissected, stomach removed, added into a pre-weighed 1.5 ml micro centrifuge tube and weighed. The collected gut tissue was then homogenised in sterile NSS (0.1 g sample: 900 µL NSS) using a sterile tissue homogenizer plastic pestle (Sigma-Aldrich Singapore). One hundred µL of 10^{-1} and 10^{-2} dilutions were prepared from the tissue homogenate and spread onto Vibrio Chromogenic Agar (CONDAlab Pronadisa) solid media for *V. parahaemolyticus* enumeration. For every dilution 3 culture plates were used to quantify bacterial growth. For the enumeration of gut total aerobic bacteria dilutions of 10^{-3} and 10^{-4} tissue homogenate solution were prepared and spread-plated onto Nutrient agar solid media (CONDAlab Pronadisa). The plates were incubated at 30°C and colonies were counted 24-48 hours after incubation. The bacterial counts were reported as cfu g^{-1} gut tissue.

Feeding Trial and Shrimp Growth Evaluation

To assess the effect of the isolated HbT on overall shrimp growth performance, a 45 day feeding trial was conducted using 0.2±0.02 g shrimp stocked in 6 circular concrete tanks of $1m^3$ at a density of 100 shrimps per tank. These shrimp were divided into two treatment groups that were run in triplicate. Shrimp were fed 15% of the average body weigh 3 times daily with the control and the HbT supplemented diet at 10^6 cfu g^{-1} feed, feeding ration was adjusted every sampling. To quantify the feed intake, uneaten feeds were removed 2 hours after feeding. Water change was 50% daily, and optimum water quality was maintained (salinity: 30±1 ppt; pH: 8.0±0.2; dissolved oxygen: 6±1ppm; temperature 27±1°C) throughout the trial period. Feed preparation and gut bacterial quantification were done similar to the methods described in the gut colonization and *V. parahaemolyticus* exclusion test as described above. Gut bacterial content evaluation was performed every 3 days. Following the feeding trial, shrimp under the different treatment groups were weighed to evaluate the growth performance in terms of survival, % weight gain (WG), feed conversion ratio (FCR) and protein efficiency ratio (PER).

***Vibrio parahaemolyticus* Infection Challenge**

Upon finishing the feeding trial, 20 shrimps from each treatment group were collected and used in the infection challenge test with pathogenic *V. parahaemolyticus*. The concentration of

V. parahaemolyticus used in the infection challenge test was based on a previously optimized LC50 dose of the pathogen (Dabu et al., 2017). The test shrimp were stocked in 30 L plastic tanks containing UV filtered seawater and provided with gentle aeration. Optimal culture water parameters were maintained (salinity: 30 ppt; temperature: 28°C) throughout the trial. Shrimp in the treatments were fed with diets containing *V. parahaemolyticus* at 10^6 cfu g^{-1} diet in the morning and control diet was used in the afternoon. The shrimp conditions, water parameters, and mortalities were monitored daily. At the end of the experiment, remaining shrimp were collected and *V. parahaemolyticus* content in shrimp gut was evaluated following the methodology described above. To confirm that the mortalities observed is caused by the *V. parahaemolyticus*, moribund shrimp were collected, dissected and the presence of *V. parahaemolyticus* in the hepatopancreas was documented and quantified using the *V. parahaemolyticus* chromogenic media as described above (Traifalgar et.al., 2009).

Statistical Analysis

Statistical analyses were done using SPSS16 with significance level set at 0.05. Results were analyzed using independent samples t-test. Pathogenicity test data was subjected to oneway analysis of variance (ANOVA) with significance level set at 0.05. Tukey's test was used for post-hoc analysis.

Results and Discussion

The use of Tilapia green water has been previously documented as an effective strategy to suppress the population of pathogenic *Vibrios* in culture water of aquatic animals including shrimp (Sampollo et al., 2018; Cadiz et al., 2016; Corre et al., 2005) but the active component involved is not fully elucidated. Our present findings in TGW indicate the presence of a red orange colored gram positive cocci, exhibiting a potent antimicrobial activity against the shrimp pathogenic *V. parahaemolyticus*. These bacteria may be a part of a complex component in TGW that are involved in the suppression of *Vibrios* in this aqueous culture system. To identify the potential probiotic bacterial isolate 16s rDNA gene sequence analysis was performed. 16s rDNA sequence analysis shows that the isolate exhibited 100% sequence similarity with *Salinicoccus hispanicus* KR137717.1 and the sequence was deposited in GenBank database with accession number OL638305.

Salinicoccus hispanicus, previously known as *Marinococcus hispanicus,* is a gram positive non-motile cocci, non-spore forming and oxidase and catalase positive. It is a moderate halophilic bacteria that grows at salt concentration between 0.5% to 25% (w/v) (Marquez et al., 1990). As far as we know, the present study is the first to document the presence *S. hispanicus* in TGW as well as the findings on its inhibitory activity against *Vibrio parahaemolyticus*. Moreover, little or no information is known about *S. hispanicus* being used as a probiotic in shrimp culture. Our study of the pathogenicity test showed no mortalities in all treatments, including treatments exposed to the highest concentration of 10^8 cfu ml^{-1}, suggesting that our isolate *S. hispanicus* HbT is not pathogenic to shrimp and can be used as a probiotic.

Our present results suggest that this bacterium secretes a compound that is soluble in ethyl acetate and exhibits an inhibitory activity against *V. parahaemolyticus* (Figure 1). Though there have been limited information on the antibacterial activity of the genus *Salinoccocus*, some species have been reported to exert potent antibacterial activities against bacterial pathogens. Similar to our findings it was shown that

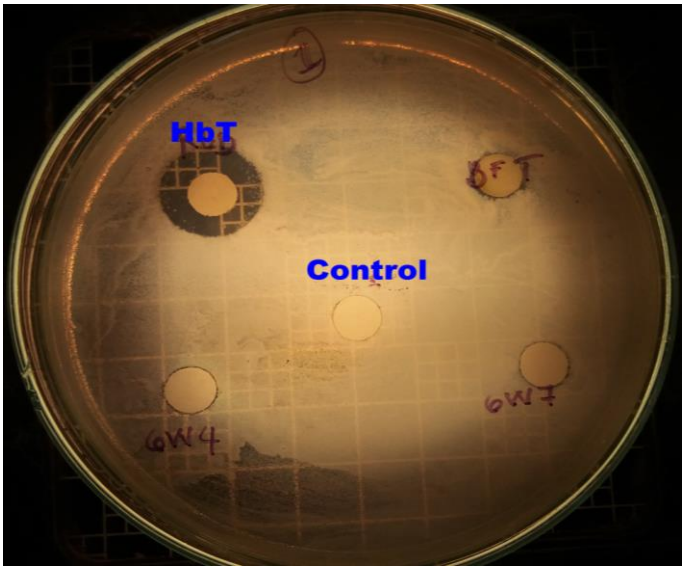

Figure 1. *Vibrio parahaemolyticus* zone of inhibition (14.5±0.3 mm) of the ethyl acetate extract of *Salinococcus hispanicus*.

Salinicoccus sesuvii MB597 isolated from a salt range soil exhibited a potent inhibitory activity against several species of bacteria and fungi including *Bacillus subtilis, Bacillus pumilus, Enterococcus faecalis, Bacillus cereus, Klebsiella pneumoniae, Alcaligenes faecalis, Pseudomonas geniculata, Enterococcus faecium, Aspergillus fumigatus, Aspergillus flavus, Fusarium solani,* and *Mucor* spp (Fariq et al., 2019). Another study found a *Salinococcus* sp. isolated from marine coastal soil with potent antibacterial activity against several human bacterial pathogens comprising of *Proteus vulgaris, Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa* (Srilekha et al., 2017). Our present results also coincide with the earlier findings of Anand et al. (2006) showing that an ethyl acetate extract of *Salinicoccus roseus* exhibited an antibiosis effect against *Bacillus subtilis*. Further, an ethyl acetate extract of a metabolite of *Salinicoccus halodurans* isolated from a salt evaporation pond was also shown to exhibit a potent antibacterial activity against *Aspergillus niger* and *Fusarium oxysporum* (Chen et al., 2009). In these earlier reports the antibiosis effects of the *Salinicoccus* isolates have been linked to the secretion of low molecular weight and pigment related compounds produced by these bacteria. The molecules, involved in the inhibition of *V. parahaemolyticus,* produced by *S. hispanicus* HbT were not purified and elucidated in the present study and this aspect needs to be done in future studies. Antagonistic activity of *S. hispanicus* HbT could be due to secretions of inhibitory compounds such as antibiotics, bacteriocins, proteases, and lysozymes (Verschuere et al., 2000).

The capacity to adhere, colonize gut surfaces, and produce antibacterial substances to prevent the proliferation of opportunistic pathogens are the major criterion in the selection of potential probiotics for

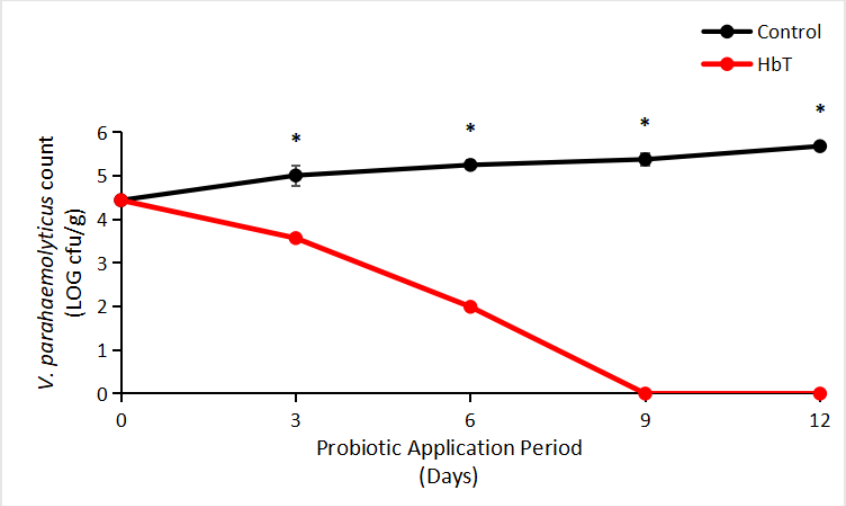

Figure 2a. *Vibrio parahaemolyticus* count in shrimp gut during gut colonization assay. Data is presented as mean ± SEM.* signifies significant statistical difference at α=0.05

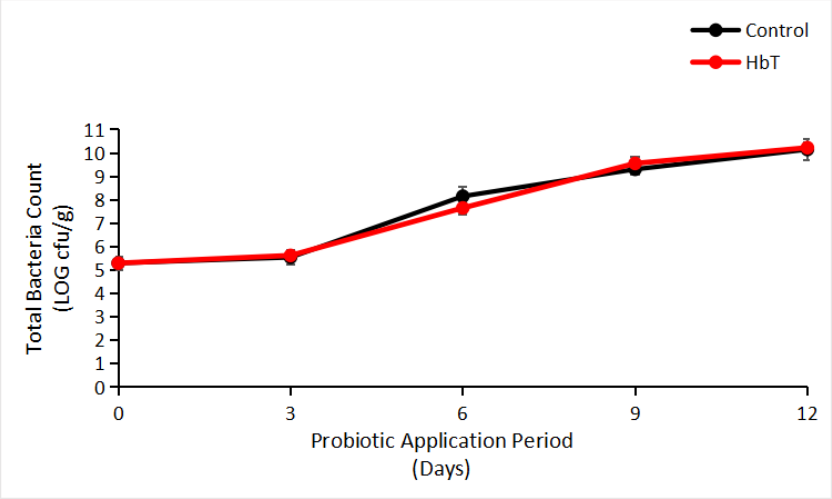

Figure 2b. Total aerobic bacterial count in shrimp gut during gut colonization assay. Data is presented as mean ± SEM. .* signifies significant statistical difference at α=0.05

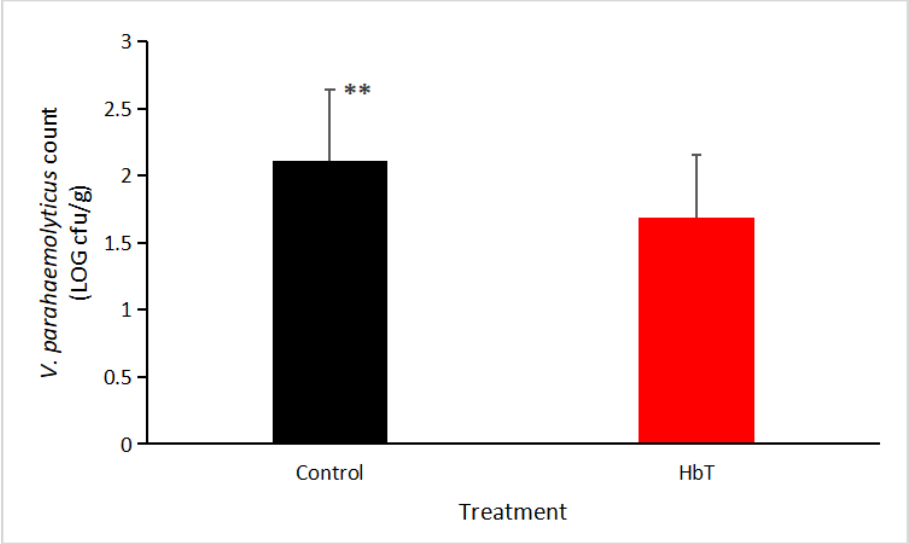

Figure 3. Mean *Vibrio parahaemolyticus* count in shrimp gut (cfu/g) during the feeding trial. Data is presented as mean ± SEM. ** signifies significant statistical difference at α=0.05

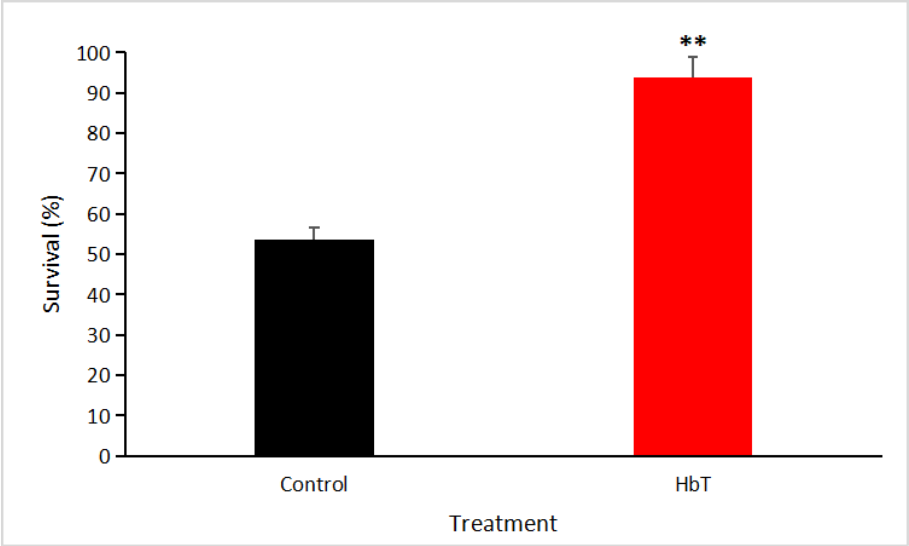

Figure 4. Survival after *Vibrio parahaemolyticus* infection challenge test. Data is presented as mean ± SEM. ** signifies significant statistical difference at α=0.05

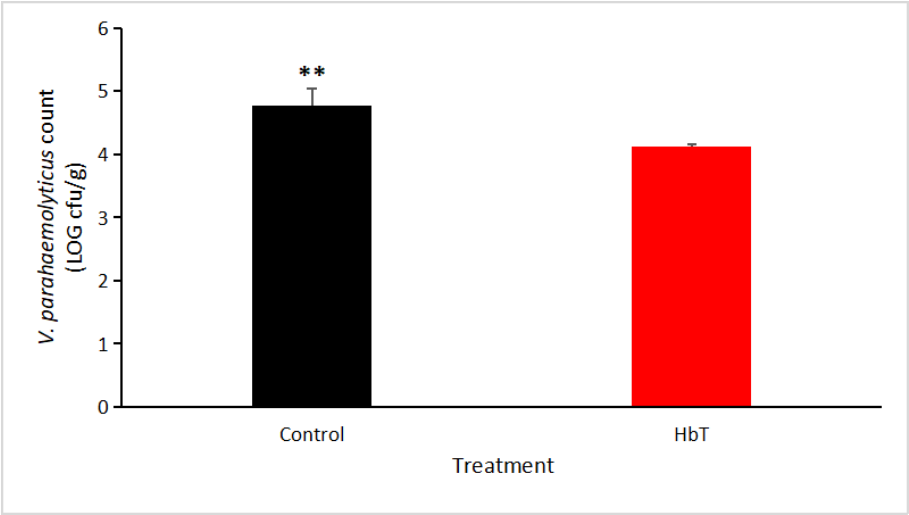

Figure 5. Mean *Vibrio parahaemolyticus* count in shrimp gut during infection challenge. Data is presented as mean ± SEM.** signifies significant statistical difference at α=0.05.

aquaculture use (Vine et al., 2004; Verschuere et al., 2000). In the present study, *V. parahaemolyticus* gut colonization test shows that supplementation of *S. hispanicus* HbT to shrimp, significantly decreased the numbers of *V. parahaemolyticus* in shrimp gut. A significant decline in the counts *V. parahaemolyticus* in shrimp gut was observed starting from day 3 and this pathogen was already undetectable in the shrimp gut on the 6th day of the test period (Figure 2a). Further it is worthy to mention that though gut *V. parahaemolyticus* counts are decreased in the treated group but the total aerobic bacterial counts remains similar in the two treatment groups (Figure 2b).

The genus *Salinicoccus* belongs to the family Staphylococcaceae under the phylum Firmicutes and this bacterial group has been known for their natural ability to colonize animal digestive tracts (Ventousa et al., 1990; Rosenstein and Götz, 2012). Several species of *Salinococcus* have been previously documented as part of the normal bacterial gut microbiota of mice (Cheng et al., 2020) and humans (Zhang et al., 2015). Similarly, in fish, *Salinicoccus* was also documented by metagenomic analysis to be present in the gut of large-mouth bass (Larsen et. al., 2014) and a novel *Salinicoccus cyprini* was reported to be present in the digestive tract of mirror carp, *Cyprinus carpio var. specularis* (Talwar et. al., 2020). In addition, *Salinicoccus* was also documented to be a component of the natural microbiota of benthic marine invertebrates including sea anemone, *Stichodactyla haddoni* (Williams et al., 2007) and marine sponges (Anand et al., 2006). Similar to our findings these *Salinicoccus* bacteria associated with these marine invertebrates were documented to exhibit antibacterial activities against human and fish bacterial pathogens. Our present results conform to these earlier reports suggesting that *S. hispanicus* HbT could also prevent or inhibit the gut colonization of *V. parahaemolyticus* in shrimp gut that could be linked to its capacity to secrete *Vibrio* inhibitory compounds.

Further, our potential probiotic isolate was tested in a feeding trial to assess its influence on shrimp growth performance. Results show that dietary supplementation of *S. hispanicus* HbT supplementation has no significant effect on the overall growth performance, feed conversion efficiency, protein efficiency ratio and survival of *Penaeus vannamei* (Table). Though the difference in terms of growth and feed conversion ratio between the control and the probiotics treated group were not significant but the numerical values in the probiotic treated growth were found higher than the control group. Moreover, the daily mean *Vibrio parahaemolyticus* count in the gut during the 45-day feeding trial was significantly lower in treatments fed with the probiotic isolate (Figure 3). This shows the capacity of *S. hispanicus* HbT to reduce if not exclude the growth of *Vibrios* in the gut.

The protective effect of *S. hispanicus* HbT on shrimp against *V. parahaemolyticus* infection was evaluated through an infection challenge test. Results shows that HbT dietary supplementation could result to a two-fold improvement in survival following a *V. parahaemolyticus* infection challenge test as compared to the control (Figure 4). This improvement in survival is further supported by the decreased numbers of *V. parahaemolyticus* in the gut of the shrimp receiving HbT supplemented diets (Figure 5). High survival in HbT-supplemented diet could be attributed to the ability of this probiotic isolate to secrete compounds that are inhibitory to *V. parahaemolyticus* as shown in Figure 1. Our results agree with earlier reports on the antibacterial activities of *Salinicoccus* species comprising the natural microbiota of aquatic invertebrates. Similar to our findings *Salinicoccus* sp. isolated from the sea anemone, *Stichodactyla haddoni* was reported to exhibit strong inhibitory activities against several species of fish bacterial pathogens including *V. parahaemolyticus* (Williams et al., 2007). It was also reported that an isolate of *Salinicoccus roseus* from marine sponges exhibited a potent antibacterial activity against *Bacillus subtilis* (Anand et al., 2005). Other species of *Salinicoccus* isolated from the environment have been also documented to elicit a broad spectrum of antibacterial activities against bacterial and fungal pathogens (Fariq et al., 2019; Srilekha et al., 2017; Chen et al., 2009). These earlier findings support our results on the efficiency of *S. hispanicus* HbT as a probiotic to prevent *V. parahaemolyticus* infection in *P. vannamei*.

Table 1. Growth performance of *Penaeus vannamei* fed with commercial feeds and probiotic supplemented diet

	Control	HbT
Initial Weight (g)	1.76±0.11	1.67±0.23
Final Weight (g)	7.20±0.01	7.88±1.36
Survival (%)	86.00±1.00	74.5±0.05
Weight Gain (%)	310.66±25.10	369.55±16.77
Specific Growth Rate (%/day)	3.13±0.13	3.43±0.08
Feed Conversion Ratio (FCR)	1.47±0.12	1.22±0.06
Protein Efficiency Ratio (PER)	1.97±0.16	2.35±0.11

Weight gain (WG) = $\frac{Wf-Wi}{Wi}(100)$; Specific growth ratio (SGR, % body weight. day -1) = $\frac{\ln Wf-\ln Wi}{t}(100)$; Feed conversion ratio (FCR) = $\frac{Wd}{WG}$; Protein efficiency ratio (PER) = $\frac{WG}{Pc}$; Survival (%) = $\frac{(Fi-Ff)}{Fi}(100)$; Where Wf is the final body weight in grams, Wi is the initial body weight in grams, Wd is the weight of the diet consumed in grams, and t is the experimental duration in days. Fi is the initial number of fish, Ff is the final number of fish at the end of the trial, Pc is the total protein consumed in grams dry weight.

Conclusion

Salinicoccus hispanicus HbT isolated from saline tilapia green water has a potent *Vibrio parahaemolyticus* antimicrobial activity and can decrease numbers of this bacterial pathogen in shrimp gut. This bacterial isolate is not pathogenic to shrimp, does not affect growth performance and can be used as a potential probiotic to prevent infection caused by *V. parahaemolyticus* in *Penaeus vannamei* culture.

Ethical Statement

Not applicable

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Author Contribution

Conceptualization: EJGM, REC, RFMT, Formal Analysis: EJGM, REC, RFMT, Funding Acquisition: RFMT Investigation: EJGM, REC, Methodology: EJGM, REC, RFMT, Project Administration: RFMT, Resources: EJGM, REC, RFMT, Supervision: RFMT, Validation: RFMT, Visualization: EJGM, REC, RFMT, Writing – Original Draft Preparation: EJGM, REC, RFMT, Writing – Review & Editing: EJGM, REC, RFMT

Conflict of Interest

The authors declare that they have no conflict of interest.

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