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RESEARCH ARTICLE

Protective effects of indigenous lactic acid bacteria in *Artemia salina* challenged with *Vibrio parahaemolyticus*: an *in vitro* and *in silico* approach

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ABSTRACT

Aquaculture is one of the fastest-growing sectors of the food industry and vibriosis is a major disease affecting finfish and shellfish production capabilities. In recent years, probiotics specifically lactic acid bacteria (LAB) have proven to be an environmentally friendly alternative to antibiotics to maintain aquatic animal health. In this study, five strains of indigenous LAB isolated from traditional and non-traditional sources were evaluated for their potency in the prevention of vibriosis in brine shrimp, Artemia salina as a model organism. The LAB were well tolerated at all doses, and no negative effects on the hatching ability of brine shrimps were observed when exposed to Lactiplantibacillus plantarum KCFe63 and Limosilactobacillus fermentum NCCu21. Furthermore, all the tested LAB were able to protect the brine shrimp from the pathogen Vibrio parahaemolyticus under co-exposure and pre-exposure conditions. Molecular docking analysis revealed a high binding affinity of common probiotic metabolites lactic, butyric and propionic acids to the PirAvp and PirBvp proteins of V. parahaemolyticus which can prevent toxin formation and thereby acute hepatopancreatic necrosis disease (AHPND). Overall results suggest that two strains, L. plantarum KCFe63 and L. fermentum NCCu21, are suitable candidates to reduce the incidence of vibriosis and AHPND during brine shrimp cultivation.

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Introduction

Global food demand has increased as a result of the continuously growing human population. Many government and private organizations are focusing on fish farming or aquaculture to meet this demand for protein sources in a sustainable and cost-effective manner (Mustapha et al. 2021). Aquaculture is a vital source of income and food security in developing countries, and hence, periodic advancement in the aquaculture industry through the employment of recent technologies is of utmost importance (El-Saadony et al. 2021). The aquaculture industry has always experienced the emergence and spread of diseases by opportunistic pathogens (Fernandes et al. 2021).

The Indian aquaculture industry is growing steadily, and *Artemia* spp. (brine shrimp) are an important live feed source. However, they are also a potential risk factor for carrying and spreading diseases (Quiroz-Guzmán et al. 2018). The pathogen *Vibrio parahaemolyticus* remains a major threat to the health of animals as well as humans and leads to major economic losses (Ninawe et al. 2017). It is known to cause the devastating acute hepatopancreatic necrosis disease (AHPND) in shrimps. *Vibrio parahaemolyticus* contains a plasmid encoding the binary Photorhabdus insect-related (Pir) toxin genes PirA/PirB (Zheng et al. 2021). Several chemical and antibiotic treatment strategies have been employed to combat AHPND. However, antimicrobial resistance, accumulation of chemicals and environmental pollution pose potential risks to both the aquatic ecosystem and public health (El-Saadony et al. 2021). Extensive research is being conducted, and various strategies to safeguard aquaculture have been undertaken. One such

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environmentally friendly strategy suggested was the usage of probiotics (Wang et al. 2008).

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit to the host' (FAO/WHO 2002). A large group of probiotics are lactic acid bacteria that can utilize carbohydrates to produce organic acids. These metabolites not only maintain intestinal health but are also used in several industrial processes (Lee et al. 2020). Metabolites especially butyric, propionic and valeric acids have been tested against various fish pathogens including *V. cholerae* (You et al. 2019). Probiotic species isolated from traditional sources provide benefits to aquatic organisms that include improved growth rate and health status, disease resistance and immunomodulation (Merrifield et al. 2010). The underlying mechanism of action is strain-dependent and can be broadly attributed to antimicrobial compound production, competition for receptor and adherence sites as well as nutrition, modulation of host immune responses and manipulating quorum sensing to obstruct virulence factors production (Balcázar et al. 2007). The potential probiotics should tolerate changes in the gastrointestinal transit and colonize the tract to impart benefits to the host health. Probiotics are known to alter the gut microbiota and modify the immune responses (Pradhan et al. 2020). Almost all of the strains belonging to the genus *Lactobacillus* (old nomenclature) and a few belonging to the genus *Lactococcus* are categorized as 'generally recognised as safe' (GRAS) by the US Food and Drug Administration (FDA) (Žuntar et al. 2020; Borase et al. 2022). Since novel strains are continuously being isolated and commercialized, the risk that they provide should be assessed using *in vitro* as well as *in vivo* safety assessment methods.

With this background the LAB of five indigenous strains, *Limosilactobacillus fermentum* AlFe1 (GenBank ID MZ048397), *Pediococcus pentosaceus* BRDb27 (MT968036), *Lactiplantibacillus plantarum* ACFe58 (MT994635), *Lactiplantibacillus plantarum* KCFe63 (MT982170) and *Limosilactobacillus fermentum* NCCu21 (MT974572), that were isolated from traditional and non-traditional sources were used in this study (unpublished data). These lactic acid bacteria strains have been newly isolated and have not yet been studied for aquaculture applications and probiotic effect. Therefore this study was carried out with the aim to verify their safety and determine their protective ability against vibriosis and AHPND. Furthermore, *in silico* analysis was employed to analyse the binding affinities of common short-chain fatty acids (SCFAs) produced by probiotics against the $PirA^{vp}$/$PirB^{vp}$ toxin complex.

Materials and methods

Chemicals and reagents

Analytical grade biochemical reagents and chemicals were procured from SRL Pvt. Ltd, Mumbai (India), while microbiological media used in the study were procured from HiMedia Laboratories Pvt. Ltd, Mumbai (India).

Bacterial strains and maintenance

Indigenous probiotics were previously isolated, identified and characterized (unpublished data). Isolates *Limosilactobacillus fermentum* AlFe1 (GenBank ID MZ048397), *Pediococcus pentosaceus* BRDb27 (MT968036), *Lactiplantibacillus plantarum* ACFe58 (MT994635), *Lactiplantibacillus plantarum* KCFe63 (MT982170) and *Limosilactobacillus fermentum* NCCu21 (MT974572) showed excellent probiotic activities such as resistance to gastric acid and bile, NaCl and phenol tolerance, adhesion to epithelium and antimicrobial properties, and hence were selected for *in vivo* toxicity analysis. All bacterial isolates were maintained at −80°C in 20% glycerol for long-term storage. Pure cultures were grown in De Man, Rogosa and Sharpe (MRS) broth for 16–18 h at 37°C. Similarly, *Vibrio parahaemolyticus* MTCC 451 (used as a positive control for toxicity analysis) was grown in nutrient broth (NB) supplemented with 3% NaCl and incubated for 16–18 h at 37°C. All strains were harvested by centrifugation at 3000 rpm for 10 min, the bacterial pellet thus obtained was resuspended in saline solution and densities were determined according to McFarland standards.

Maintenance and hatching of Artemia

The *Artemia* cysts (O.S.I PRO brand) procured from a local dealer in Chennai (India) were disinfected and decapsulated by sodium hypochlorite (NaOCl) treatment to generate bacteria-free cysts according to Quiroz-Guzmán et al. (2018). Briefly, a very small quantity of cysts (50 mg) was treated with 1.5 ml of 0.5% (v/v) NaOCl followed by vortexing for 3 min. The cysts were then washed thrice with 25 ml of sterile distilled water before transferring into sterile tubes containing 20 ml brine solution (3% NaCl; pH 8.7) and exposed to illumination (2000 lux) and shaking (24 h, 150 rpm at 28°C). Post hatching (14 h) the sterile nauplii (instar 1) were transferred to 25 ml flasks containing 3% NaCl. Rates of hatching and survival were

continuously monitored during the hatching period (14 h) by observation under 40× magnification using an inverted microscope (RTC-5; Radical Scientific Equipments, India). The changes in developmental stages of brine shrimp were observed and developmental deformities such as the differences in morphological appearance, body length, survival, and abnormal behaviour were also noted. The death of larvae was confirmed by an absence of opercular movement or reaction to external stimuli. To avoid any bacterial contamination, the dead nauplii were removed immediately.

Effect of live lactic acid bacteria on the hatching and early survival of Artemia

The effects of live bacteria on the hatching and survival of *Artemia* were evaluated according to Quiroz-Guzmán et al. (2018) and Giarma et al. (2017). Approximately 10–30 cysts were exposed to three different cell concentrations of a single strain of probiotic bacteria (10^4, 10^6, 10^8 CFU/ml) and incubated at 28°C in shaking conditions for 24 h under continuous light (2000 Lux). The rates of hatching and survival were monitored up to 14 and 24 h, respectively. *Vibrio parahaemolyticus* MTCC 451 was considered as a disease control and axenic cysts served as negative controls. All experiments were conducted in triplicate, and the data are shown as mean ± SD.

Effect of the cell-free supernatant (CFS) of lactic acid bacteria on hatching and early survival of Artemia

The effects of cell-free supernatants (CFS) on the hatching and early survival of brine shrimp were evaluated according to Quiroz-Guzmán et al. (2018). Approximately 10–30 cysts were exposed to different concentrations of the CFS (1:10, 1:1, undiluted) and incubated at 28°C in shaking conditions for 24 h under continuous light. The survival rate was monitored up to 24 h. CFS of *V. parahaemolyticus* was used as a disease control, while negative controls (axenic cysts) were also maintained.

Effects of co-incubation of lactic acid bacteria and pathogen V. parahaemolyticus *on the survival of* Artemia *nauplii*

To evaluate the antimicrobial effect and determine the capacity of competitive exclusion and colonization, disinfected cysts were exposed to 10^4 CFU/ml of different probiotics along with 10^8 CFU/ml *V. parahaemolyticus* for 24 h at 28°C in a rotary shaker at 150 rpm. The survival rates were monitored for 24 h. Cysts exposed to *V. parahaemolyticus* alone were considered as disease controls (Quiroz-Guzmán et al. 2018).

Protective effect of pre-exposure of lactic acid bacteria against pathogen V. parahaemolyticus

The cysts were enriched with probiotic bacteria (10^4 CFU/ml) for a period of 14 h. Post-enrichment, they were aseptically transferred to flasks containing 100 µl of *V. parahaemolyticus* (10^8 CFU/ml) (Quiroz-Guzmán et al. 2018) along with brine solution. This suspension was further incubated as previously mentioned and the survival of nauplii was recorded for 24 h. Flasks that contained axenic cysts up to 14 h and then exposed to *V. parahaemolyticus* acted as disease control.

Molecular docking studies

Short-chain fatty acids (SCFA) such as lactic acid, propionic acid and butyric acid, formed by the action of probiotic bacteria were selected for molecular docking. $PirA^{vp}$ (Protein Data Bank (PDB) code: 3X0T) and $PirB^{vp}$ (PDB code: 3X0U) were obtained from the RCSB PDB, and used as targets for docking analysis (Ong et al. 2021). Molecular docking was carried out using Autodock Vina, and pose analysis was performed with BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, BIOVIA Discovery Studio Visualizer, Version 20.1.0.192, San Diego: Dassault Systèmes, 2020). The SMILES (simplified molecular-input line-entry system) of the ligands were generated and visualized on the BIOVIA visualizer tool and then saved in .pdb format. Similarly, the two proteins were also downloaded in .pdb format. The ligands and proteins were prepared for docking (converted to .pdbqt format) using the AutoDock 4.2 software. The conformation of the ligand with the significant binding score was selected. Three-dimensional (3D) structures of the protein–ligand complexes were visualized by using BIOVIA Discovery Studio Visualizer.

Statistical analysis

All the analyses were carried out using IBM SPSS Statistics Version 23.0 (IBM Corporation, New York, USA). One-way analysis of variance (ANOVA) was applied to study the variation of % survival between different probiotic strains, between co-exposure and between post-exposure experiments. A Dunnett's test was also performed post-hoc to compare the difference between individual exposure groups and the control. Data were considered to be statistically significant at three levels of significance ($^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$).

Results

Effect of live lactic acid bacteria on hatchability of Artemia *cysts*

The effects of lactic acid bacteria on the hatchability of *Artemia* cysts were studied. The various developmental stages of the brine shrimp are shown in Figure 1. Effects of LAB on the hatching success of brine shrimp cysts revealed that none of the isolates showed visible detrimental effects on the hatching ability and body length (Figure 2). Furthermore, the LABs neither inhibited the hatching success of cysts nor induced deformity in brine shrimp (Table I). However, when exposed to the pathogen *V. parahaemolyticus*, the cysts showed delayed or no hatching compared with the control group (Axenic cysts) (Figure 4). A few cysts were infested which correlates that they were colonized by the pathogen. Administration of the LAB at different cell concentrations revealed varying hatching rates in a dose-dependent manner. No significant difference ($P > 0.001$) in the hatching rate was observed when exposed to all five strains compared with control (axenic cysts). Nonetheless, a strain-specific activity was observed where amongst the two *L. fermentum*, cysts exposed to isolate NCCu21 had higher (57.69%, $F = 2.746$) hatching ability at the lowest exposed concentration (10^4 CFU/ml). *Lactiplantibacillus plantarum* isolate ACFe58 had better effects at all exposed cell concentrations (63.54%, 67%, 58.22% at 10^4, 10^6, 10^8 CFU/ml respectively, $F = 0.166$) compared with the axenic cysts control (62.69%). Similarly, higher hatching ability was observed in the cysts exposed to *P. pentosaceus* BRDb27 at 10^4, 10^6 CFU/ml with 57.71% and 48.27% ($F = 1.885$) hatching rate, respectively.

Effect of live lactic acid bacteria on the early survival of Artemia *nauplii*

Administration of the bacteria revealed a concentration-dependent effect of isolate *L. fermentum* AIFe01 on the early survival of nauplii. The survival rates of nauplii exposed to different concentrations of the isolates *L. fermentum* NCCu21 (67.46%, 63.48% and 85.93%) and *L. plantarum* KCFe63 (66.15%, 37.08% and 54.68%) did not show any significant changes when compared with control (axenic cyst) (Figure 3). However, a significant reduction in the survival rate of nauplii exposed to all concentrations of isolates AIFe01 ($P < 0.01$), BRDb27 ($P < 0.05$) and ACFe58 ($P < 0.01$) was observed. Delay in hatching and development was observed when the cysts were exposed to *V. parahaemolyticus*. There was no hatching even at 24 h, wherein although the organisms were detached, they could not develop further (Figure 4). The administration of *L. plantarum* KCFe63 resulted in better hatching and survival of nauplii as they reached the nauplius (instar II) stage (Figure 1) earlier whereas the control (axenic cysts) was still in the umbrella or metanauplius stage. Administration of *L. plantarum* KCFe63 and *L. fermentum* NCCu21 resulted in better survival and fitness of the nauplii determined by the rate of movement.

Effects of co-incubation of lactic acid bacteria and V. parahaemolyticus *on the survival of* Artemia salina *nauplii*

The cysts were treated with lactic acid bacteria strains and *V. parahaemolyticus* simultaneously in order to

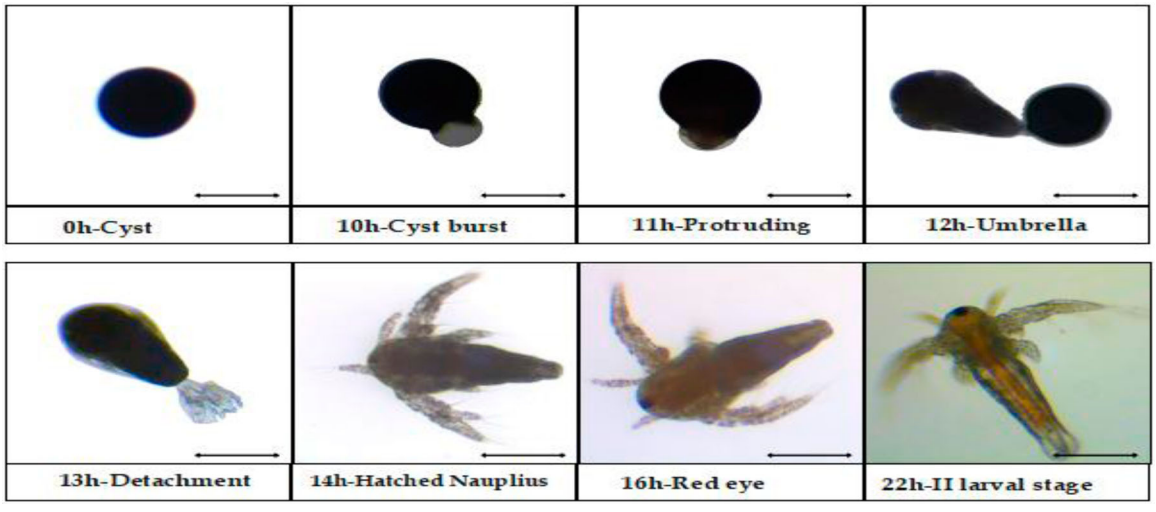

Figure 1. Developmental stages of *Artemia salina* under 40× magnification (RTC-5 Radical Inverted microscope). Scale bar represents 200 µm.

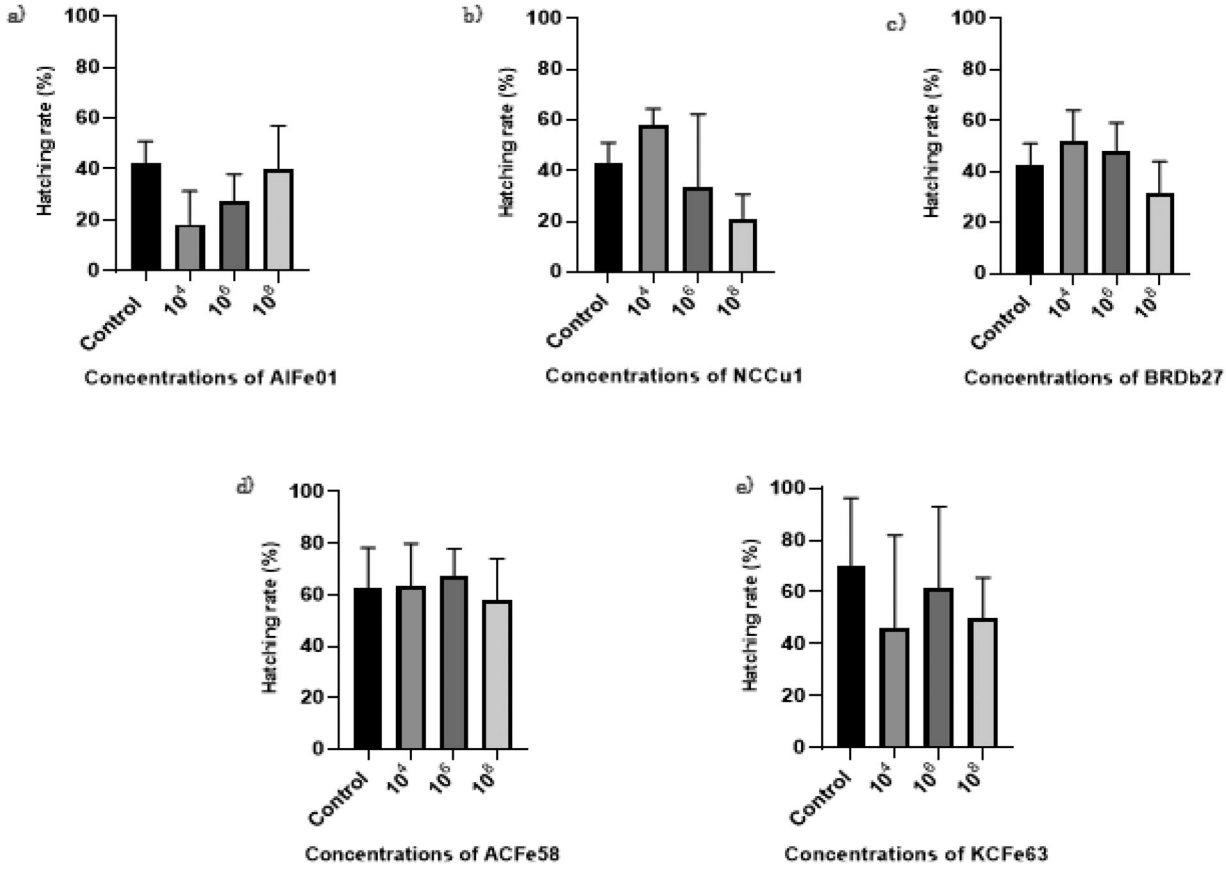

Figure 2. Hatching rate (%) of cysts exposed to different concentrations (10^4 10^6, 10^8 CFU/ml) of potential probiotics. (a) Isolate AlFe01, (b) Isolate NCCu21, (c) Isolate BRDb21, (d) Isolate ACFe58, (e) Isolate KCFe63. Data are represented as mean + SD ($n = 3$). (Control = axenic cysts).

evaluate the antimicrobial effect and determine the capacity of competitive exclusion and colonization. An increase in the survival of nauplii was seen in all sets compared with the pathogen-exposed group at low doses, as shown in Figure 5. There were significant differences ($F = 5.381$, $P < 0.01$) between and among the exposed lactic acid bacteria and higher survival of nauplii was observed when exposed to isolate AlFe01 (77.83%).

Table I. Developmental changes in cyst and nauplii exposed to the lactic acid bacteria and *V. parahaemolyticus 451* (Vp 451).

	Developmental deformities		
Exposure conditions	Morphology of cyst	Morphology of II instar larva	Body length (mm)
Control (anexic cyst)	No disintegration		0.70 ± 0.02
AlFe01		No structural abnormality or malformation of antenna, naupliar eye or tail	0.61 ± 0.03
NCCu21			0.67 ± 0.03
BRDb27			0.68 ± 0.01
ACFe58			0.66 ± 0.03
KCFe63			0.69 ± 0.02
Vp 451 (Disease control)	Slight disintegration of cyst	Not hatched	Not hatched

Protective effects of lactic acid bacteria pre-exposure against V. parahaemolyticus

To evaluate the protective effects of lactic acid bacteria on the survival of nauplii, the cysts were exposed to a low dose (10^4 CFU/ml) of all the five lactic acid bacteria prior to the challenge with the pathogen *V. parahaemolyticus* (Figure 6). Nauplii exposed to isolates AlFe01 (72.71%), ACFe58 (48.89%), NCCu21 (74.24%) and BRDb27 (41.33%) had lower survival rates than controls, but they were still significantly higher than those exposed to the *V. parahaemolyticus* directly after 14 h. Isolates of KCFe63 showed significant protective effects with a survival rate of 90%.

Molecular docking

It is important to identify compounds that can inhibit the toxin complex that $PirA^{vp}$ and $PirB^{vp}$ form as

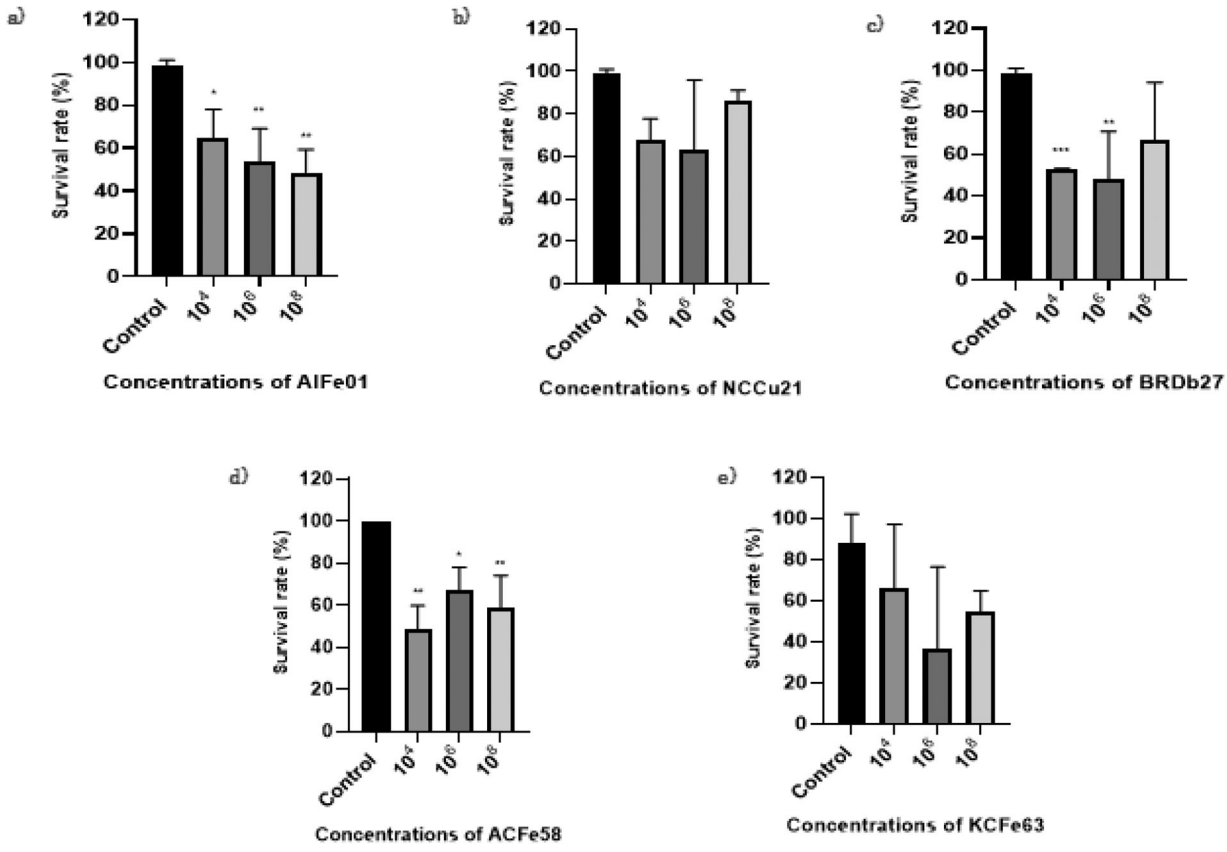

Figure 3. Survival rate (%) of nauplii exposed to different concentrations (10^4, 10^6, 10^8 CFU/ml) of potential probiotics. (a) Isolate AIFe01, (b) Isolate NCCu21, (c) Isolate BRDb21, (d) Isolate ACFe58, (e) Isolate KCFe63. Data are represented as mean + SD. Asterisk represents statistical difference between negative control and treatment groups. *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$).

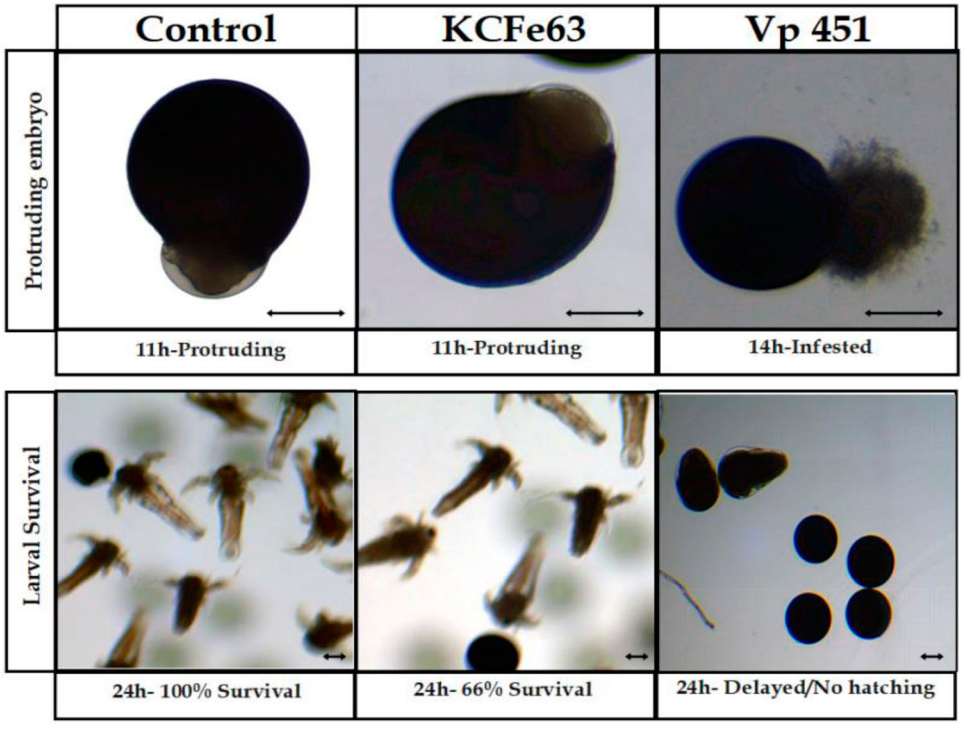

Figure 4. Representative image of the effect of *L. plantarum* KCFe63 and *V. parahaemolyticus* MTCC 451 on survival of brine shrimp nauplii. The images were taken at 40× magnification (RTC-5 Radical Inverted microscope). Scale bar represents 100 µm. (Control = axenic cysts).

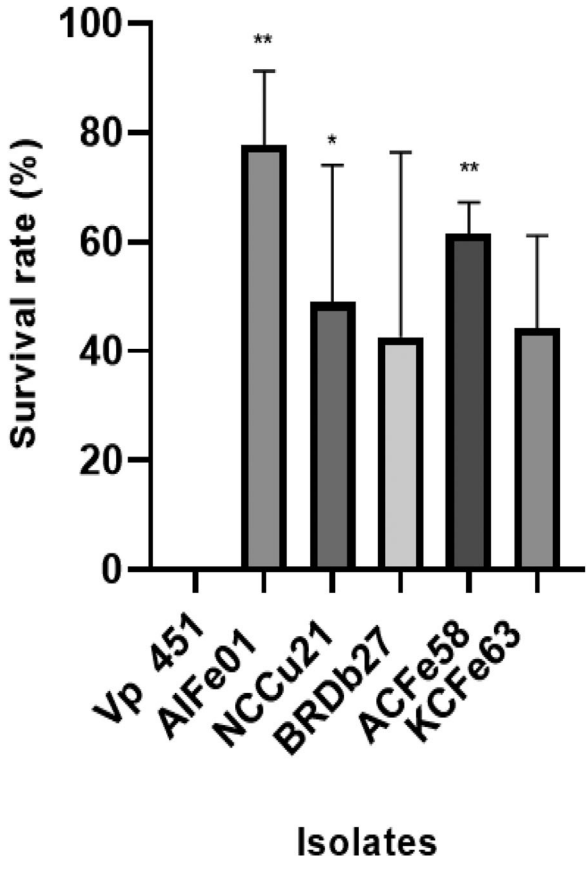

Figure 5. Survival rates (%) of nauplii exposed to potential probiotics and pathogen simultaneously. Data are represented as mean + SD. Asterisk represents statistical significance between disease control Vp 451 and probiotics. *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$).

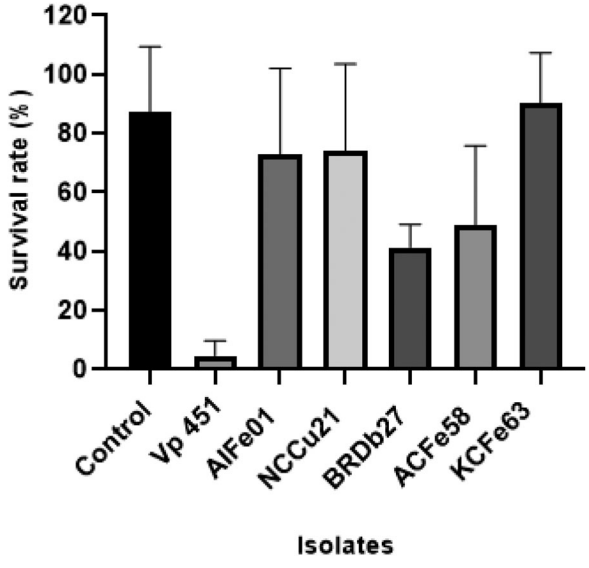

Figure 6. Survival rate (%) of nauplii treated with potential probiotics before exposure to Vp 451. Data represented as mean + SD. No significant difference between negative control and probiotics exposed groups.

these two proteins are the key factors involved in the pathogenesis of acute hepatopancreatic necrosis disease (AHPND) (Lin et al. 2017). To check the binding affinity of the three common probiotic metabolites, namely lactic acid, propionic acid and butyric acid, molecular docking was performed and the results are presented in Figures 7 and 8. The 2D and 3D interactions were studied with BIOVIA Discovery Studio Visualizer (Version 20.1.0.192). Among the three, butyric acid showed significant binding affinity (binding energy −4.6 kcal/mol) as compared with those of lactic (−4.5 kcal/mol) and propionic acid (−4.0 kcal/mol). Similar results were observed when these three ligands were docked with PirB (3X0U). Lactic acid showed a higher binding score of −4.5 kcal/mol compared with that of butyric (−4.1 kcal/mol) and propionic acid (−3.8 kcal/mol). In the case of PirA, lactic acid shows conventional hydrogen bonds with Trp 57, Ala 88 and Asn 87 residues, whereas both butyric and propionic acid showed conventional hydrogen bonds with the Trp 57 and Asn 87 residues. Similarly, lactic acid forms hydrogen bonds with Arg 305, Asn 299 and Met 300 residues of the target PirB. These three amino acid residues were also seen to be involved in hydrogen bond/interactions with butyric and propionic acid (Figures 7 and 8).

Discussion

Pathogens such as *Vibrio parahemolyticus* are known to cause vibriosis and acute hepatopancreatic necrosis disease (AHPND) in shrimps and extensive antibiotic usage has led to the development of antimicrobial drug resistance in fish pathogens (Miller and Harbottle 2018). Therefore, the focus should be shifted towards alternative and prophylactic treatments. Probiotics are known to have beneficial effects, however, they produce potent bioactive compounds which might have several negative effects (Anadón et al. 2021). Although all three genera of lactic acid bacteria (*Limosilactobacillus*, *Pediococcus*, *Lactiplantibacillus)* selected in this study have GRAS status by the FDA, there can be strain-dependent toxicity profiles. Hence, the safety of these strains *in vivo* was determined in this study using *Artemia salina* as a eukaryotic model for safety studies. *Artemia* spp. have previously been studied as model organisms to determine the toxicity of probiotics, where metabolites from some strains were lethal to the organism (Neu et al. 2014). Furthermore, the protective role of LAB as potential probiotics and their ability to prevent vibriosis and AHPND was also assessed.

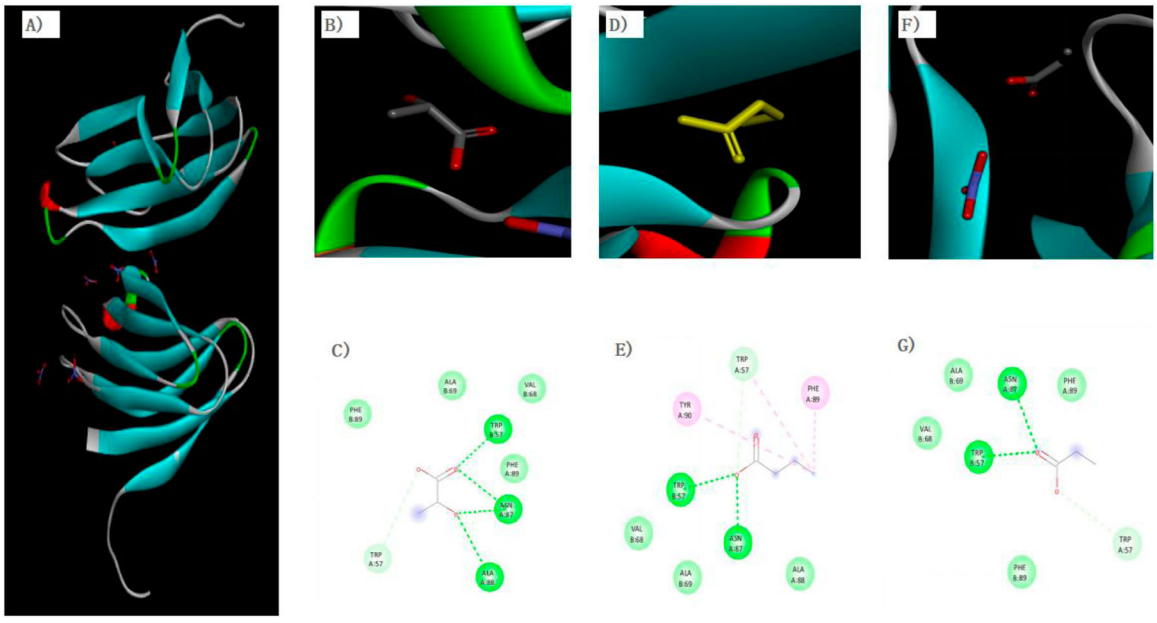

Figure 7. The molecular docking study of PirA^{VP}; (A) PirA (PDB: 3X0T); (B) 3D model of lactic acid (grey and red); (C) 2D model of lactic acid, amino acid residues with bond length Ala88-3.22 Å, Trp57-2.24 Å, Asn87-2.12, 2.16, 2.58 Å; (D) 3D model of butyric acid (yellow); (E) 2D model of butyric acid, amino acid residues with bond length Trp57-2.26 Å, Asn87-2.55 Å, Asn87-2.97 Å, Trp57-4.08 Å, Trp57-4.00 Å; (F) 3D model of propionic acid (grey and red); (G) 2D model of propionic acid, amino acid residues with bond length Asn87-2.57 Å, Trp57-2.25 Å, Trp57-3.86 Å.

The effects of live lactic acid bacteria and their cell-free supernatant on hatching and survival rates of *Artemia salina* was assessed. It was evident that all five bacterial strains had no adverse effects as no significant differences in hatching rates were observed compared with the control group. The lethal effects of *V. parahaemolyticus* on *Artemia* seen in this study are in line with Neu et al. (2014). The greater survival rates of nauplii exposed to LAB isolates AIFe01, ACFe58, KCFe63 at higher doses used (10^8 CFU/ml)

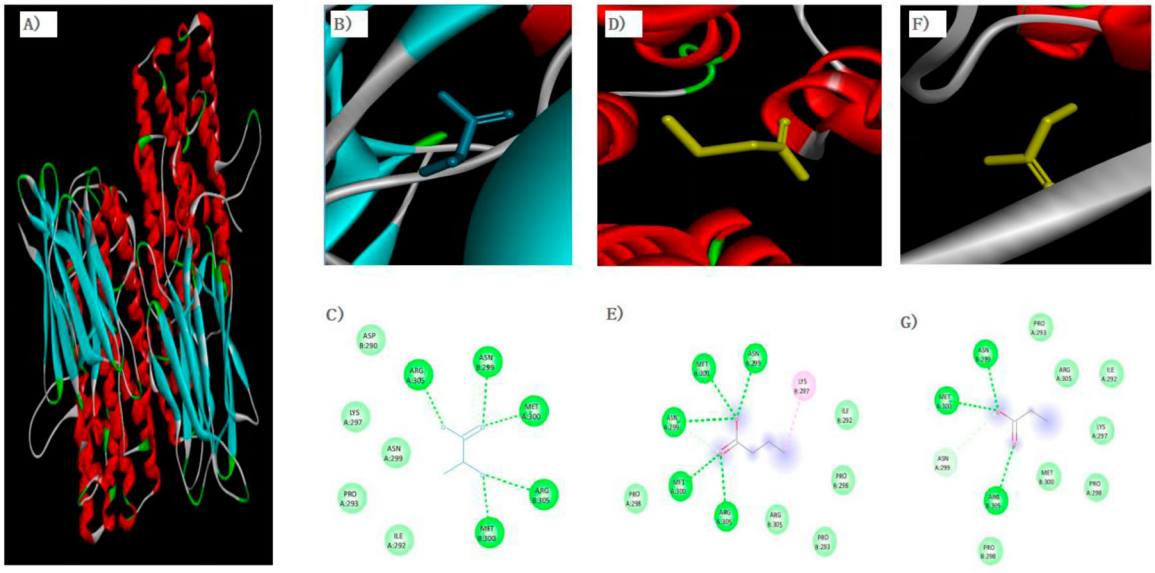

Figure 8. The molecular docking study of PirB^{VP}; (A) PirB (PDB: 3X0U); (B) 3D model of lactic acid (blue); (C) 2D model of lactic acid, amino acid residues with bond length Arg305-2.03 Å, Arg305-2.50 Å, Asn299-2.33 Å, Met300-1.90 Å; (D) 3D model of butyric acid (yellow); (E) 2D model of butyric acid, amino acid residues with bond length Arg305-2.40 Å, Asn299-3.25 Å, Asn299-3.75 Å, Asn299-2.44 Å, Met300-2.15 Å, Met300-2.38 Å; (F) 3D model of propionic acid (yellow); (G) 2D model of propionic acid, amino acid residues with bond length Arg300-2.40 Å, Asn299-3.53 Å, Asn299-2.22 Å, Met300-2.32 Å.

demonstrate their non-toxic activity. Nonetheless, isolates NCCu21 and BRDb27 showed lower survival rates which could potentially be due to increased production of the organic acids. Furthermore, the reduction in hatching rate and mortality of *Artemia* could be attributed to higher bacterial densities and thereby depletion of oxygen (Farahi et al. 2011; Touraki et al. 2012). The cell-free supernatant of LAB led to zero hatching and survival rates that indicate the potency of antimicrobial compounds produced by all LAB studied (Touraki et al. 2012).

The LAB used in this study have been found to increase *Artemia* survival when exposed to the pathogen *V. parahaemolyticus*. The LAB could significantly inhibit the growth of *V. parahaemolyticus* without inducing any deformities. Similar findings involving the ability of *Bacillus* strains to protect brine shrimp against *V. alginolyticus* have been reported by Mahdhi et al. (2011).

According to Giarma et al. (2017), the administration of *L. plantarum*, *Lactococcus lactis* and *B. subtilis* increased antioxidant enzymes and decreased oxidative damage in shrimp exposed to *V. anguillarum*. Similarly, Quiroz-Guzmán et al. (2018) reported that brine shrimp infected with *V. parahaemolyticus* and *V. harveyi* were more likely to survive when given a probiotic consortium made up of ten different strains. According to several other studies, e.g. Niu et al. (2014) and Touraki et al. (2013), probiotics' protective effects were only noticed when given prior to the pathogenic challenge. Our results showed that the LAB were able to antagonize *V. parahaemolyticus* as well as provide protective effects. According to Verschuere et al. (2000) and Touraki et al. (2013), pathogenesis can be controlled using optimum concentrations of probiotics.

In our study, the administration of a low dose of LAB (10^4 CFU/ml) provided beneficial anti-*V. parahaemolyticus* protective effects while exhibiting no potential toxic effects. The hatching and survival rate of *Artemia* is influenced by several abiotic factors including temperature, salinity, pH and dissolved oxygen (Bahr et al. 2021). The difference in hatching rate when exposed to LAB could be attributed to the different concentrations of SCFAs secreted by the organisms (Quiroz-Guzmán et al. 2018). Live bacteria are being used as alternatives to antibiotics, however, postbiotics (microbial metabolites), have gained immense attention as alternative disease management agents (Sudhakaran et al. 2022). Further studies should focus on the quantification of metabolites such as organic acids using mass spectrometry to get insights into the dynamics of metabolic processes. Probiotic bacteria are also used as feed additives as they are known to benefit the host by increasing growth, immunity and gut health. Probiotics can additionally help to improve the water quality (El-Saadony et al. 2021) and hence would be economically viable options.

Previous studies suggest that probiotics protect organisms through multiple mechanisms (Lauzon et al. 2014; Pérez-Sánchez et al. 2014; Quiroz-Guzmán et al. 2018). Probiotics could be competing for nutrients essential for metabolism and adhesion sites or create an unfavourable environment for the pathogenic organisms by producing their antimicrobial substances (Touraki et al. 2012). Results from this study show that when co-incubated the LAB could competitively exclude *V. parahaemolyticus* and thereby increase the survival rates of nauplii. All five strains of lactic acid bacteria used in this study were able to provide protective effects which could be attributed to the colonization capacity indicating their probiotic effect. Probiotics have the ability to colonize cysts after three hours and in turn, prevent the adhesion and virulence factors of pathogenic bacteria (Balcázar et al. 2007; Quiroz-Guzmán et al. 2018).

The molecular docking results suggest that butyric acid, propionic acid and lactic acid are promising leads as they could bind to the toxin proteins of *V. parahaemolyticus* and thereby could potentially inhibit the $PirA^{vp}/PirB^{vp}$ toxins. Ong et al. (2021) reported similar results where 28 such peptides were able to interact with oilseed proteins and reported to potentially mitigate vibriosis.

Our study showed that *L. plantarum* KCFe63 and *L. fermentum* NCCu21 were non-toxic and led to better hatching of *Artemia*. Moreover, they could also reduce the abundance and pathogenic effect of *V. parahaemolyticus in vitro*. The metabolites from the potential probiotic LAB could serve as alternative anti-AHPND agents. This implies that the administration of probiotics along with or before the infection proved to be beneficial to the host organism.

Probiotic administration in aquaculture is an intriguing but under-used strategy. Certain probiotics and their peptides can have a positive impact on the physiological aspects of hatching, growth and survival of the organisms. Hence, using probiotics as an alternative treatment to antibiotics can result in reduction of the antimicrobial resistance of pathogens (Reverter et al. 2020; Fernandes and Jobby 2022). The application of these potential probiotic lactic acid bacterial strains could prove beneficial against infection by pathogenic bacteria, minimize antibiotic resistance risks and provide significant value to animal and

human health. This study provides strong evidence that indigenous lactic acid bacteria isolates could be used as an antibiotic-free prophylactic method against vibriosis and AHPND.

Disclosure statement

No potential conflict of interest was reported by the author (s).

Data availability statement

All data generated or analysed during this study are included in this article and its supplementary information file.

Ethical approval

The care and use of animals for scientific research governed by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests (Animal Welfare Division), Govt. of India were adopted in the study.

Authors' contribution

The idea of the project and the design of the work was conceived by Abigail Fernandes who carried out the literature search, experimentation, and analysis and wrote the manuscript. Avelyno D'Costa carried out the statistical analysis, helped in the original draft preparation and editing. Santosh Jathar provided resources and helped in analysis. Akhil Nair carried out the molecular docking analysis and helped with writing the original draft and editing. Anoop Kumar Yadav analysed the images and helped with writing the original draft. Dr Pamela Jha helped provide resources, in interpretation of data and editing the draft. Dr Vinothkannan helped in molecular docking, critical revision and editing the draft. Renitta Jobby supervised the work, provided resources, interpreted the findings, and edited the original draft and helped in critical revision. All authors provided feedback and helped shape the research, analysis and manuscript.

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