RESEARCH PAPER

Isolation and screening of antibacterial activity and probiotic potential of bacteria isolated from Fenneropenaeus indicus shrimp.

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ABSTRACT

The present work aimed to identify probiotic bacterial strain from Fenneropenaeus indicus (normal shrimp). Among the isolated lactic acid bacterial strains LPGUS1 showed resistance to low pH, tolerance to bile salts, and sodium chloride. The selected strain was exhibited to antimicrobial activity against E. coli (MTCC40), Bacillus cereus (MTCC430), Klebsiella pneumoniae (MTCC 432), Proteus vulgaris (MTTC 1771), Streptococcus pyogenes (MTTC 442), Vibrio harveyi (MTCC 7771), Salmonella typhi (MTCC 3216) and Staphylococcus aureus (MTCC 7443). Cell-free supernatants of LPGUS1 exhibit maximum antimicrobial activity against Vibrio harveyi and Streptococcus pyogenes of 0.8 cm, Staphylococcus aureus, E. coli, Bacillus subtills and Proteus vulgaris activity of 0.7 cm. The probiotic bacterial strain LPGUSI was tested for susceptibility against the antibiotics such as Sulpha trimethoprim (25µg), Kanamycin (30µg), Erythromycin (15µg) and Penicillin (10µg) using Kirby-Bauer disc diffusion method and exhibit (0.5 cm) inhibition zones for Sulphatrimethoprim and (1.1 cm) Erythromycin, while Kanamycin and Penicillin show 0.1–1 cm zones, the strain LPGUS1- demonstrate a pattern of broad resistance to Penicillin. The selected strain LPGUS1 was identified by 16S RNA genome sequencing as Lactococcus gravieae. This present study provides valuable insights into the probiotic nature of this strain, underscoring the safe utilization of Lactococcus gravieae..

Keywords: Probiotics, marine shrimp, 16S rRNA sequencing, antimicrobial activity, Lactococcus gravieae, aquaculture sustainability...

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INTRODUCTION

Aquaculture, an excellent food-producing sector, is touted to be a major source of high-quality protein for the world's booming population in the near future. However, the recent decades saw a decrease in aquaculture production accompanied by the emergence of deadly diseases (Thitamadee *et al.,* 2016). In order to prevent disease outbreaks and promote growth in shrimp farming, the industry witnessed the abuse of antibacterial drugs, pesticides and disinfectants in aquaculture. Probiotics have gained significant attention in recent years due to their potential applications in sustainable aquaculture practices, human health, and food preservation (Amin *et al*., 2022). The Food and Agriculture Organization and the World Health Organization define probiotics as live microorganisms that confer health benefits on their hosts when ingested in an adequate concentration (Salminen *et al*., 2021). Over the last decades, studies on probiotics have expanded tremendously. Numerous in vivo studies have found that, when adequately administered, probiotics modulate the gut microbiota by promoting the growth of beneficial microorganisms in the gastrointestinal tract (GIT) (Vadopalas *et al*., 2022). Most strains of lactic acid bacteria (LAB) are commonly used as probiotics in foods (Zapassnik *et al*., 2022). LAB are a group of bacteria that include genera such as *Lactobacillus, Lactococcus, Pediococcus, Enterococcus, and Streptococcus,* are Gram-positive cocci or rods, and are acid-tolerant, non-respiring but aerotolerant bacteria (Shokryazdan *et al*., 2017). They are naturally present in fermented foods, composts (Tran *et al*., 2019), GIT (Marchwinnska and Gwiazdowska, 2021), vaginal tract (Silva *et al*., 2022), plant surfaces (Yu *et al.,* 2020), and silages (Bohn *et al*.,2017).

Among the probiotics discovered, lactic acid bacteria (LAB) and *Bacillus* sp. have been proven to possess the capabilities of a long- lasting shelf life, production of antimicrobial substances as a secondary metabolite (non-pathogenic and non- toxic) and resistance to the conditions of extreme pH and temperature. In addition, probiotics are considered as safe additives, to provide health benefits to the cultured fish by enhancing growth and immunity, improving feed utilization rate, increasing digestive enzyme activity, maintaining water quality, controlling diseases, modulating microbial colonization in the intestine and pre-digestion of anti- nutritional factors present in the feed

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(Amoah *et al.,* 2025). However, the successful application of probiotics in shrimp farming relies on the isolation, characterization, and molecular identification of beneficial bacterial strains with proven probiotic properties, such as antimicrobial activity, adhesion capabilities, and tolerance to gastrointestinal conditions. Further, bacterial strains with probiotic potential isolated from marine samples have demonstrated vital functions. Research has focused on isolating and molecularly identifying proteolytic bacteria from various marine sources, such as vaname shrimp ponds (Fitriadi *et al*., 2023).

The criteria for the selection of probiotic strains are considered important before their use in animal and/or human studies. The most important feature of a probiotic is its potential health effect and safety. Desirable properties of probiotics include their ability to survive in the GIT, their antimicrobial activity against pathogenic microorganisms (Reuben *et al.,* 2020). Exploring the target functions and technological applications, screening, selecting, and evaluating new probiotic bacteria, the candidates should produce extracellular antimicrobial ability by converting carbohydrates, proteins, and other minor compounds into important substances that can inhibit pathogenic bacteria or by competing for nutrients, aggregating with pathogens, and stimulating the immune system. Hence, to obtain new bacterial strains with promising probiotic potential, we collected gonad dissections from the marine shrimp *Fenneropenaeus indicus* and isolated potential bacterial strain and assessed antimicrobial activity, stress tolerance, safety characteristics, and growth performance were assessed. Our study will provide new probiotic bacterial candidates for further probiotic development and industry.

2. Materials and methods

Isolation of probiotic bacterial strains from marine shrimp *Fenneropenaeus indicus*

2.1.a Collection of samples

The marine shrimp sample *Fenneropenaeus indicus* (normal shrimp) (Fig 1), was collected from Vizhinjam port, Thiruvananthapuram (8.021°N, 70.0°E) during the month of January 2023. The sample was collected in clean, sterile, wide-mouthed disposable plastic container, without disinfectant and detergent residue and tight-fitting leak-proof. Immediately after collection, the sample was transferred to the laboratory for further analysis and stored aseptically in low temperature (−4°C) refrigerator to protect from contamination and deterioration.

2.1.b Culture Media

The standard MRS media was used for the isolation of probiotic bacterial strains from the shrimp gut samples. Additionally, 0.05% cysteine was added to improve the specificity and pH of this medium was adjusted to 6.5 ± 0.2 for isolation of probiotic bacterial strain.

2.1.c Isolation of probiotic bacteria

The collected sample shrimp was rinsed thoroughly in 70% ethanol prior to dissection as surface sterilization. Using sterile forceps, the shrimp gonad sample (Fig 2) were extracted aseptically and homogenized in phosphate-buffered saline (10 mM PBS, pH 7.2). The samples were serially diluted up to 10^{-7} dilutions, following this, 100 microliters of the serially diluted sample were serially spreaded on MRS agar plates and incubated for 48-72 hours at 37°C. By repeated subculturing the distinct colonies were selected and purified using MRS agar medium and stored for future use at 4°C.

Fig 1: Marine Shrimp *Fenneropenaeus indicus* (Normal)

Fig 2: Gonad sample dissected from *Fenneropenaeus indicus.*

Identification of probiotic bacterial strain

The isolated colonies from MRS agar media were subjected to repeated subculturing and identified phenotypically and genotypically. The identification was performed according to Bergey's manual of determinative of bacteriology. The isolated culture was stored at 4 °C for further studies.

2.2.a Morphological characterization of probiotic bacterial strain

Morphological characterization of isolated bacterial isolates was studied based on the methodology of Cappuccino and Sherman (2014). Gram staining, motility and colony characteristics such as shape, size, elevation, margin and colour was noted.

2.2.b Biochemical characterization of probiotic bacterial strain

The isolated probiotic bacterial strains were subjected to biochemical tests such as indole, methyl red, voges proskauer, citrate utilization, hydrogen sulfide test, catalase, oxidase, triple sugar iron (TSI), fermentation test, urease test, gelatin hydrolysis and casein hydrolysis and the bacterial strains were identified based on Bergey's manual of systematic Bacteriology, (Hammes *et al*., 2009).

Screening of probiotic potential of isolated probiotic bacterial strain

2.3.a. Low pH tolerance

To evaluate the efficacy of using these probiotics for oral application, the tolerance to low pH in the stomach was evaluated according to (Oh *et al*., 2018). The probiotic bacterial strains were inoculated in MRS broth with varying pH levels of 2, 3, 4, and 5, in which the medium was adjusted by adding HCl or NaOH. The inoculated tubes were incubated at 37ºC for 24 hours and optical density was measured at 600 nm. Cultures grown on MRS broth without pH served as controls.

2.3.b Bile salt tolerance assay

The ability of probiotic strain to survive the bile salt concentration in the intestine environment was determined using the bile salt tolerance assay as reported (Manovina *et al.,* 2022). 0.1-1%w/v bile salt containing nutrient broth was prepared and an inoculum of probiotic bacterial strain in its exponential growth phase was added and incubated for 24 hours at 37°C. After incubation, the optical density was measured at 600 nm. Cultures grown on MRS broth without bile salt served as controls.

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2.3.c NaCl tolerance assay

Testing the salinity tolerance of probiotic bacteria was essential to understand their viability and functionality under different environmental conditions was assessed following Putri *et al.,* (2020). MRS broth was prepared with different concentrations of NaCl, such as 0.5%, 1%, 1.5%, 2%, 2.5%, and 3% and inoculated with 1 % (v/v) fresh overnight inoculum of selected probiotic bacterial strain. After incubation at 37°C for 24-48 h, growth was measured using a UV spectrophotometer at OD at 620 nm. Cultures grown on MRS broth without sodium chloride served as controls.

2.3.d Gelatinase activity

The ability to degrade gelatin by the probiotic bacterial strain was investigated using nutrient media containing 3% (w/v) gelatin according to the method reported by Daliri *et al*., (2022). The probiotic sample was inoculated on the plates and incubated for 24 hours. *Staphylococcus aureus* MTCC 7443 was used as a reference for quality control and was grown on tryptone soya broth (TSB) containing 3% (w/v) gelatin. Coomassie blue stain was added to the culture plates to evaluate gelatin-degrading ability by the presence of a clear zone around the bacterial colony.

2.3.e Hemolytic activity

The probiotic bacterial strains were further subjected to hemolytic assay by inoculating them in blood agar medium. The inoculated plates were incubated for 24-48 hours at 37°C and the hemolytic zones were observed. Depending upon the mode of hemolysis the probiotic bacterial strains were subsequently classified as alpha, beta or gamma hemolytic strains. To be considered safe the probiotics must display gamma hemolysis (Yasmin *et al.,* 2020).

Molecular identification of probiotic bacteria by 16S rRNA sequencing

a) Isolation of genomic DNA from probiotic bacterial isolates

Genomic DNA from selected probiotic bacterial isolates were isolated by the method proposed by Smoker and Barnum *et al.,* 1988. Overnight broth culture of 1.5ml was taken in a 2 ml microcentrifuge tube and centrifuged at 8000 rpm for 5 minutes. After centrifugation, the supernatant was discarded, and the pellet was suspended in 200µl of 1X TE buffer and 100µl of 10% SDS and mixed by vortexing. The tubes were kept in a water bath at 60°C for 20 minutes. After 20 minutes of incubation, 300µl of Phenol: Chloroform: Isoamyl alcohol mixture (24:25:1) was added to extract the DNA and mixed by vortexing. Again, the tubes were centrifuged at 10000 rpm for 10 minutes to separate the phases. The aqueous phase containing the DNA was carefully removed and transferred to new tubes. Equal volume of 100% isopropanol was added to the tubes containing the aqueous phase and mixed by inverting the tubes 3 to 4 times and the tubes were centrifuged at 10000 rpm for 10 minutes to pellet the DNA. The supernatant was discarded and to the collected pellet, 200µl of 70% ethanol was added and centrifuged at 10000 rpm for 10 minutes and the pellet was airdried to get purified DNA. Purified dried DNA pellet was resuspended with 20 µl of TE buffer and dissolved by tapping. The obtained DNA pellets were purified by agarose gel electrophoresis (Lastauskienee *et al*., 2021).

b) 16S rRNA gene sequencing of probiotic bacterial isolates

Fragments of the 16S rRNA genes of each isolated bacterial strain were separately amplified using the eubacterial universal primers 27F (5′ CAGGCCTAACACATGCAAGTC 3′) and 1492R (5′TACGGYTACCTTG TTACGACTT3′). Each vial contained 5 µl of 2X PCR master mix, 0.25 µl of each primer, isolated DNA 1 µl and 4 µl of distilled water. The constituted reaction was denatured at 95°C for 5 minutes. Cycling began with denaturing at 95°C for 30 seconds, annealing at 60°C for 40 seconds, extension at 72°C for 60 seconds and final extension for 7 minutes at 72°C and cycle was repeated for a total of 30 cycles was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Bio systems). The reaction was then purified on sepheadex plate (Edge Biosystems) unbound labelled and unlabelled nucleotides and salts were removed by centrifugation.

c) Phylogenetic Analysis

Consensus sequence of 16SrRNA gene isolates were generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using the neighbour joining and Kimura 2parameter method. The topologies were evaluated by performing bootstrap analysis of 1000 sets by using MEGA 7 (Varghese

2012). The assembled complete 16S rRNA sequences of twelve distinguishable probiotic bacterial strains were deposited in NCBI Gene Bank using the BANKIT sequence submission tool (Narasimman *et al*., 2021).

2.5 Antibiotic Susceptibility Test

The susceptibility of the isolated probiotic bacterial strains to antibiotics was tested according to Wang *et al.,* 2021. The selected probiotic bacterial strains were spread on the surface of Muller Hinton agar plates and tested the susceptibility against the following antibiotics: Sulpha trimethoprim (25µg), Kanamycin (30µg), Erythromycin (15µg) and Penicillin (10µg) using Kirby-Bauer disc diffusion method. The inoculated plates were incubated at 37° C for 24-48 h and were examined for the presence or absence of zones of inhibition in cm.

2.6 Antimicrobial Activity

For antimicrobial activity, eight pathogenic strains were used to investigate the activity of isolated probiotic bacterial strains according to Balouiri *et al.,* 2016. The pathogenic bacteria include *E. coli* (MTCC40), *Bacillus cereus* (MTCC430)*, Klebsiella pneumoniae* (MTCC 432), *Proteus vulgaris* (MTTC 1771)*, Streptococcus pyogenes* (MTTC 442)*, Vibrio harveyi* (MTCC 7771), *Salmonella typhi (*MTCC 3216) and *Staphylococcus aureus* (MTCC 7443) were separately inoculated in their respective growth media and incubated at 37º C for 24-48 hrs. The antimicrobial activity was performed using agar-well diffusion method in which the pathogenic bacterial strain was spread on the agar surface uniformly using a spreader and the probiotic bacterial cell free supernatant were placed in 7mm diameter well on Muller–Hinton-agar plates. The plates were incubated at 37º C for 24-48 hrs. After incubation the plates were observed for zone of inhibition and diameter of inhibition zone was measured in cm. The inhibition zones of the probiotic bacteria are compared with controls to evaluate their antimicrobial activity. Inhibition was measured and classified as sensitive (<20mm), moderate (10-20mm) and low or resistant (>10mm).

2.7 Statistical analysis

All the experiments were performed in triplicate. Results were statistically analyzed and expressed as mean ± standard deviation calculated at a 95% confidence level. All statistical analyses were performed using SPSS version 24.0.

3. Results and discussion

3.1 Isolation, Morphological and Biochemical screening of bacteria

Probiotics in shrimp aquaculture have emerged as sustainable alternatives to antibiotics, offering benefits such as enhanced growth, improved immunity, disease resistance, and better water quality. Recent research has focused on isolating and identifying probiotic bacteria from shrimp and their culture environments.

In the present study the bacterial strain LPGUS1 with probiotic activity was selected from the gonad sample of large shrimp *Fenneropenaeus indicus* using MRS agar plates. The gonad of healthy shrimp is a greater reservoir for endogenous probiotics, which are well adapted to colonize the host's digestive system and provide benefits like pathogen inhibition and nutrient digestion. The probiotic bacterial strain LPGUS1 was gram positive with cocci shaped microscopic observation which are non-motile in nature in which size of the colonies were pinpoint with circular shape, creamy, raised and entire edged in morphology. *Lactococcus gravieae* is a gram positive, spore forming, aerobic/facultative anaerobic bacterium in the genus Bacillus (family *Bacillaceae*). The isolated probiotic bacterial strain shows positive indication to methyl red and catalase and negative observation for rest of all biochemical studies. The results were tabulated in Table 1 and colony morphology of the strain was given in Figure 3. The strains morphology seen to be pinpoint, circular, white, smooth, raised and entire. The bacterial strains isolated from soil and used in animal feed (Jimnez *et al*., 2013) which can tolerate GIT conditions, enabling survival through gastric transit (Williams *et al*., 2019). In fact, the isolation of probiotics from shrimp has been shown to be a valid practice for shrimp farming, enhancing the control or inhibition of pathogenic bacteria, zootechnical performance, digestive enzyme activity and host immune responses against pathogens or physical stress (Li *et al.,* 2020).

Table 1: Morphological and Biochemical characterization of the probiotic bacterial strain isolated from the marine shrimp *Fenneropenaeus indicus.*

Sl. No	Test	LPGUS1
1	**Morphological studies**	
	Gram staining	Gram +ve
	Shape	Cocci
	Motility	Non motile
2	**Colony Morphology**	
	Size	Pinpoint
	Shape	Circular
	Colour	White
	Elevation	Raised
	Margin	Entire
3	**Biochemical Characterization**	
	Indole	-ve
	Methyl red	+ve
	Vogus proskaeur	-ve
	Urease	-ve
	Nitrate reduction	-ve
	Gelation hydrolysis	-ve
	Casein Hydrolysis	-ve
	Catalase	+ve
	Sugar Fermentation	-ve

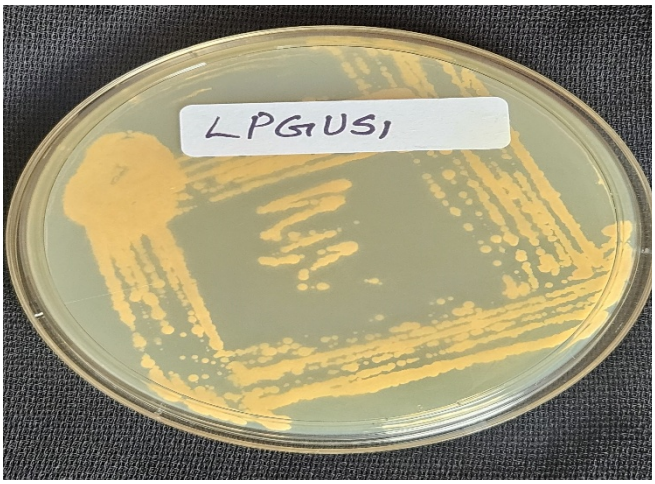

Fig 3: Colony morphology of Probiotic bacterial strain isolated from the marine shrimp *Fenneropenaeus indicus*.

Molecular Identification of the species

The 16s rRNA sequencing BLAST homology indicated that bacterial strain LPGUS1 was identified as prospective probiotics, *Lactococcus gravieae* respectively with 99% similarities with the probiotic strains. The similar sequences are subjected for phylogenetic tree construction which was shown in Fig 4.

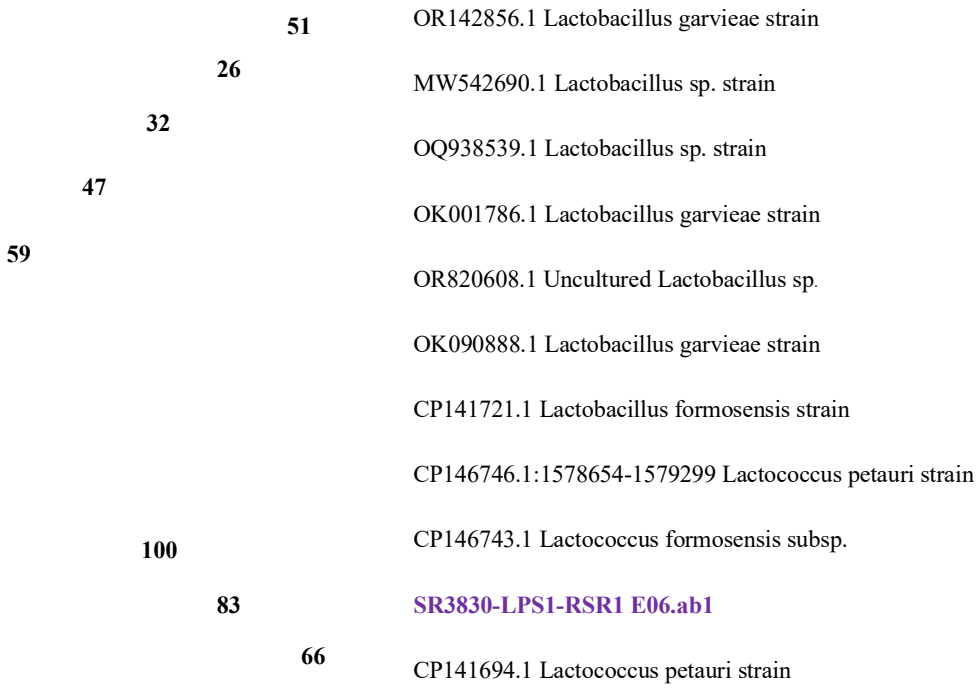

Fig 4: Phylogenetic tree constructed showing similarities of the isolated strains with probiotic strains

In vitro characterization of probiotic properties of the marine shrimp *Fenneropenaeus indicus*

Low pH tolerance assay

Low pH plays a key role in the digestion of proteins by activating digestive enzymes, which together break down the long chains of amino acids of proteins. At pH 2, LPGUSI shows the highest activity of 0.499 ± 0.0020 and slightly lower at the pH 4 of 0.342±0.0037. At pH 3, LPGUSI shows the mild activity of 0.095 ± 0.0020 and at the pH of 5 the activity was slightly higher than that of pH 3 and proven to be maximum activity at the pH of 2. The results were given in the Table 2. *L. garvieae* (LPGUS1) showed poor acid tolerance, particularly at pH 2–3, with survival dropping below 50% after 3 hours, consistent with *Lactococcus garvieae* and *Lactobacillus* strains from cheeses (Baig *et al.,* 2022; Zommara *et al*., 2023) and the strain may require protective measures like encapsulation (Wang *et al*., 2015).

Bile salt tolerance assay

Bile salts are steroid acids produced in the liver and stored in the gallbladder that help in the digestion of fats. The effect of bile salts on probiotic strains confirmed the survival at the tested concentrations and thus proved safe for oral applications. The isolated probiotic bacterial strain was found to be tolerant to bile concentrations from 0.5 to 2%. The results were given in Table 4. The bacterial strain LPGUS1 showed maximum activity of 0.207±0.0016 at 2% concentration and showed mild activity at 1.5% concentration of 0.189±0.0020 and low activity at the concentration of 0.5 and 1%. The results were tabulated in Table2. In contrast, *L. garvieae* (LPGUS1) showed weaker bile tolerance, particularly at higher concentrations (1.5–2%). Proteomic analysis of *Lactococcus garvieae* identified

47 differentially expressed proteins under bile stress, with 22 upregulated, suggesting adaptive mechanisms but limitations at higher exposures (Baig *et al.,* 2022). *Lactobacillus* strains from cheeses showed survival rates of 42.25–85.25% at 0.3% bile salts, with an inverse relationship to increasing bile concentrations (Zommara *et al*., 2023).

3.3.3 NaCl tolerance assay

The sodium chloride tolerance of probiotic bacterial strains was checked across six different concentrations of NaCl (0.5%, 1%, 1.5%, 2%, 2.5%, and 3%). At 2.5%, LPGUS1 exhibits the highest activity (0.179±0.0012), indicating strong tolerance at high sodium chloride levels. It was proven that bacterial strain LPGUS1 can tolerate high salt concentration with high activity. It was proven that as concentration of sodium chloride increases there activity also increases and gets start to decrease above to the concentration of 2.5%. The results were tabulated in Table 2. LPGUS1 (*Lactococcus gravieae*) displays low initial growth but a sharp increase and thrive at 3% NaCl, indicating a potential preference for higher salinity, a characteristic of moderate halophiles. While information on *Lactococcus gravieae* is less common than on its closely related genus *Lactococcus garvieae*, certain lactic acid bacteria, including *Lactococcus* species, are known to tolerate or even grow optimally in saline conditions, which can be linked to their presence in fermented foods where salting is a preservation method (Tanasupawat *et al*., 2008). Therefore, the behavior of LPGUS1 suggests a strong halotolerant or possibly halophilic nature, a physiological adaptation that highlights the diverse niche-specific characteristics within this group.

Table 2: Bile salt, pH and NaCl tolerance of the probiotic strain LPGUS1.

Probiotic bacterial strain (LPGUS1)	**Bile salt concentration (Percentage)**					
	0.5%	**1%**	**1.5%**	**2%**		
	0.102±0.0028	0.10±0.0020	0.189±0.0020	0.207±0.0016		
	pH					
	2	**3**	**4**	5		
	0.499±0.0020	0.095±0.0020	0.342±0.0037	0.123±0.0029		
	Concentration of NaCl					
	0.5%	**1%**	**1.5%**	**2%**	**2.5%**	**3%**
	0.069±0.0008	0.129±0.0024	0.081±0.0024	0.113±0.0012	0.179±0.0012	1.241±0.0024

3.3.4 Gelatinase activity

Gelatin is a natural biomacromolecule derived from collagen in animal skin, bones, and connective tissues. Gelatinase is an enzyme produced by several bacteria that is capable of degrading gelatin. The probiotic bacterial strain LPGUS1 indicate no zone formation around the colony which indicates that the strains did not have the property of breaking down gelatin, thus, they were considered safe to use. The gelatin degradation plates were given in Figure 5. *L. garvieae* (LPGUS1) lack of gelatinase activity is notable, as some *L. garvieae* strains are associated with fish pathogenicity and may produce gelatinase. However, *L. garvieae* C47 from camel milk identified probiotic strains lacking gelatinase, suggesting strain-specific differences and supporting LPGUS1 safety for probiotic use (Baig *et al.,* 2022). The positive control, *S. aureus* MTCC 7443, exhibited expected gelatinase activity, consistent with its known virulence. *S. aureus* strains confirmed gelatinase as a common virulence factor, with clear zones of hydrolysis in 90% of isolates, validating its role as a positive control (Smith *et al.,* 2017).

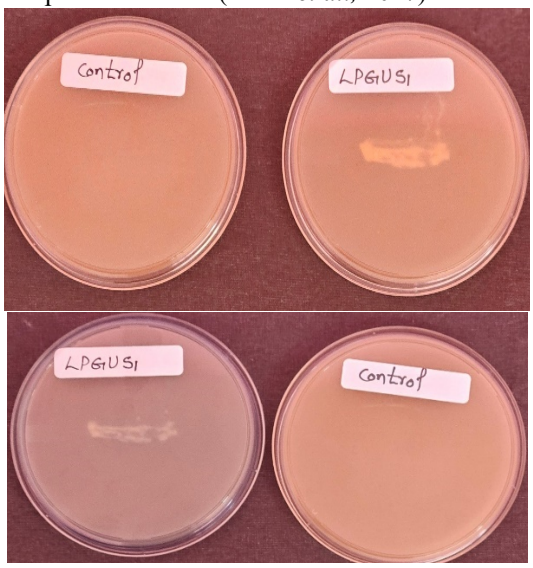

Fig 5: Gelatinase activity of the probiotic strains and the respective control.

3.3.5 Hemolytic activity

The probiotic strains LPGUS1 showed no hemolytic zone (γ-hemolysis) as indicated by the absence of a zone diameter and distance measurement. This suggests these strains do not produce haemolysis, making them potentially safe for probiotic applications. This proves the non-virulent property of the selected probiotic strains and thus safe for consumption. *L. garvieae* (LPGUS1) lack of hemolytic activity is notable, as some *L. garvieae* strains are associated with fish pathogenicity and hemolysis. *L. garvieae* C47 from camel milk identified non-hemolytic probiotic strains, suggesting strain-specific safety and supporting LPGUS1 potential for safe applications (Baig *et al*., 2022).

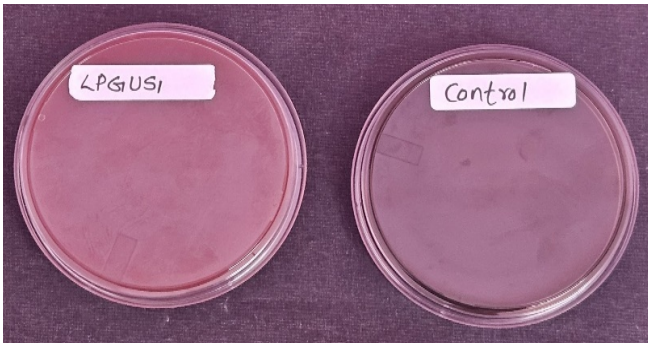

Fig 6: Hemolytic activity of the probiotic strains and the respective control.

Antibiotic Sensitivity

The bacterial strains LPGUS1 was tested for antibiotic activity using antibiotic disc such as Sulpha trimethoprim (25µg), Kanamycin (30µg), Erythromycin (15µg) and Penicillin (10µg). The results of present study were shown in Table 3 and Fig 7. The bacterial strain LPGUS1 exhibit small inhibition zones (0.5 cm) for Sulphatrimethoprim and Erythromycin (1.1 cm), suggesting weak or intermediate sensitivity, while Kanamycin and Penicillin show minimal effect (0.1–1 cm zones or no zone). The probiotic bacterial strains exhibited strong adhesion and can effectively colonize in the gut region and provide antibiotic property. *Lactococcus gravieae* LPGUS1- demonstrate a pattern of broad resistance to Penicillin, consistent with intrinsic mechanisms observed in many *Lactobacillus* and *Enterococcus* species, where the absence of sensitive penicillin-binding proteins or low-affinity targets prevents effective β-lactam inhibition, as documented in studies on commercial probiotic isolates showing universal Penicillin G resistance rates exceeding 50% (Seyirt *et al.,* 2023).

Table 3: Antibiotic susceptibility assay of probiotic bacterial strains

Probiotic bacterial strain	Zone of Inhibition (cm)			
	Sulphatrimethoprim (25µg)	Kanamycin (30µg)	Erythromycin (15µg)	Penicillin (10µg)
LPGUS1	0.5	No Zone	1.1	No Zone

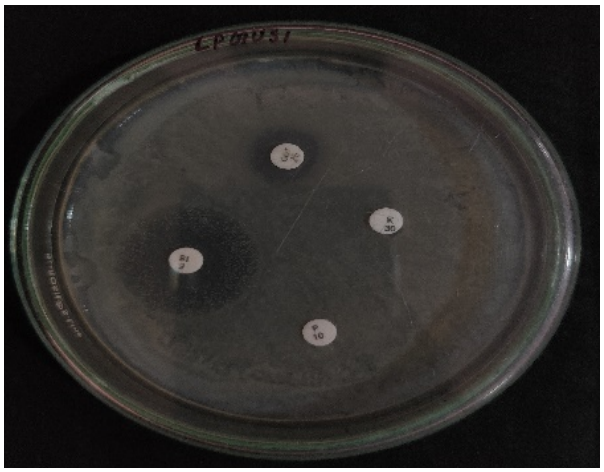

Fig 7: Antibiotic Susceptibility assay of probiotic bacterial strains

Antimicrobial Activity

Probiotics produce metabolites that can be useful for the host. Bacteriocin is a metabolite that hinders and/or suppresses the growth of pathogenic bacteria in the gonad. To investigate their antimicrobial property, eight pathogenic bacteria (*E. coli*, *Bacillus cereus, Klebsiella pneumoniae*, *Proteus vulgaris, Streptococcus pyogenes, Vibrio harveyi, Salmonella typhi* and *Staphylococcus aureus*) were treated with the CFS of the selected two probiotic bacterial strain. The probiotic bacterial strains LPGUS1 exhibit maximum antimicrobial activity against *Vibrio harveyi* and *Streptococcus pyogenes* of 0.8 cm, *Staphylococcus aureus, E. coli, Bacillus subtills* and *Proteus vulgaris* activity of 0.7 cm and hence the probiotic bacterial strain LPGUS1 showed good activity on selected pathogenic bacterial strains. The results were given in Table 4. Hutt *et al.,* (2006) on *Lactobacillus* strains reported larger zones (15–20 mm) for bacteriocin-mediated inhibition of *E. coli* and *S. aureus*, suggesting that these probiotics may have lower bacteriocin production or rely on other mechanisms like pH reduction.

Table 4:Antimicrobial activity of isolated probiotic bacterial strain

Pathogens	Zone of inhibition (cm) LPGUS1
Staphylococcus aureus	0.7 ± 0.1
Salmonella typhi	0.6 ± 0.1
Klebsiella pneumoniae	0.9 ± 0.1
E. coli	0.7 ± 0.2
Bacillus subtills	0.7 ± 0.1
Vibrio harveyi	0.8 ± 0.1
Streptococcus pyogenes	0.8 ± 0.2
Proteus vulgaris	0.7 ± 0.1

CONCLUSION

The present study confirmed the successful isolation of bacterial strains with prospective probiotic properties. The isolated strains were characterized using morphological and biochemical tests. Molecular identification of the isolated strains confirmed that the bacterial strain was *Lactococcus gravieae*. This result was validated using BLAST analysis and construction of phylogenetic tree. Screening evaluation of the probiotic properties was carried out to prove the potential of the isolated probiotic strains for various applications. All the selected strains were safe for consumption as well as safe for treatment of different diseases caused to varieties of shrimp itself. Indeed, as the development of eco-friendly probiotics is a key aspect of aquaculture health management, these findings hold significant promise for future applications.

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