JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

IN-VITRO ASSESSMENT OF PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM NATURALLY FERMENTED RICE GRUEL OF SOUTH INDIA

Mariyappan Kowsalya[1], Kattakgounder Govindaraj Sudha[1], Saheb Ali[1], Thangavel Velmurugan[1], Gopalu Karunakaran[2], Mohan Prasanna Rajeshkumar[1]*

Address(es):
[1]K.S. Rangasamy College of Arts and Science (Autonomous), Department of Biotechnology, K.S.R. Kalvi Nagar, Tiruchengode, 637215, Namakkal, Tamil Nadu, India.
[2]Biosensor Research Institute, Department of Fine Chemistry, Seoul National University of Science and Technology (Seoul Tech), Gongneung-ro 232, Nowon-gu, Seoul, 01811, Republic of Korea.

*Corresponding author: prasanna4d@gmail.com; prasanna@ksrcas.edu

https://doi.org/10.55251/jmbfs.4908

ARTICLE INFO

Received 14. 6. 2021
Revised 6. 5. 2022
Accepted 13. 5. 2022
Published 1. 8. 2022

Regular article

OPEN ACCESS

ABSTRACT

Fermented foods prepared traditionally involves natural fermentation by numerous beneficial bacteria. The scientific evidence for the presence of specific probiotic strains in fermented rice gruel is lacking. The objective of our study is to identify the lactic acid bacteria involved in the fermentation of rice gruel and to evaluate their probiotic characteristics. From the fermented rice gruel, *Pediococcus pentosaceus* strain PRK1 (MT019527) and *Lactobacillus plantarum* strain PRK7 (MT022517) and *Lactobacillus plantarum* strain PRK11 (MT022576) were identified by 16s rRNA gene sequencing and their sequence was submitted to NCBI. The *invitro* probiotic characterization methods like autoaggregation, co-aggregation, tolerance to acidic pH, bile, Nacl, antibacterial activity, antibiotic susceptibility and safety assessment were analyzed in this study. The evaluated *invitro* probiotic properties of the isolates were tolerance to pH 2; autoaggregation is 91%, 83 %, and 89%; co-aggregation is 36.1%, 37.1%, and 42.5%; the percentage of suppression at 0.3% of bile is 88%, 86.5%, and 81.3% and tolerance to 4% of NaCl. The isolates exhibited antibacterial activity against the clinical pathogens *E. coli*, *P. aeroginosa*, *Proteus* sp., *K. pneumoniae*, *Salmonella* sp., *S. aureus*, *Staphylococcus haemolyticus*, *Enterococcus faecium*, and *S. epidermidis.* The safety assessment of the isolates exhibits no hemolytic activity and it is also sensitive to most of the tested antibiotics. Further, nutrition profiling of fermented rice gruel shows increase in micronutrients and the presence of vitamin B9 and B12. Hence, this study provides evidence for the presence of lactic acid bacteria in the fermented rice gruel an indigenous food which can be developed as functional food in future.

Keywords: Lactic acid bacteria; Probiotic activity; Fermented rice gruel; Nutrition parameter

INTRODUCTION

India is rich in diverse culture with many religions, varied climatic environments, hence, it has innumerable fermented foods prepared by ethnic knowledge of ancestors **(Sathe & Mandal, 2016)**. Fermented foods and fermented beverages were an essential feature of their heritage. The tribal people use the available raw materials (grains, milk, plant leaves, roots, etc.) to prepare fermented foods with a pleasant flavor, consistency, and color. Fermented foods prepared traditionally are olden biotechnological processes involving microorganisms or starter cultures to preserve the perishable foods for a longer duration **(Ray *et al.*, 2016)**. In fermented foods, probiotics are used to enhance the nutrients and to maintain a healthy gut **(Singh & Rao, 2021)**. The beneficial microbes in food, especially the lactic acid bacteria are considered as probiotics. Lactic acid bacteria and starter cultures convert the biochemical and organoleptic characteristics of substrate into metabolites and enhance the ample variety of micronutrients like vitamins, minerals, essential amino acids **(Giri *et al.*, 2018)**. It also produces several bioactive compounds contributing to health benefits, like antioxidant, anti-inflammatory, and immunomodulatory effects **(Chugh & Kamal-Eldin, 2020)**. The bioactive compounds produced by probiotics include bacteriocin, organic acids, exopolysaccharides, short chain fatty acids, amino acids and peptides, cholesterol removal, vitamins, gamma aminobutyric acid, beta-galactosidase and lactase. **(Chugh & Kamal-Eldin, 2020).**

The lactic acid bacteria (LAB) are Gram-positive, catalase-negative, nonmotile, non-spore-forming with rod- or cocci-shape in morphology **(Yu *et al.*, 2018)**. The autoaggregation, coaggregation, hydrophobicity, tolerance to low pH, Nacl and bile, antimicrobial substances and resistance to antibiotics are the invitro tests used to evaluate the probiotic characteristics of lactic acid bacteria **(Arumugam & Govindaraj, 2021).** Nowadays, fermented foods are becoming functional foods. The lactic acid bacteria are the predominant group of microorganisms that are accepted as safe for consumption. They play a key role in the fermentation of foods and as a preservative **(Patil *et al.*, 2019)**. During fermentation, probiotics enhance the minerals and B-group vitamins and facilitates the colonization of beneficial microbes in the gut **(Ray *et al.*, 2016)**. The probiotic bacteria exhibit therapeutic properties in curing the ailments like intestinal bowel syndrome, ulcerative colitis **(Pacheco *et al.*,2021)**, maintenance of gut-brain axis **(Margret *et al.*, 2021)**. Therefore, fermented foods have many health benefits to the host. The probiotics are identified in fermented foods like takrarishta **(Chopade *et al.*, 2019)**, Tunisian fermented foods **(Abid *et al.*, 2018)**, pomegranate **(Gajbhiye, 2016)**, Dahi products **(Nurmiati *et al.*, 2018)**, yogurt **(Mohanty *et al.*, 2019)**, goat and cow milk **(Kondrotiene *et al.*, 2018)**. These served as evidence for the presence of probiotics in fermented foods. The lactobacillus bacteria present in fermented foods have various technological applications in the food industry **(Giri *et al.*, 2018)**. From traditional beverage Haria (Rice Beer) a probiotic strain *Bifidobacterium* was isolated with probiotic properties has application in fortification of cereal-based foods **(Petrova & Petrov, 2020)**. However, there were only very few research articles on the analyzes of fermented rice gruel. Further, the research on the presence of specific strains of probiotics involved in the fermentation of rice gruel is very less compared to that on kefir, yogurt, cheese, curd, miso, kombucha, and tempeh. Hence, fermented rice gruel was selected as a sample to explore the probiotic bacteria in the fermented rice gruel. The objective of our study is to explore the lactic acid bacteria involved in the fermentation of rice gruel and to analyze the nutritional factor and to evaluate the probiotic characteristics, antibacterial, antibacterial susceptibility, safety assessment of the isolates from rice gruel.

MATERIAL AND METHODS

Sample preparation

The fermented rice sample was prepared by cooking Ponni variety rice (*Oryza sativa*). First, 100 g rice was soaked in water for half an hour and cooked in 250ml of plain water with the addition of salt, and the excess water was drained off. The rice was cooled down to room temperature (30 ºC) followed by the addition of sterile water. Then, the rice was allowed to ferment in an earthen pot overnight. The pH of the fermented rice gruel sample was observed as 6.5. Then, the sample was aseptically transferred to the laboratory for further studies.

Isolation of lactic acid bacteria in fermentation rice gruel

The de Mans, Rogosa, and Sharpe (MRS) broth was used to isolate LAB. The one ml of fermented rice gruel sample was diluted in 9 ml sterile distilled water and serially diluted up to 10^{-7}. Then, 0.1 ml of sample from each dilution was spread on the MRS agar plate with respective dilutions. Thereafter, the plates were incubated anaerobically at 35 ºC for 24–48 h in an anaerobic jar with an Anaero Gen gas-pack system. After incubation, the isolates were cultured on the MRS broth with 30% glycerol and stored at –20 ºC **(Bin Masalam *et al*., 2018)**.

Identification of lactic acid bacteria

Phenotypic characterization

The morphological characterization of the isolate was carried out by considering its appearance, colony shape, Gram's staining, catalase test, and motility. The oxygen requirement was determined by inoculating the isolates in thioglycolate broth and incubating them at 35ºC for 24h. The biochemical characterization (indole, methyl red, Voges-Proskauer, Simmon's citrate) of the isolate was performed **(Chowdury *et al*., 2012)**.

Genotypic characterization

From 24h culture, genomic DNA was isolated and evaluated on 1.0% agarose gel electrophoresis. Then, amplification was carried out with 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers. Forward and reverse DNA sequencing reactions of PCR amplicon were carried out with forward and reverse primers using BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. The BLAST was performed for the 16S rDNA gene sequence with the NCBI database and the phylogenetic tree was constructed using MEGA 7 software **(Tallapragada *et al*., 2018; Dowarah *et al*., 2018)**.

Probiotic characterization of the isolate

Acid tolerance

The isolates obtained from the fermented rice gruel sample were subjected to low pH. Briefly, the MRS broth was modified to pH 3, pH 4.5 with 1 N HCL solution, and the MRS broth with a normal pH of 5.5 was used as control. 10µl of 24h culture was inoculated into MRS broth with distinct pH and incubated at 35ºC in anaerobic condition. Then their optical density was measured at 600 nm **(Ayyash *et al*., 2018)**.

Autoaggregation

The isolates were grown in MRS broth and incubated anaerobically at 35ºC for 24h. Then the lactic acid bacterial cultures were centrifuged at 5000 rpm for 10 minutes. The obtained cell pellet was suspended in PBS at pH 7.4 and incubated at 37°C. The initial absorbance (A_0) was taken at 600nm and then after incubation of 3h and 24h, the absorbance (A_t) was again taken. The experiment was performed in triplicate. The percentage of autoaggregation was calculated with the formula $(1– [A_t/A_0]) \times 100$, where and A_0 represents the initial absorbance at $t = 0$ and A_t represents absorbance after incubation at time t **(Chopade *et al*., 2019)**.

Co-aggregation

The co-aggregation attributes of the selected isolates were studied against seven clinical pathogens *Escherichia coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Proteus* sp., *Salmonella* sp., *Staphylococcus aureus*, and *Streptococcus epidermidis* obtained from Alpha Diagnostics Centre, Erode, Tamil Nadu, India. The pathogens were inoculated in tryptic soy broth and incubated at 37°C for 24h. The lactic acid bacteria obtained from fermented rice gruel was inoculated into MRS broth and incubated at 35°C for 24h in anaerobic condition. After incubation, the pathogens grown in tryptic soy broth were centrifuged at 5000g for 10 minutes and the pellet was suspended in 3ml of phosphate-buffered saline (PBS- pH 7.4). Similarly, the 24h culture of isolated lactic acid bacteria in MRS broth was centrifuged and the pellet was suspended in 3ml of phosphate-buffered saline (pH 7.4). Then, the PBS suspension of clinical pathogens and lactic acid bacterial isolates were mixed in a 1:1 ratio, vortexed for 1 min, and incubated at 35 ºC for 4h. Afterwards the OD was measured at 570 nm. The experiments were performed in triplicate and the percentage of co-aggregation was calculated with the formula $([A_{Isolate} + A_{pathogen}] – [A_{mix} (A_{isolate} + A_{pathogen})])/2 \times 100$, where $A_{Isolate}$, $A_{pathogen,}$ and A_{mix} represent the absorbance of lactic acid bacterial isolates, clinical pathogens, and the mixture of lactic acid bacterial isolate and pathogen, respectively **(Prabhurajeshwar & Chandrakanth, 2019)**.

Cell surface hydrophobicity

The 24h culture of lactic acid bacteria in MRS broth was centrifuged at 5000g for 10 minutes and the pellet was dissolved in PBS (pH 7.4). To 3ml bacterial suspension, 1 ml hexadecane/xylene was added and then vortexed well for 1 min and incubated at 37 ºC for 2h for phase separation without agitation. The aqueous phase was gently removed to measure its absorbance at 620 nm **(Botthoulath *et al*., 2018)**.

Bile tolerance

The lactic acid bacterial isolates were tested for tolerance to bile at different concentrations, by using bile at 0.1% and 0.3% in MRS broth. The 18h culture was inoculated into MRS broth with bile and incubated anaerobically at 35ºC for 24h; then, growth was determined by absorbance at 540 nm **(Shehata *et al*., 2016)**. The bacterial culture inoculated into MRS broth without bile served as control. The suppression of growth by bile salt was determined by the formula (Growth in control broth/[Growth in control broth – Growth in bile broth]) × 100 and expressed in percentage.

NaCl tolerance

The isolates were tested at different concentrations of NaCl (2%, 4%, and 6%) in MRS broth. The isolated lactic acid bacteria were inoculated into MRS broth with NaCl and incubated anaerobically at 37°C for 18h; growth of bacteria was observed by measuring the absorbance at 620 nm. The isolates inoculated into MRS broth without NaCl served as control **(Prabhurajeshwar & Chandrakanth, 2019)**.

Carbohydrate profile

The carbohydrate fermentation profile of the isolate was determined by the HiLacto identification kit (HiMedia, India). First, 100µl freshly prepared culture was aseptically inoculated in the API strip and incubated at 35°C. The results are observed for fermentation of sugar after 24–48h of incubation **(Alsona *et al*., 2019)**. For the Esculin hydrolysis test, the cream color turns to black, which indicates a positive result. The different carbohydrates present in the API strip are arabinose, cellobiose, galactose, maltose, mannose, melibiose, raffinose, sucrose, trehalose, and xylose, which turn from purple to yellow, indicating that the isolates have used the carbohydrates.

Antibacterial activity

The antibacterial activity was studied by well diffusion method by using clinical pathogens *E. coli*, *P. aeroginosa*, *Proteus* sp., *K. pneumoniae*, *Salmonella* sp., *S. aureus*, *Staphylococcus haemolyticus*, *Enterococcus faecium*, and *S. epidermidis* obtained from Alpha Diagnostics Centre. The lactic acid bacteria isolated from fermented rice gruel was inoculated into MRS broth and incubated anaerobically at 35°C for 24 h. Then, the bacterial culture was centrifuged at 4500g for 10 minutes and the supernatant was collected and filtered. The clinical pathogens were inoculated into tryptone soy broth the plates were incubated at 37°C for 18 h; then each clinical pathogen was swabbed over a separate Muller- Hinton Agar plate and a 7 mm well was made with a sterile cork borer. Then 100 µl of filtered supernatant of each lactic acid bacterial isolate was loaded to the well and incubated at 37°C for 24h aerobically **(Mohanty *et al*., 2019)**. The antibacterial activity of the isolates was tested against nine clinical pathogens.

Safety Assessment

Hemolytic activity

The hemolytic activity was measured in Columbia Blood Agar, the bacterial isolates were streaked over the blood agar plates and incubated at 37 ºC for 48h to observe the zone of inhibition. The hemolytic activity was characterized based on α-hemolytic (halo zone in green), β-hemolytic (clear zone), and γ-hemolytic (no zone) **(Ayyash *et al*., 2018)**.

Antibiotic susceptibility test

The antibiotic susceptibility of the identified LAB was determined by the disc diffusion method with the Dodeca G-III-Plus disc obtained from HiMedia, India, which consists of 12 clinically prevalent antibiotics namely Penicillin-G, oxacillin, erythromycin, clindamycin, linezolid, co-trimoxazole, vancomycin, ciprofloxacin, tetracycline, cefotaxime, chloramphenicol, and gentamicin. The 18-h-old bacterial culture was swabbed on the MRS agar plate and the Dodeca discs were placed over it and incubated at 37 ºC for 24 h. The zone of inhibition was measured and the isolate was characterized into a distinct group as sensitive(S), intermediate (I), and resistance (R) to antibiotics, based on the Clinical and Laboratory Standards Institute (CLSI) standards.

Organic Acid Profile

The organic acid profile of the lactic acid bacteria was detected by HPLC method. The 24h bacterial isolates were inoculated in MRS broth with 10% of lactose and allowed to ferment at 35º C for 3 days. Then it was centrifuged at 10,000g for 5 minutes. The cell free supernatant was obtained through filtering through 0.45 45µm syringe filter. Then 20µl of sample was injected into HPLC analyzer with flow rate of 1.5ml per minute, 30º C, acetonitrile as mobile phase, with analysis time of 15 minutes with UV absorbance detection in range of 210-230nm **(Nuryana *et al.*, 2019)**.

Nutrition Profiling

The nutrient parameter of fermented rice gruel was analyzed by standard methods. The energy was determined by CMUA&F/NI/SOP/01. Protein, total carbohydrates, and total fat were analyzed by AOAC 2001.11, AOAC 986.25, AOAC 950.52 methods respectively. The minerals sodium, calcium, iron, potassium, magnesium, phosphorous, and zinc were determined by AOAC 2011.14. The vitamin B12 (cyanocobalamin) was detected by AOAC 2011.09 method and the vitamin B9 (Folic acid) was detected by CMUINS/SOP/61 method. Further, the total bacterial count of bacteria in fermented rice gruel was rested by IS5402-2012 (Reaff:2018) method; the yeast and moulds were tested by IS:5403-1999 (Reaff:2018) method and the presence of enteric pathogen salmonella was detected by IS 5887 Part 3-1999 (Reaff:2018).

Statistical analysis

The data were collected in triplicate experiments, and to determine the significant differences in the mean, one-way analysis of variance and independent samples *t*-test were used to statistically analyze the results using SPSS, version 20 software.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria in fermented rice gruel

Almost 70 isolates were obtained from fermented rice gruel. The colonies were selected based on preliminary screening, Gram staining, and catalase test. Among them, 12 isolates were gram-positive, cocci and rod-shaped, nonmotile, and catalase-negative in nature. The biochemical characterization (indole, MR-VP, Simmons citrate, TSI) of the isolates revealed that the bacteria belonged to the LAB group. The oxygen requirement of the isolate showed that most of the isolates belonged to anaerobic bacteria and were microaerophilic. Only three isolates showed good growth in the MRS medium consistently. Hence, among these 12 isolates, only 3 isolates were selected for further studies (Table 1; Fig. 1). LAB is well established in nutrient-abundant habitats and efficiently grown on raw foods. They utilize carbohydrates and synthesize essential amino acids, vitamins, and minerals **(Kondrotiene *et al*., 2018)**.

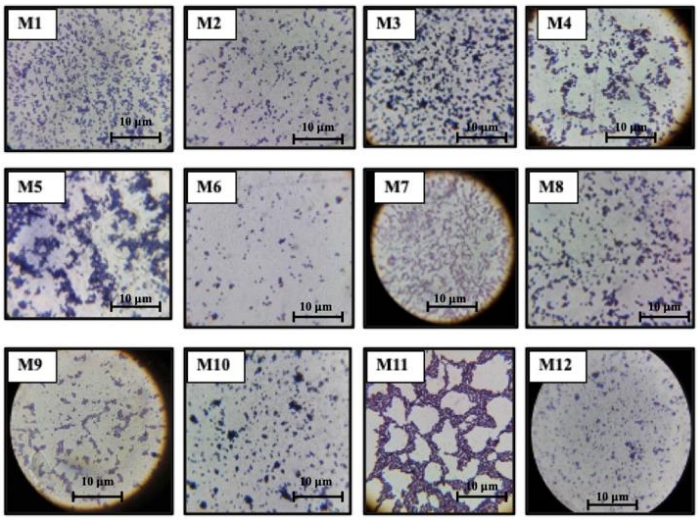

Figure 1 Microscopic examination under light microscope (400x) shows the gram positive isolates either with spherical or rod in shape.

Table 1 Morphological, biochemical characteristics and carbohydrate fermentation profile of lactic acid bacteria isolated from fermented rice gruel.

CHARACTERISTICS	*P. pentosaceus* strain PRK1	*L. plantarum* strain PRK7	*L. plantarum* strain PRK11
MORPHOLOGICAL CHARACTERISTICS			
Shape	Cocci	Rod	Rod
Stain	Gram positive	Gram positive	Gram positive
Catalase	-	-	-
Motility	Nonmotile	Nonmotile	Nonmotile
BIOCHEMICAL CHARACTERISTICS			
Indole	-	-	-
Methyl red	-	-	-
Voges-Proskauer	-	-	-
Simmon's citrate	-	-	-
O_2 REQUIREMENTS	Microaerophilic	Anaerobe	Anaerobe
CARBOHYDRATES FERMENTATION			
Arabinose	+	++	++
Cellobiose	+	+++	+++
Esculin hydrolysis	+	+	+
Galactose	+++	+++	+++
Maltose	+	+++	+++
Mannose	+++	+++	+++
Mellibiose	ND	+	+++
Refinose	ND	ND	+
Sucrose	ND	ND	++
Trehalose	++	+	+
Xylose	ND	ND	ND

+++, excellent fermentation; ++, moderate fermentation; +, less fermentation; ND, no reaction; –, negative.

Genotypic characterization of the isolate

The extracted genomic DNA was amplified with the universal primer. All the isolates were run in agarose gel (2%) with 100bp ladder. The PCR amplicon size was around 1500bp. The obtained PCR product was sequenced in ABI PRISM genetic analyzer and compared with NCBI Blast (Fig. 2). The Isolate 1 was identified as *Pediococcus pentosaceus* strain PRK1, whereas isolates 2 and 3 were identified as *Lactobacillus plantarum* strain PRK7 and *L. plantarum* strain PRK11. The phylogenetic tree of all the isolates was constructed using the MEGA-7 software for *P. pentosaceus* PRK1 (Fig. 3), *L. plantarum* PRK7 (Fig. 4), and *L. plantarum* PRK 11 (Fig. 5). The 16S rDNA sequence of the isolates *Pediococcus pentosaceus* strain PRK1, *Lactobacillus plantarum* strain PRK7, *L. plantarum* strain PRK11 were submitted to the National Centre for Biotechnology Information (NCBI) and assigned with accession numbers MT019527, MT022517, and MT022576, respectively. The bacteria isolated from fermented rice gruel belonged to lactic acid bacteria, which are generally regarded as safe for consumption. Molecular characterization is one of the predominant methods used to confirm a species belongs to lactic acid bacteria. The phylogenetic tree was constructed by using NCBI nucleotide BLAST tool, the neighbor-joining method. From the rice gruel sample, it was found that the isolates belonged to *Pediococcus pentosaceus* and *Lactobacillus plantarum*, which are listed in the QPS standard (Qualified Presumption of Safety) controlled by the Food Safety Authority **(Pinto *et al.*, 2020)**. Previous studies reported that *L. paraplantarum* and *P. pentosaceus* could be obtained from rice bran, sourdough, pickle, fermented sausages, and olive fermentation brine. It has been reported that in east-central India, a popular rice beer called *haria*, which is a tribal beverage, possesses lactic acid bacteria *Lactobacillus* sp., *Bifidobacterium* sp., *Lactococcus* sp., and *Bacillus* sp **(Ray *et al.*, 2017)**.

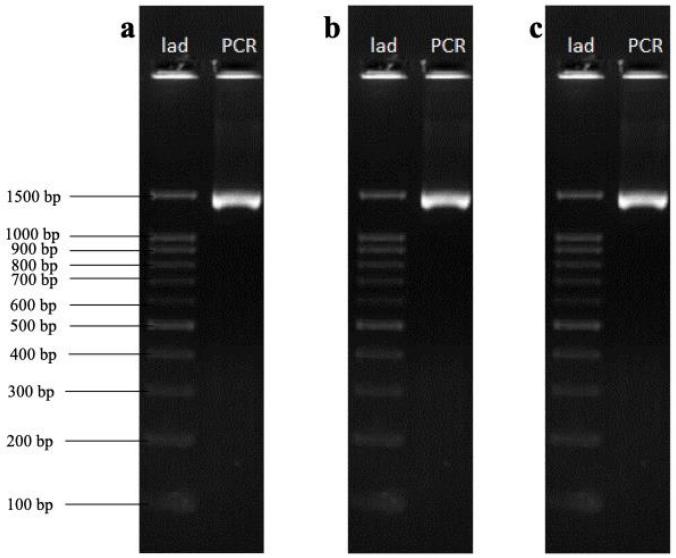

Figure 2 The PCR amplicon product (1500 bp) in 2% agarose gel (**a**) *Pediococcus pentosaceus*; (**b**) *Lactobacillus plantarum* strain PRK7; (**c**) *Lactobacillus plantarum* strain PRK11.

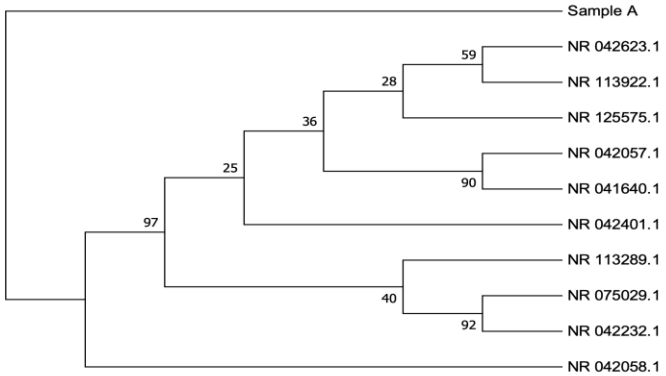

Figure 3 The phylogenetic tree for *Pediococcus pentosaceus* strain PRK 1 was constructed by using the neighbor-joining method in MEGA 7 software.

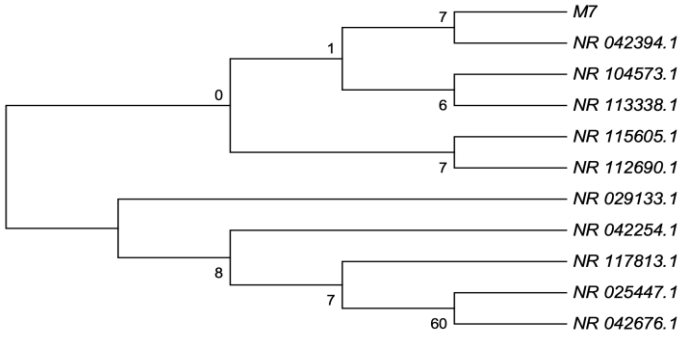

Figure 4 The phylogenetic tree for *Lactobacillus plantarum* strain PRK7was constructed by using the neighbor-joining method in MEGA 7 software.

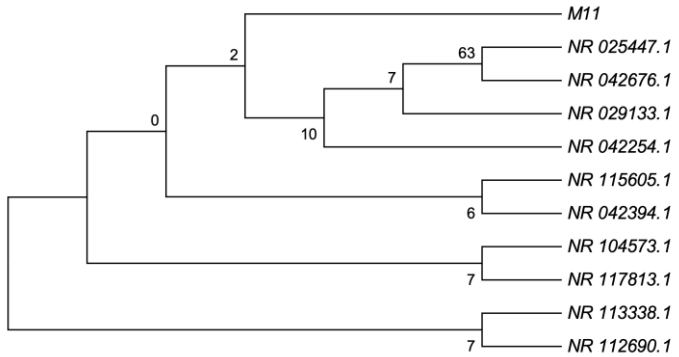

Figure 5 The phylogenetic tree for *Lactobacillus plantarum* strain PRK11 was constructed by using the neighbor-joining method in MEGA 7 software.

Probiotic characterization of the isolates

pH Tolerance

To be consider as a probiotic, the strain should survive at low pH condition as in the stomach. In this study, all the isolates showed good growth at pH 4 compared to control (pH 5.5). All three isolates showed fair growth at pH 3 (Fig. 6). In the figures isolates were represented as PP 1 (*Pediococcus pentosaceus* strain PRK1), LP7 (*Lactobacillus plantarum* strain PRK 7) and LP 11 (*Lactobacillus plantarum* strain PRK 11). Acid tolerance is an important criterion used for the selection of probiotic bacteria. In this study, the isolated bacteria were perceived to live under the acidic environment of pH 3 for 3 h. Previous research has showed that LAB can survive at pH 2–4, which is a significant parameter to be used as potential probiotic bacteria **(Giri *et al*., 2018)**.

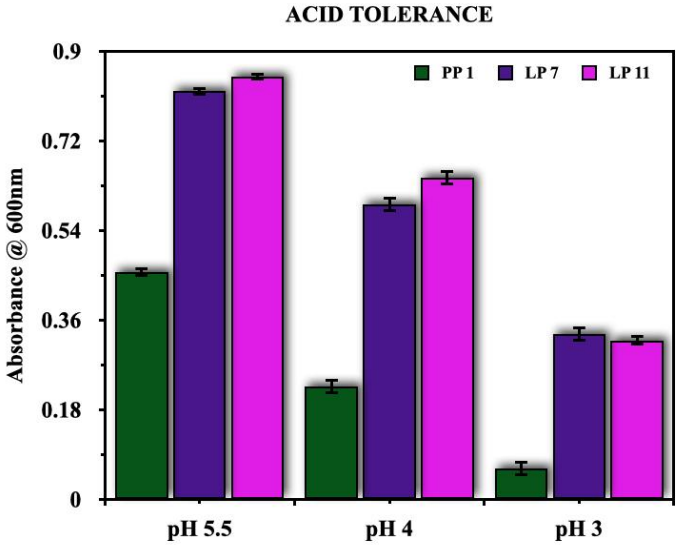

Figure 6 The ability of the isolate to grow in pH 5.5 (Control), pH 4, and pH 3 in MRS broth. The mean ± SD values were significant at $p < 0.05$.

Autoaggregation

The autoaggregation percentage of isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 was 68%, 40.25%, and 42.25 %, respectively, at 3 h of incubation. Whereas at 24 h of incubation, isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 showed 91%, 83 %, and 89% autoaggregation. This study shows that the autoaggregation percentage of isolates increases with an increase in the incubation time (Fig. 7). Autoaggregation experiment helps to detect the colonization of probiotic isolates to the gastrointestinal epithelial cells, which prevents the pathogens' colonization in the gut. The greater the percentage of autoaggregation, the greater the ability of probiotic bacteria to bind with epithelial cells and stimulate health benefits **(Menezes *et al*., 2019)**. An alternative for adhesion assay was found to be the autoaggregation test, which was demonstrated to be more authentic and inexpensive compared with cell lines, which were challenging in both procedure and costly **(Sriraj *et al*., 2017)**.

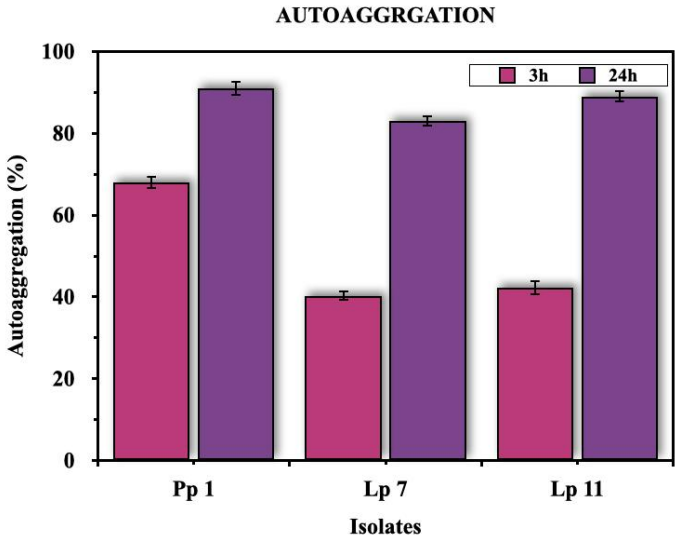

Figure 7 The isolates autoaggregation percentage after 3h and 24 h of incubation. The mean ± SD values were significant at $p < 0.05$.

Coaggregation

The highest co-aggregation percentage was found to be against *S. aureus* for isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 at 36.1%, 37.1%, and 42.5%, respectively. The lowest percentage of co-aggregation was observed against *P. aeroginosa* for isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 at 28.33%, 30.5%, and 30.8%, respectively (Fig. 8). It is vital to evaluate these characteristics for the probiotic bacterial ability to colonize the gut in addition to inhibiting the formation of biofilm and attachment of pathogens in the intestine **(Vasiee *et al.*, 2020)**. The infection caused by pathogens colonizing the intestine can be minimized by probiotics **(Ayyash et al., 2014)**. The LAB isolated from goat milk showed less co-aggregation activity with *Salmonella enteritis* **(Reuben *et al.*, 2019)**. However, the isolates from fermented rice gruel have shown reasonable co-aggregation activity against *Salmonella* sp. In our study, the co-aggregation percentage of isolates was below 40%, which indicated that these isolates possess good probiotic property as the minimal level of co-aggregation among pathogen can prevent the formation of biofilm and colonization of pathogens in the gastrointestinal tract **(Mohanty *et al.*, 2019)**.

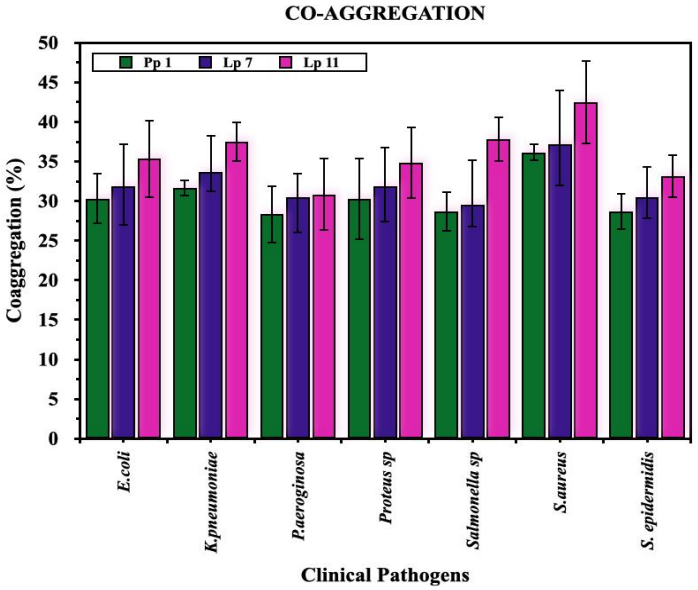

Figure 8 Percentage of co-aggregation of the isolates against clinical pathogens. The mean ± SD values were significant at $p < 0.05$.

Cell surface hydrophobicity

The cell surface hydrophobicity for isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 is shown in Fig. 9. The range of adhesion was 6.77%–18.64% and 0%–20% for hexadecane and xylene, respectively. The outer membrane of bacteria generally has hydrophobic components and due to net negative charge, bacteria act hydrophobically.

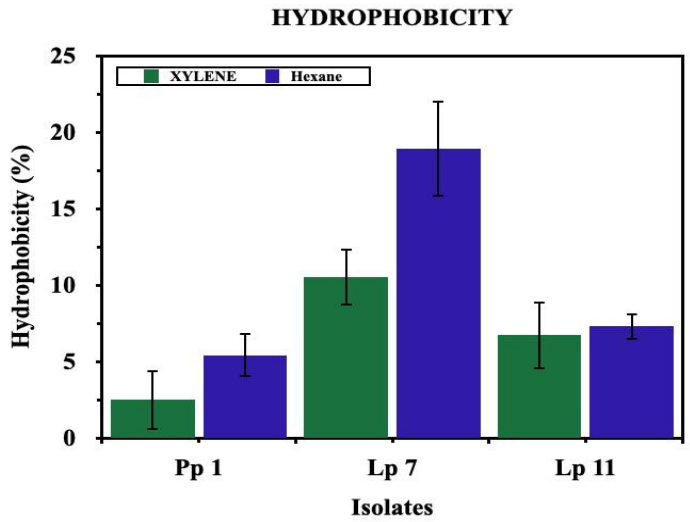

Figure 9 The percentage of hydrophobicity exhibits the cell adhesion ability of the isolates in xylene and hexadecane. The mean ± SD values were significant at $p < 0.05$.

The hydrophobic interaction plays a key role in the adherence of bacteria to the epithelial cell. The hydrophobicity of the bacterial cell is influenced by media composition, bacterial culture growth rate, structure of the bacterial surface, and changes in protein composition due to stress from the environment **(Botthoulath *et al.*, 2018)**. Further, the hydrophobicity property is beneficial to bacterial strains in battling with other gastrointestinal bacteria **(Yerlikaya, 2019)**. In this study, the isolates showed good hydrophobicity property toward hexadecane compared to xylene.

Bile Tolerance

The isolates were tested at different concentrations of bile in MRS broth and their percentage of suppression was calculated. In this study, the isolates were able to survive at 0.1% bile. However, at 0.3% bile, the growth of isolate *P. pentosaceus* strain PRK1 was suppressed to 88% whereas that of isolates *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 was found to be 86.5% and 81.3%, respectively (Fig. 10). The capability of the isolate to tolerate various concentrations of bile salt helps the lactic acid bacteria maintain a balanced intestinal microflora by reaching the small intestine and colon **(Shehata et al., 2016)**. For this, the bacteria should withstand a human intestinal bile concentration of 0.3% **(Prabhurajeshwar & Chandrakanth, 2019)**. Resistance to bile is one of the exceedingly important criteria to be used as probiotics because it decides their ability to remain alive in the gastrointestinal tract, to involve in its functional character, and to colonize in the intestine **(Ali et al., 2020)**.

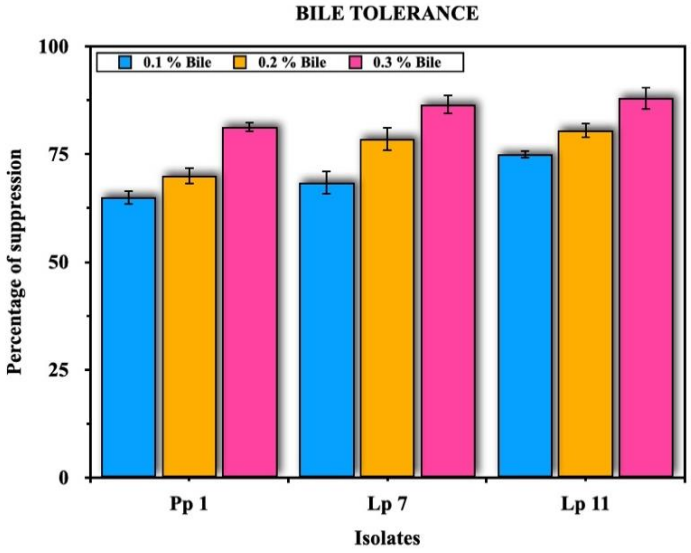

Figure 10 Percentage of suppression of the isolates in different concentrations of bile (0.1%–0.3%). The mean ± SD values were significant at $p < 0.05$.

NaCl tolerance

The ability of isolates to survive at 1%–6% NaCl concentration was studied. The isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK11 showed a good growth rate of up to 4% NaCl in MRS broth. At 6% NaCl, the isolate exhibited fair growth (Fig. 11).

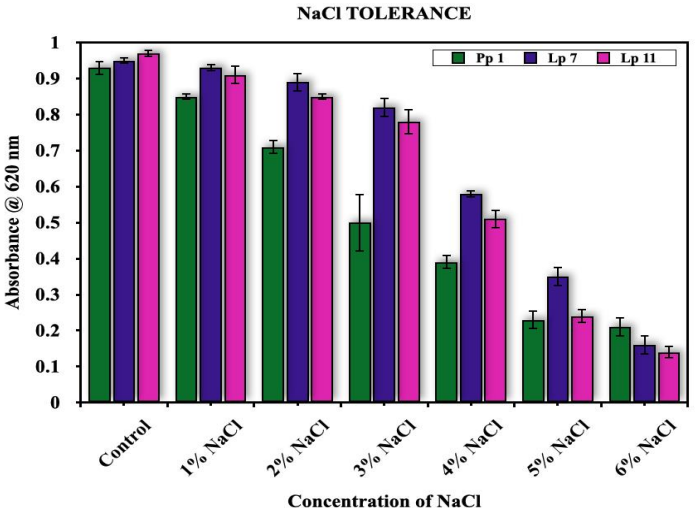

Figure 11 The ability of the isolates to tolerate different concentrations of NaCl (1-6%) and MRS broth without NaCl serving as a control. The mean ± SD values were significant at $p < 0.05$.

Table 2 Antibacterial activity of bacteria isolated from fermented rice gruel against clinical pathogens

Test Pathogen	Zone Of Inhibition (mm)			
	***P. pentosaceus* strain PRK1**	***L. plantarum* strain PRK7**	***L. plantarum* strain PRK11**	**Positive Control**
Escherichia coli	-	-	-	10
Psuedomonas aeroginosa	13.5 ± 0.5	11 ± 0.5	12 ± 0.0	15
***Proteus* sp**	10.5 ± 0.5	10 ± 0.5	10.5 ± 0.5	15.5
Klebsiella pneumonia	-	-	9.5 ± 0.5	14
***Salmonella* sp**	-	-	-	14
Staphylococcus aureus	11.25 ± 0.25	-	12 ± 0.0	16.5
Staphylococcus haemolyticus	-	-	10 ± 0.5	16
Enterococcus faecium	13 ± 0.25	12.5 ± 0.5	16 ± 0.5	17
Streptococcus epidermidis	-	-	-	17

–, no zone of inhibition; ±, mean SD; Positive control is streptomycin (1 mg/ml)

The isolates from the fermented rice gruel sample showed better growth up to 4% of NaCl. The growth rate was decreased by 6% of NaCl. This is following the result of a previous report in which isolates from yogurt samples tolerated up to 3% concentration of NaCl **(Chowdury *et al*., 2012)**.

Carbohydrate fermentation

The isolates were able to use different carbohydrates (Table 1). *L.plantarum* strain PRK11 was able to ferment most of the carbohydrates used in this study compared to *P. pentosaceus* strain PRK1 (Fig. 12). The obtained results were in accordance with the biochemical characterization of isolates belonging to lactic acid bacteria **(Prabhurajeshwar & Chandrakanth, 2019)**. It has also been reported that *Lactobacillus plantarum* fermented glucose, sorbose, maltose, arabinose, xylose, and rhamnose **(Muthusamy *et al*., 2020)**.

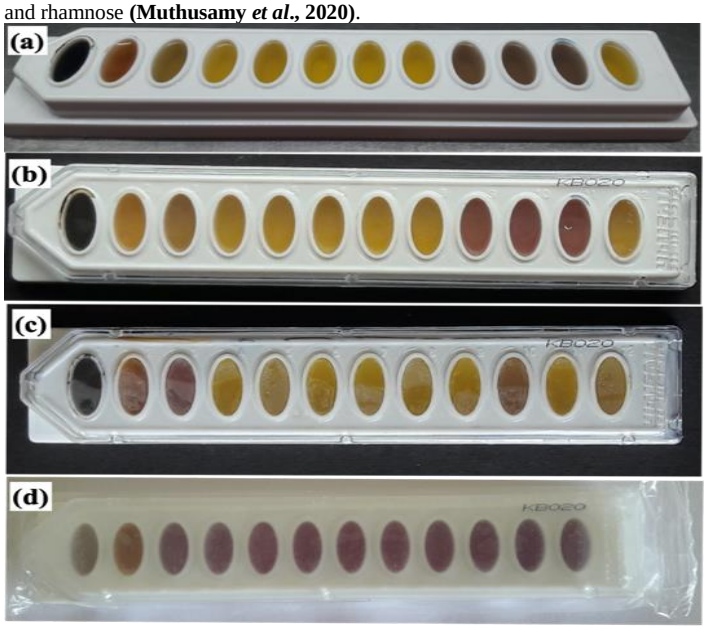

Figure 12 The carbohydrate fermentation profile of (**a**) *Pediococcus pentosaceus*; (**b**) *L. plantarum* strain PRK 7; (**c**) *L. plantarum* strain PRK11 and (**d**) The Control API strip.

Antibacterial Activity

The antibacterial activity of the isolates were tested against clinical pathogens (Table 2). All the isolates possess antibacterial activity either through the production of bacteriocin or organic acids. *L.plantarum* strain PRK11 shows excellent antibacterial activity compared to *P. pentosaceus* strain PRK1 and *L.plantarum* strain PRK7. All the tested isolates does not inhibit *E. coli* and *S. epidermidis* but inhibits *E. faecium*. These isolates have greater inhibitory activity than the previous research data in which the isolates from sourdough (*L. farraginis* No. 206, *P. pentosaceus* No. 183, and *P. acidolacti* No. 29) didn't inhibit *E. faecium* **(Bartkiene *et al*., 2019)**. Also, another report shows that *L. plantarum* D1 and *L. plantarum* D2 inhibited *Salmonella* but the isolates in our study did not show any inhibition against clinical *Salmonella* sp. **(Hu *et al*., 2020)**. The antibacterial activity of *Pediococcus pentosaceus* was due to inhibiting compound like bacteriocin **(Azevedol *et al*., 2020)**. The antibacterial activity could also occur due to organic acids such as lactic acid, acetic acid, and propionic acid, which effectively inhibit the growth of pathogens and are used as additives/preservatives in food industries **(Desniar *et al*., 2020)**.

Safety Assessments

Haemolytic activity

The isolates *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 were tested for hemolytic activity and none of them has showed α- and β-hemolytic activities. All the isolates exhibited γ-hemolytic activity (i.e.) no zone was observed around the isolates in blood agar plates (Fig. 13). For the selection of probiotic bacteria, one of the safety assessment is the absence of hemolytic activity **(Kong *et al*., 2020; Pavli *et al*., 2019, Silva *et al*., 2019; Amin *et al*., 2019)**. Some previous reports showed that *L. plantarum* M2 and *L. plantarum* KO9 strain exhibited γ-hemolytic activity in which no clear or halo green zone was formed on agar plates **(Kolestac *et al*., 2021)**.

Figure 13 The γ- haemolytic activity exhibited by (**a**) *Pediococcus pentosaceus* (**b**) *Lactobacillus plantarum* strain PRK7; (**c**) *Lactobacillus strain* PRK11.

Antibiotic susceptibility test

All three isolates, *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7 and *L.plantarum* strain PRK 11 were observed to be sensitive to chloramphenicol, cefotaxime, tetracycline, and linezolid, which inhibit the bacterial cell wall synthesis and protein synthesis. All the isolates showed similarity in resistance to ciprofloxacin and intermediate resistance to penicillin G (Table 3; Fig 14). In previous study, the isolates showed resistance to ciprofloxacin **(Yerlikaya, 2019; Xu *et al*., 2019)** which inhibits the bacteria by inhibiting DNA synthesis. Further, *P. pentosaceus* SC28 and *L. brevis* KU15151 were resistant to gentamycin, streptomycin, kanamycin, and ciprofloxacin and sensitive to tetracycline, chloramphenicol, ampicillin, and doxycycline, which is in accordance with our present study **(Yang *et al*., 2020)**.

Table 3 Antibiotic susceptibility test for bacteria isolated from fermented rice gruel

ANTIBIOTICS	SYMBOL	CONCENTRATION	ISOLATE		
			***P. pentosaceus* strain PRK1**	***L. plantarum* strain PRK7**	***L. plantarum* strain PRK11**
Penicillin G	P	10 Units	R	R	I
Oxacillin	OX	1 µg	S	R	R
Erythromycin	E	15 µg	S	S	I
Clindamycin	CD	2 µg	S	S	I
Linezolid	LZ	30 µg	S	S	S
Co-Trimoxade	CoT	25 µg	R	S	S
Vancomycin	VA	30 µg	R	R	R
Ciprofloxacin	CIP	5 µg	R	R	R
Tetracycline	TE	30 µg	S	S	S
Cefotaxime	CTX	30 µg	S	S	S
Chloramphenicol	C	30 µg	S	S	S
Gentamycin	GEN	10 µg	S	R	I

S, sensitive (≥ 20 mm); I, intermediate (13–19 mm); R, resistant (≤11 mm)

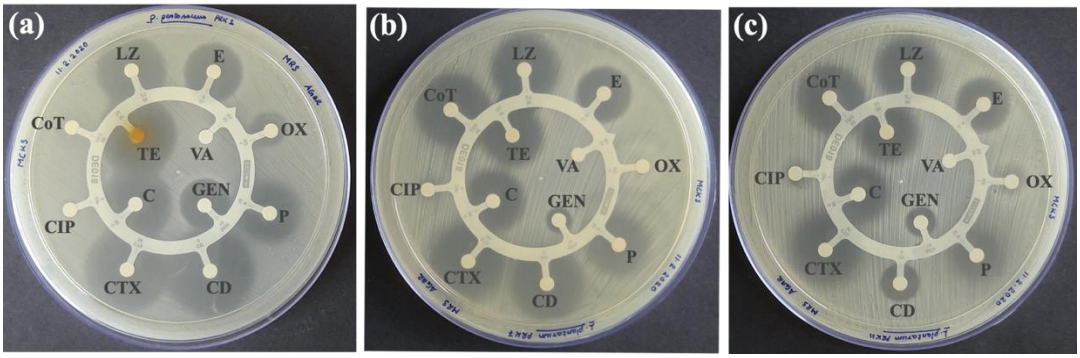

Figure 14 The antibiotic susceptibility of (**a**) *Pediococcus pentosaceus* strain PRK1 (**b**) *Lactobacillus plantarum* strain PRK7 (**c**) *Lactobacillus strain* PRK11.

Organic acid profile

The Organic acid produced by L. plantarum strain PRK 7 and strain PRK 11 was depicted in Fig 15. HPLC separation of organic acid of the isolate shows the presence of lactic acid and acetic acid. The retention time of lactic acid was 2.092 and 2.095 with peak area 17482021 and 19035013 for the isolates *L.plantarum* strain PRK 7 and *L.plantarum* strain PRK 11 respectively. The retention time (RT) of acetic acid was 2.375 and 2.391 with peak area 15347062 and 1294745 respectively.

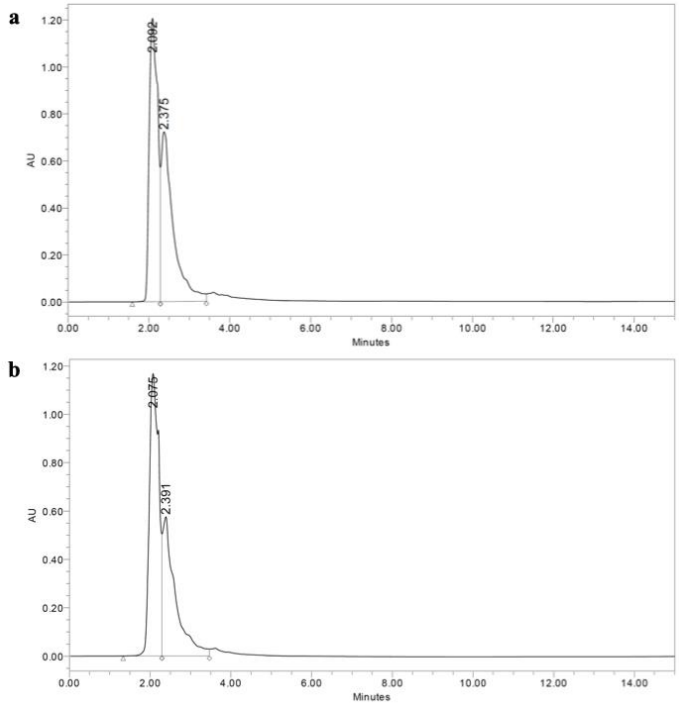

Figure 15 Organic acid profile of isolates by HPLC analysis (**a**) The peak shows the presence of lactic acid (RT -2.092) and acetic acid (RT- 2.375) in *L. plantarum* strain PRK 7 (**b**) The lactic acid (RT -2.075) and acetic acid (RT- 2.391) produced by *L. plantarum* strain PRK 11.

The HPLC analysis of organic acid from *Pediococcus pentosaceus* strain PRK 1 was not detected. The retention time was similar to the retention time of lactic acid and acetic acid in the chromatography analysis of standard organic acids **(Reuter, 2015)**. The *L.plantarum* strain PRK 7 and strain PRK 11 was heterofermentative as it produces lactic acid as end product. One of the characteristics of lactic acid bacterial fermentation of carbohydrates to produce lactic acid as end product enhances the flavor and aroma of the food **(Llano *et al.*, 1996)**. Further, the organic acid produced during fermentation also acts a preservative for food and possess the antibacterial activity. It has a wide range of industrial applications hence the bacteria producing lactic acid as end product during fermentation can be used as starter culture in production of dairy products **(Desniar *et al.*, 2020)**. Probiotic bacteria exhibit high antagonistic activity against pathogens due to the production of organic acids (**Evivie *et al.*, 2019).**

Nutrition Profiling

The nutritional parameters, minerals, and vitamins in fermented rice gruel were analyzed. The test results of nutritional parameters per 100g of sample. The mineral contents in the fermented rice gruel sample and the concentration of vitamin B12 (Cyanocobalamin) and B9 (Folic acid) in fermented rice gruel were quantified and represented in Table 4. Lactic acid bacteria play a key role in breaking down anti-nutritional compounds in rice and improve the bioavailability of nutrients and minerals such as iron, potassium, and calcium in fermented foods, which can be easily absorbed and serves much healthier than whole grains. Furthermore, the microbial analysis of the sample reveals that the sample has bacteria, yeast, molds, and the absence of *salmonella* (Table 5). The bacteria, yeast, and molds could ferment the rice naturally. The microbes utilize the rice substrate and synthesize vitamins (B6, B12, B9, and K), minerals, bacteriocins, short-chain fatty acids (reduce the cholesterol), essential amino acids, exopolysaccharides into the gruel (Fig.16). Fermentation enhances the bioavailability of nutrients that can be easily absorbed by our digestive system. It also reduces the antinutritive compounds like phytic acid, tannins, and polyphenols **(Rawat *et al.*, 2020)**. The magnesium and selenium increase bone strength; potassium reduces blood pressure.

Table 4 Nutritional profiling of fermented rice gruel

S. No.	PARAMETERS	RESULTS
	Nutritional parameters	
1	Energy	12kcal
2	Protein	0.88g
3	Total carbohydrates	2.0g
4	Total fat	Less than 0.1g
	Minerals	
5	Sodium	41.5 mg
6	Calcium	0.91 mg
7	Iron	0.2 mg
8	Potassium	6.32 mg
9	Magnesium	1.74 mg
10	Phosphorus	2.81 mg
11	Zinc	0.08 mg
	Vitamins	
12	Cyanocobalamin	0.5 µg
13	Folic acid	2.0 µg

Table 5 Microbial analysis of fermented rice gruel

S. No.	Test Parameters	Result
1	Total Bacterial count	66,82,000 CFU/g
2	*Escherichia coli*	16,000 CFU/g
3	Yeast	64,000 CFU/g
4	Molds	14,000 CFU/g
5	*Salmonella*	Absent/25g

CFU/g, Colony forming unit per gram

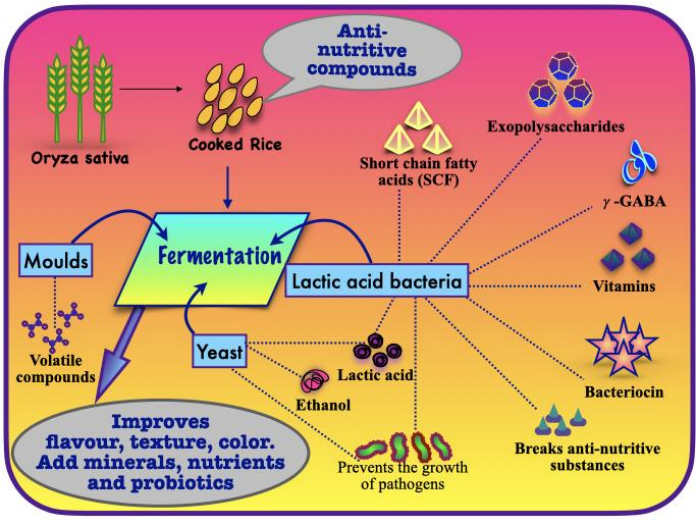

Figure 16 Schematic representation of fermentation process involved by lactic acid bacteria to enrichich the bioavailability of nutrients during fermentation of rice gruel.

CONCLUSION

The fermentation of rice gruel involves the lactic acid bacteria which exhibits probiotic characteristics. As the isolated *P. pentosaceus* strain PRK1, *L. plantarum* strain PRK7, and *L. plantarum* strain PRK11 from fermented rice gruel, which was tolerant to low pH, bile (0.1%–0.3%), and NaCl (1%–6%). Further, antibacterial activity shows its ability to inhibit clinical pathogens. Among the isolates, *L. plantarum* strain PRK 11 showed excellent antibacterial activity compared to the other two isolates in this study. Besides, the safety assessment of the isolates confirmed that all three isolates were non-hemolytic. Also, these isolates were sensitive to most of the tested antibiotics. The nutrition profiling of fermented rice gruel shows that the minerals and vitamins have increased. It also has less fat and total carbohydrates. The fermented rice gruel has enhanced the bioavailability of nutrients compared to non-fermented rice. Thus, this study showed the association of lactic acid bacteria in the fermentation of rice gruel have probiotic properties which can be utilized as a starter culture in formulating functional foods in future. Though, these isolates from fermented rice gruel have the probiotic properties further in vivo studies are needed to confirm their safety for human consumption and also their stability in the GI tract is still need be investigate in in-vivo models.

Acknowledgments: The author is deeply grateful to the management of K. S. Rangasamy College of Arts Science (Autonomous). Department of Biotechnology is greatly acknowledged for the facilities provided to carry out this research. The author expresses sincere gratitude to the research supervisor for the continuous guidance for the successful completion of this study and extend the gratitude to all who have supported this work.

REFERENCES

Abid, Y., Casillo, A., Gharsallah, H., Joulak, I., Lanzetta, R., Corsaro, M.M., Attia, H., Azabou, S. (2018). Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. *Int. J. Biol. Macromol*, 108: 719-728. http://doi.org/10.1016/j.ijbiomac.2017.10.155.

Ali, F.S., Zayed, G., Saad, O.A.O., Salwa, A., Gharib, H. (2020). Antimicrobial activity and Probiotic Properties of Lactic Acid Bacteria Isolated from Traditional Fermented Dairy Products. *J. Mod. Res*, 2: 40-48. https://doi.org/10.21608/jmr.2020.22931.1015.

Alsona, S., Carmen Castro, M., Bersasco, M., de la Blanda, I.G., Moreno-Ventas, X., Rojas, A.H. (2019) Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fish for potential applications as probiotics in aquaculture. *Probiotics Antimicrob. Proteins* 11: 569-579. https://doi.org/10.1007/s12602-018-9439-2.

Amin, M., Adams, M.B., Burke, C.M., Bolch, C.J.S. (2020). Isolation and screening of lactic acid bacteria associated with the gastrointestinal tracts of abalone at various life stages for probiotic candidates. *Aquacult. Rep*, 17: 100378. https://doi.org/10.1016/j.aqrep.2020.100378

Arumugam, N., Govindaraj, D. (2021). Characterization of yeast isolated from different sources as probiotics. *J. Microbiol. Biotechnol. Food sci*, 11 (1). https://doi.org/10.15414/jmbfs.937.

Ayyash, M., Abushelaibi, A., Al- Mahadin, S., Enan, M., El-Tarabily, K., Shah, N. (2018). In-vitro investigation into probiotic characterization of *Streptococcus* and *Enterococcus* isolated from camel milk. *LWT- Food Sci. Technol*, 87: 478-487.https://doi.org/10.1016/j.lwt.2017.09.019.

Azevedol, P.O.S., Mendoncal, C.M.N., Moreno, A.C.R., Bueno, A.V.I., Almeida, R.Y., Seibert, L., Converti, A., Watanabe, I-S., Gierus, M., Oliveiera, R.P.S. (2020). Antibacterial and antifungal activity of crude and freeze-dried bacteriocin-like inhibitory substance produced by *Pediococcus pentosaceus. Sci. Rep*, 10: 12291. https://doi.org/10.1038/s41598-020-68922-2.

Bartkiene, E., Lele, V., Ruzauskas, M., Domig, K.J., Starkute, V., Zavistanaviciute, P., Bartkevics, V., Pugajeeva, I., Klupsaite, D., Juodeikiene, G,….Rocha, J.M. (2019). Lactic Acid Bacteria Isolation from Spontaneous Sourdough and their caracterization including antimicrobial and antifungal properties evaluation. *Microorganisms*, 8 (1): 64. https://doi.org/10.3390/microorganisms8010064.

Bin Masalam, M.S., Bahieldin, A., Alharbi, M.G., Al-Masaudi, S., Al-Jaouni, S., Harakeh, S.M., Al-Hindi, R.R. (2018). Isolation, Molecular characterization and Probiotic potential of lactic acid bacteria in Saudi Raw and Fermented milk. *Evid.-Based Complementary Altern. Med*, 2018: 1-12. Article ID 7970463. https://doi.org/10.1155/2018/7970463.

Botthoulath, V., Upaichit, A., Thumarat, U. (2018). Identification and In vitro assessment of potential probiotics characteristics and antibacterial effects of Lactobacillus plantarum subsp. Plantarum SK119, a bacteriogenic strain isolated from Thai fermented pork sausage. *J. Food Sci. Technol*, 55 (7): 2774-2785. https://doi.org/10.1007/s13197-018-3201-3.

Chopade, L.R., Paradeshi, J.S., Amrukthar, K.P., Chaudhari, B.L. (2019). Finding out potent probiotic cultures from Ayurvedic formulation *Takrarishta* through in-viitro probiotic characterization and principal component analysis. *LWT- Food Sci. Technol*, 100: 205-212. doi: http://doi.org/10.1016/j.lwt.2018.10.061.

Chowdury, A., Hossain, N., Mostazir, N.J., Fakruddin, M., Billah, M., Ahmed, M.M. (2012). Screening of *Lactobacillus spp*. From buffalo youghurt for probiotic and antibacterial activity. *J. Bacteriol. Parasitol*, 3: 156.

Chugh, B., Kamal-Eldin, A. (2020). Bioactive compounds produced by probiotics in food products. *Curr. Opin. Food Sci*, 32: 76-82. http://doi.org/10.1016/j.cofs.2020.02.003.

Desniar, Rusmana, I., Suwanto, A., Mubarik, N.R. (2020). Organic acid produced by lactic acid bacteria from bekasam as food bio preservatives. *IOP Conf. Series: Earth and Environmental Science*, 414: 012003. https://iopscience.iop.org/article/10.1088/1755-1315/414/1/012003/pdf.

Dowarah, R., Verma, A.K., Agarwal, N., Singh, P., Singh, B.R. (2018). Selection and characterization of probiotics lactic acid bacteria and its impact on growth, nutrient, digestibility, health and antioxidant status in weaned piglets. *Plos one*, 13 (3): e0192978.

Evivie, A.E., Abselazez, A., Li, B., Bian, X., Li, W., Du, J., Huo, G., & Liu, F. (2019). In vitro Organic acid production and In vivo food pathogen suppression by probiotic *S.thermophilus and L.bulgaricus. Front. Microbiol*, 10. https://doi.org/10.3389/fmicb.2019.00782.

Gajbhiye, M.H. (2016). Probiotic traits of lactic acid bacteria isolated from Ariel surfaces of pomegranate. *Int. J. bioassays* 5(8): 4733-4738. http://dx.doi.0rg/10.21746/ijbio.2016.08.002

Giri, S.S., Sen, S.S., Saha, S., Sukumaran, V., Park, S.C. (2018). Use of a Potential Probiotic, *Lactobacillus plantarum* L7, for the Preparation of a Rice-Based Fermented Beverage. *Front. Microbiol*, 9: 473. https://doi.org/10.3389/fmicb.2018.00473.

Guimaraes, J.T., Balthazar, F., Silva, R., Esmerino, E.A., Silva, M.C., Sant'Ana, A.S., Durate, M.C.K.H., Freitas, M.Q., Cruz, A.G. (2020). Impact of probiotics and prebiotics on food texture. *Curr. Opin. Food Sci*, 33: 38-44. https://doi.org/10.1016/j.cofs.2019.12.002.

Hu, C-H., Ren, L-Q., Zhou, Y., Ye, B-C. (2020). Characterization of Antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy foods. *Food Sci Nutr*, 7: 1997-2005. https://doi.org/10.1002/fsn3.1025.

Kolestac, D., Geric, M., Gajski, G., Markov, K., Domijan, A.M., Canak, I., Jakopovic, Z., Svetec, I.K., Zunar, B., Frece, J. (2021). Lactic acid bacteria isolated from equid milk and their extracellular metabolites show great probiotic properties and anti-inflammatory potential. *Int. Dairy J*, 114: 104828. https://doi.org/10.1016/j.idairyj.2020.104828

Kondrotiene, K., Kasnauskyte, N., Serniene, L., Gölz, G., Alter, T., Kaskoniene, N., Maruska, M. (2018). Characterization and application of newly isolated nisin producing *Lactococcus lactis* strains for control of *Listeria monocytogens* growth in fresh cheese. *LWT-Food Sci. Technol* 87: 507-514. http://doi.org/10.1016/j.lwt.2017.09.021.

Kong, Y., Li, M., Li, R., Shan, X., Wang, G. (2020). Evaluation of cholesterol lowering property and antibacterial activity of two potential lactic acid bacteria isolated from the intestine of snakehead fish (*Channa argus). Aquac. Rep*, 17: 100342. https://doi.org/10.1016/j.aqrep.2020.100342

Llano, G.D., Rodriguez, A., Cuesta, P. (1996). Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening-analysis by HPLC. *J. Appl. Bacteriol*, 80(5), 570–276. https://doi.org/10.1111/j.1365-2672.1996.tb03259.x.

Menezes, A.G.T., Ramos, C.L., Cenzil, G., Melon, D.S., Dias, D.R., Schwan, R.F. (2019). Probiotic Potential, Antioxidant Activity, and Phytase Production of Indigenous Yeasts Isolated from Indigenous Fermented Foods. *Probiotics Antimicrob. Proteins*, 12: 280-288. https://doi.org/10.1007/s12602-019-9518-z.

Margret, A.A., Sukanya, M., Christinal, J., Subbiah, K., Nagendran, N.A. (2021). Facilitating the Gut-brain axis by probiotic bacteria to modulate neuroimmune response on lead exposed zebra fish models. *Acta Sci. Health Sci*. 43.

Mohanty, D., Panda, S., Kumar, S., Ray, P. (2019). In Vitro evaluation of adherence and anti-infective property of probiotic *Lactobacillus planetarium* DM 69 against *Salmonella enterica. Micro. Pathog*, 126: 212-217. http://doi.org/10.1016/j.micpath.2018.11.014.

Muthusamy, K., Soundharrajan, I., Srisesharam, S., Kim, D., Kupusamy, P., Lee, K.D., Choi, K.C. (2020). Probiotic Characteristics and Antifungal Activity of *Lactobacillus plantarum* and Its Impact on Fermentation of Italian Ryegrass at Low Moisture. *Appl. Sci*, 10: 417. https://doi.org/10.3390/app10010417.

Nurmiati, N., Periadnadi, P., Alamsyah, F., Sapalina, F. (2018). Characterization and potential of acid fermentative and proteolytic natural microflora in several products of traditional dads from Lumbah Gumanti district West Sumatra, Indonesia. *Int. J. Curr. Microbiol. App. Sci*, 7(3): 3151-3163. https://doi.org/10.20546/ijcmas.2018.703.364.

Nuryana, I., Andriani, A., Lisdiyanti, P., Yopi. (2019). Analysis of organic acid produced by lactic acid bacteria. *IOP Conf. Series: Earth and Environmental Science*, 251, 012054. https://iopscience.iop.org/article/10.1088/1755-1315/251/1/012054/met.

Pacheco, J.R.S., Arreola, A.R., Velasco, J.A.V.R., Aguilar, J.G.S., López, J.A.A., Parra, M.L., Uscanga, B.R.A. (2021). Effect of the Intake of Probiotics Isolated from Human Milk in People with Gastritis and Irritable Bowel Syndrome. *J. Probiotics health*, 9(1), 228.

Patil, A., Disouza, J., Pawar, S. (2019). Shelf-life stability of encapsulated lactic acid bacteria isolated from sheep milk thrived in different milk as natural media. *Small Rumin. Res*, 170: 19–25. https://doi.org/10.1016/j.smallrumres.2018.09.014.

Pavli, F., Gkana, E., Adebambo, O., Karatzas, K-A., Panagou, E., Nychas, G-JE. (2019). In vitro screening of γ-Aminobutyric acid and Autoinducer-2 Signalling in Lactic Acid Bacteria Exhibiting Probiotic potential isolated from natural black conservolea olives. *Foods*, 8 (12): 640. https://doi.org/10.3390/foods8120640.

Petrova, P., Petrov, K. (2020). Lactic acid fermentation of cereals and pseudocereals: Ancient nutritional biotechnologies with modern applications. *Nutrients*, 12 (4): 1118. https://doi.org/10.3390/nu12041118.

Pinto, A., Barbosa, J., Albano, H., Isidro, J., Teixeira, P. (2020). Screening of Bacteriocinogenic lactic acid bacteria and their characterization as potential probiotics. *Microorganisms*, 8(3): 393. https://doi.org/10.3390/microorganisms8030393.

Prabhurajeshwar, C., Chandrakanth, K. (2019). Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic lactobacilli strains isolated from commercial yoghurt. *Clin. Nutr. Exp*, 23, 97-115. https://doi.org/10.1016/j.yclnex.2018.10.001.

Rawat, K., Kumari, A., Kumar, S., Gehlot, R. (2018). Traditional fermented products of India. *Int. J. Curr. Microbiol. App. Sci*, 7 (4): 1873-1883. https://doi.org/10.20546/ijcmas.2018.704.214.

Ray, M., Gosh, K., Har, P.K., Singh, S.N., Mondal, K.C. (2017). Fortification of Rice gruel into beverage and establishment as a carrier of newly isolated Bifidobacterium sp. MKK4. *Res. J. Microbiol*, 12: 102-117. https://scialert.net/abstract/?doi=jm.2017.102.117.

Ray, M., Gosh, K., Singh, S., Mondal, K.C. (2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India. *J. Ethn. Food*, 3(1): 5-18. https://doi.org/10.1016/j.jef.2016.02.002.

Reuben, R.C., Roy, P.C., Sarkar, L.S., Rubavet, A.S.M., Alam, U., Jahid, I.K. (2019). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *J. Dairy Sci*, 103 (2): 1223-1237. https://doi.org/10.3168/jds.2019-17092

Reuter, W. M. (2015). The Analysis of a Broad Range of Organic Acids by HPLC with UV Detection. PerkinElmer, Inc. www.perkinelmer.com. https://resources.perkinelmer.com/lab-solutions/resources/docs/APP_The-Analysis-of-a-Broad-Range-of-Organic-Acids-by-HPLC-with-UV-Detection-012379_01.pdf.

Sathe, G.B., Mandal, S. (2016). Fermented products of India and its implication: A review. *Asian Journal of Dairy & Food Research*, 35(1): 1-9. https://arccjournals.com/journal/asian-journal-of-dairy-and-food-research/DR-1090.

Shehata, M.G., El Sohaimy, S.A., El-Sahn, M.A., Youssef. (2016) Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Ann. Agric. Sci*, 61(1): 65-75. https://doi.org/10.1016/j.aoas.2016.03.001.

Silva, L.A., Neto, J.H.P.L., Cardarelli, H.R. (2019). Safety and probiotic functionality of isolated goat milk lactic acid bacteria. *Ann. Microbiol*, 69:1497–1505. https://doi.org/10.1007/s13213-019-01533-z.

Singh, K., Rao, A. (2021). A potential immunomodulator in COVID-19 infection management. *Nutr Res*, 87: 1-12. http://doi.org/10.1016/j.nutres.2020.12.014.

Sriraj, N.M., Sood, K., Yadav, R.N.S. (2017). Isolation and identification of potential probiotic bacteria from cattle form soil in Dibrugarh district. *Adv. microbiol*, 7, 265-279. https://www.scirp.org/journal/paperinformation.aspx?paperid=75654.

Tallapragada, P., Rayavarapu, B., Roa, P.P., Ranganath, N.N., Veerabhadrappa, P.P. (2018). Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *J. Genet. Eng. Biotechnol*, 16 (2): 357-362. https://doi.org/10.1016/j.jgeb.2018.03.005.

Vasiee, A., Falah, F., Behbahani, A.B., Tabatabaee-yazdi, F. (2020). Probiotic characterization of Pediococcus strains isolated from Iranian cereal-dairy fermented product: Interaction with pathogenic bacteria and the enteric cell line Caco-2. *J. Biosci. Bioeng*, 130 (5): 471- 479. https://doi.org/10.1016/j.jbiosc.2020.07.002.

Xu, Y., Zhou, T., Tang, H., Li, X., Chen, Y., Zhang, L., Zhang, J. (2019). Probiotic potential and amylolytic properties of lactic acid bacteria isolated from Chinese fermented cereal foods. *Food Control*, 111: 107057. https://doi.org/10.1016/j.foodcont.2019.107057.

Yang, S.E., Kim, K-T., Kim, T.Y., Pail, H-D. (2020). Probiotic properties and antioxidant activities of Pediococcus pentosaceus SC28 and Levi lactobacillus brevis KU15151 in fermented black Gamju. *Foods*, 9 (9): 1154. https://doi.org/10.3390/foods9091154.

Yerlikaya, O. (2019). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* spp. *Lactis* strains isolated from raw milk and kefir grains. *J. Dairy Sci*, 102: 124-134. http://doi.org/10.3168/jds.2018-14983.

Yu, Y-J., Chen, Z., Chen, P.T., Ng, I-S. (2018). Production, Characterization and antibacterial activity of exopolysaccharide from a newly isolated Weissella cibaria under sucrose effect. *J. Biosci. Bioeng*, 126: Issue 6769-777. https://doi.org/10.1016/j.jbiosc.2018.05.028.

Zhao, W., Liu, Y., Latta, M., Ma, W., Wu, Z., Chen, P. (2019). Probitics database: a potential source of fermented foods. *Int J Food Prop*, vol 22 (1): 198-217. https://doi.org/10.1080/10942912.2019.1579737.