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Isolation and probiotic characterization of lactic acid bacteria from traditional milk and identification of *Lactobacillus* sp. by PCR method

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Abstract
Probiotic microbiota plays a vital role in gastrointestinal health and possesses other beneficial attributes such as antimicrobial and antibiotic agents. In this study, A total of 10 traditional milk samples were collected from the Southern regions of Tamil Nadu, India. 55 bacterial isolates were isolated based on their sustainability in the gut environment and based on their antagonistic and antioxidant capacity. A series of established *in vitro* tests were conducted to determine the probiotic potential of isolates. These tests include bile salt tolerance, antibiotic resistance, ability to survive at a lower pH range. From the results 9 bacterial strains BM 3, BM 19, CU 27, CU 29, CU31, CU 40, TG 18, TM 6 and TM 37 showed good antagonistic and antioxidant activity than other strains. *Lactobacillus* bacteria were identified to genus level by using *Lactobacillus* specific primers and detected by PCR analysis. Out of these 55 beneficial strains 38 bacterial strains were identified as Lactic acid bacteria and come under the genus *Lactobacillus*.

Keywords: probiotic, characterization, lactic acid, *Lactobacillus*

Introduction
The term "probiotic" was coined by Lilly and Stillwell (1965) [18] to describe the "substances secreted by one microorganism that stimulate the growth of another" (Lilly & Stillwell, 1965) [18]. The name "probiotic" comes from the Greek word "protokos" which means "for life". According to the Food and Agriculture Organization of the United Nations (FAO) and World Organization (WHO) in 2001, probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host (Fuller, 1989). In more modern definitions, the concept of action on the gut microflora and even that of live microorganisms has disappeared. Salminen defined probiotics as "food which contains live bacteria beneficial to health" (Dal Bello *et al*., 2003) [7]. Microorganisms' efficient digestion and maximum absorption of nutrients increase the capacity of the host to exclude infectious microorganisms and prevent diseases (Williams, 2010) [31].
Live bacteria called probiotics have the potential to both treat and prevent many diseases. Consuming certain meals, beverages, and dietary supplements can provide probiotics (Daneshi *et al*., 2013) [8]. Commonly used probiotics contain microorganisms from the genus Bifidobacterium and a diverse group of lactic acid bacteria (Lactobacillus, Enterococcus) (Mathew *et al*., 2017) [20]. Lactic acid bacteria (LAB) play an important role in the production of probiotics. LAB comprises a wide range of genera and includes a diverse number of species (Van Tassell & Miller, 2011) [28].
They are a group of Gram-positive cocci or rods, non-spore-forming, catalase-negative, low pH tolerant. Lactic acid bacteria are generally considered safe in the food industry, so they are widely used in food preservation and to promote health. LAB is mostly used to produce a variety of fermented vegetables, meat, and dairy products.
Probiotic organisms are thought to promote the health of the host. Numerous pathways have been discovered in these studies to try and explain how probiotics might protect the body from internal disease (Dal Bello *et al*., 2003) [7]. By altering gut microflora, enhancing the gut mucosal barrier, preventing pathogen adherence, pathogen inactivation, altering dietary proteins by intestinal microflora, altering bacterial enzyme activity, affecting gut mucosal permeability, and regulating the immune system, they provide particular health benefits (Krasaekoopt *et al*., 2003) [17].

Lactic acid bacteria produce short-chain fatty acids, *viz*., acetic, propionic, and butyric acids. These acids lower intestinal pH and prevent the growth of harmful microbes (Cerdó *et al*., 2019) [3] Figure 1.

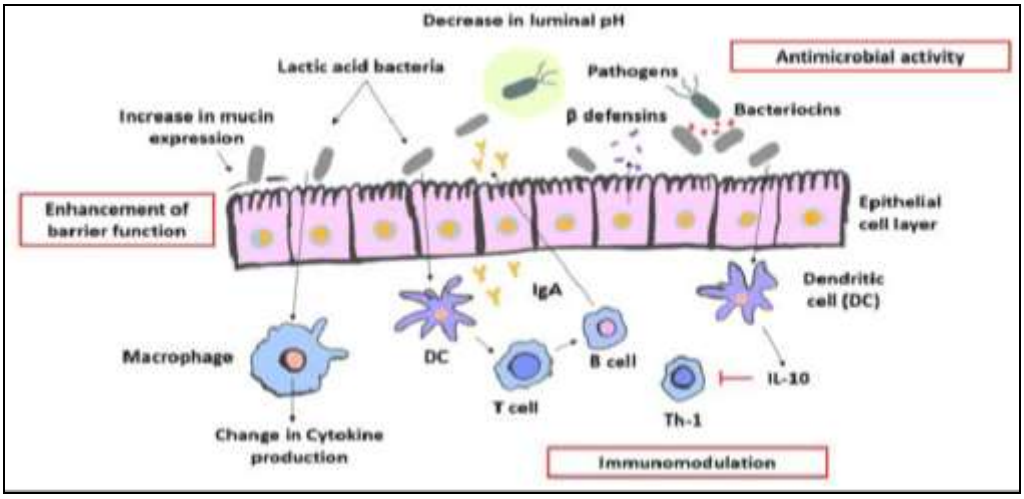

Fig 1: Potential mechanisms by which probiotic bacteria exert their beneficial roles in the intestine (Corr *et al*., 2009) [5].

Positive effects of probiotics include effects on mineral metabolism, especially bone stability, and prevention of osteoporosis, strengthening the intestinal barriers, prevention of colon cancer and urogenital infections, management of lactose intolerance, reduction of cholesterol and blood pressure, reduction of the inflammatory actions of the body, reduction of Helicobacter pylori infection, suppression, and control of pathogenic microorganism's growth (Dugas *et al*., 1999) [12].

Exopolysaccharides may be produced by several probiotic LAB (EPS) (Degeest & De Vuyst L, 1999) [10]. EPS protect cells from harmful environmental factors such as dehydration, extreme temperature, acid, osmotic stress, phagocytosis, macrophages, and antibiotics by forming layer surrounding cells (Ras *et al*., 2011) [24]. The unique structural features have made bacterial EPSs of particular interest in the fields of chemistry, medicine, and the food industry (Singh & Saini, 2017) [27]. EPS are frequently employed in the food industry as viscous, stabilising, and emulsifying agents due to their capacity to hold water (Garai-Ibabe *et al*., 2010) [14]. It improves the texture and rheological texture and sensibility of bread and dairy products such as yoghurt and cheese (Cotter *et al*., 2005) [6].

LAB can be isolated from various places including fruits, vegetables, soil, atmosphere and dairy products (Motohashi *et al*., 2002) [21]. Dairy products are an important factor of a balanced diet in our everyday routines. Due to its numerous benefits to mankind probiotic bacteria which are present in the milk samples were screened and studied for their antimicrobial and antioxidant capacity.

The purpose of the current research is to identify probiotic species from milk samples and determine their DPPH-scavenging antioxidant and antagonistic properties against gut pathogens.

Materials and Methods

Sample collection and isolation of probiotic bacteria

Traditional Milk samples were collected from the Southern regions of Tamil Nadu, India which includes Kovilpatti, Tirunelveli, Madurai, Theni and Thoothukudi. The samples were taken to the laboratory in a sterile container and a sterile condition. It was then serially diluted from 10-1 to 10-6 with sterile, deionized water. 100 µl of each dilution's aliquots were spread out on de Man Rogosa Sharpe (MRS) agar and incubated anaerobically at 37 ºC for 48–72 hours. After incubation, morphologically dissimilar colonies were selected and re-streaked on an MRS agar plate to get pure isolates. The isolated colonies were identified by colony Morphology, Gram staining, Catalase test, Oxidase test, and other biochemical tests. The identified organisms were stored at -20 ºC in a 70% glycerol stock solution for further studies.

Screening of safety and probiotic properties of LAB

Gelatinase activity: The gelatinase activity of isolates was investigated as described by Liliane, *et al*. 2019 (Bazireh *et al*., 2020) [1]. Isolated bacterial strains were streaked into tubes containing nutrient gelatin agar (peptone 5 gm/L, beef extract 3 gm/Land gelatin120 gm/L) (Himedia, Mumbai, India). The inoculated tubes were incubated at 30 ºC for 7-10 days, with liquefaction being monitored daily and refrigerated at 4 ºC for 1 h. A strain of Pseudomonas was used as a positive control.

Hemolytic activity: According to Yasmin *et al*., 2020 [32], the isolates were streaked on blood agar plates and incubated at 30 ºC for 24-48 h. They were observed for the zone of hemolysis surrounding the colonies.

Antibiotic sensitivity test: Antibiotic sensitivity of isolates was checked by disc diffusion method (Samedi & Charles, 2019) [26]. A 0.2 µl bacterial suspension was spread evenly on the surface of MRS agar plates. The inoculated plates were allowed to dry before placing the disc containing antibiotics. Standard concentrations of antibiotics belonging to 7 different classes such as Aminoglycosides (Amikacin, Tobramycin, Gentamycin), Aminopenicillins (Ampicillin), Tigecycline (Tetracycline, Trimethoprim) Quinolone (Cinoxacin), Phosphonic acid (Fosofomycin), Beta-lactams (Ceftriaxone, Amoxicillin/Clavulanicacid, cefotaxitin, cefotaxime, piperacillin/Tazobactam), Carbapenam (Ertapenem, Meropenem, Imipenem) were used.

Acid and bile salt tolerance test: The cell pellets were harvested by centrifugation and re-suspended in 10 ml of MRS broth with pH adjusted to 2.0, 3.0, 5.0 and 7.0 to

check acid tolerance. For bile salt tolerance, cells were resuspended in 10 ml MRS broth containing 0%, 0.15%, 0.3%, and 1% bile salt. The growth of viable microorganisms was enumerated at 0, 1, 2, 3, and 4 h. A UV-VIS Spectrophotometer was used to measure absorbance at 620 nm, which was used to track the growth of microorganisms.

Physiological and biochemical characterization

Growth at different NaCl concentrations: According to Samedi and Charles (Kang *et al*., 2020) [16], the 0.1 ml overnight culture of probiotic strains was inoculated in test tubes containing 10 ml MRS broth containing 2%, 4%, and 6.5% (W/V) of NaCl concentration. The appropriate amount of bromocresol purple (indicator) was added to the broth and incubated at room temperature for 7 days.

Growth at different temperatures: In the method described by Samedi and Charles in 2019 [26], the 0.1 ml overnight culture of probiotic strains was inoculated in test tubes containing 10 ml modified MRS broths. Bromocresol purple (0.04 g/l) was added to the broth and incubated for 7 days at four different temperatures; 10 ºC, 30 ºC, 37 ºC, and 50 ºC.

Resistance to phenol: According to (Yasmin *et al*., 2020) [32] the 0.1 ml probiotic strains were inoculated in 10 ml of 0.4% phenol-containing MRS broth and incubated at 37 ºC for 24h. Optical density was measured at 620 nm for 0 min immediately after inoculation and again after 24 h.

Antagonistic activity: The antagonistic activity of isolates was determined by the agar well diffusion method as described by Yasmin, *et al*. 2020 [32] with slight modification. The Four pathogenic strains such as Escherichia coli (ATCC 25922), Salmonella typhi (MTCC733), Klebsiella pneumoniae (ATCC 2146BAA) and Pseudomonas aeruginosa (ATCC PA25619) used in this study were spotted on the Muller Hinton agar plate. Prepare a bacterial suspension in 0.85% NaCl (1.505 OD at 620 nm). Then add 0.5 ml probiotic bacterial culture to each well and incubate all the plates at 37 ºC for 24-48 h in the incubator. Then observe the zone of clearance.

DPPH Free Radical Activity: Briefly, 12.5, 25, 50, and 100 µL cell-free supernatants of Lactobacillus isolates were added into the DPPH radical solution (0.05 mM). The solutions were mixed and kept in the dark for 30 min (B. Wang *et al*., 2016) [29]. The absorbance samples were measured at 517 nm. The scavenging ability was calculated as below:

Scavenging ability (%) = (1 – A sample – A blank)/A control) × 100

Identification of Lactobacillus by PCR amplification

55 isolated DNA samples were used for PCR analysis to identify the genus. Three strains of Lactobacillus plantarum (ATCC 8014) Lactobacillus rhamnosus (ATCC 9595) and Lactobacillus casei (YIT 9029) were used as positive control and, (ATCC 25922), Klebsiella pneumoniae (ATCC 2146BAA), Bacillus cerus (ATCC 9634) and Pseudomonas aeruginosa (ATCC PA25619) strains were used as negative control. A specific primer to identify the Lactobacillus genus was used.

Forward primer: LactoF: 5/-TGGAAACAGRTGCTAATACCG-3/ Reverse primer: LactoR: 5/-GTCCATTGTGGAAGATTCCC-3/

This primer sequence is designed to target highly conserved regions of the *lactobacillus* genus alone. This primer can amplify a total of 233-234 base pairs from the V2.1 to V3 region of the 16s rRNA gene, PCR settings for the amplification of DNA using a specific primer are as follows (Table. 1).

Table 1: PCR conditions for amplification using specific primer

Step	Temperature	Duration in seconds	Cycles
Initial Denaturation	95 ºC	180	1
Denaturation	95 ºC	30	
Annealing	62 ºC	30	35
Extension	72 ºC	20	
Final Extension	72 ºC	120	1

10 µl of PCR product was added with 5µl of 1X loading dye and electrophoresed in 3.5% agarose gel at 100V for 30 minutes with Thermo scientific gene ruler ULR (SM113) DNA ladder as a reference, then the gel under UV trans-illuminator. Using a gel documentation system, the gel was documented, analyzed and imaged (Byun *et al*., 2004) [2].

Results and Discussion

Isolation and Identification of probiotic bacteria

A total of 142 isolates were initially obtained from milk samples. Out of these 55 isolates exhibited all the characteristics of potential probiotic bacteria. They all have similar biochemical properties as LAB. The strains were gram-positive, short rod-shaped, and had a pinpoint, cream-coloured colony. The A5 strain appeared as a small, white colony and was gram-positive, and rod-shaped

Screening of safety and probiotic properties of LAB

Gelatinase activity: The results obtained showed that organisms do not liquefy the gelatin agar, which confirmed that both strains have gelatinase-negative activity when compared with a gelatinase-positive organism Pseudomonas aeruginosa.

Hemolytic activity: The strains were evaluated for hemolytic activity on blood agar plates and the result obtained showed that no zone of hemolysis was observed around the colony thus both strains were hemolytic negative.

Antibiotic sensitivity test: Strains were sensitive to antibiotics including streptomycin, chloramphenicol, and rifampin. None of the strains were multiple drug-resistant or extremely drug-resistant

Acid and bile salt tolerance assays: As described in the method, the optical density of the sample was measured at 620nm for different time intervals to check tolerance. From this, it is clear that the isolates were able to survive at low pH 2, 3, and 4 for 4 hours.

Physiological and biochemical characterization

Growth at different NaCl concentrations: The results showed that both A2 and A5 strains were able to survive in 2% and 4% NaCl, no growth was observed in 6.5% NaCl

concentration. The growth was visualized by the change in colour of the dye due to low pH from purple to yellow after 7 days of incubation, which indicates the growth of organisms at different NaCl concentrations.

Growth at different temperatures: During incubation at different temperatures, growth was indicated by the change in colour of the medium containing dye from purple to yellow due to a change in pH. All the strains turned yellow at 10 ºC, 30 ºC, 37 ºC. Maximum growth was observed at 37 ºC and no growth was reported at 50 ºC.

Resistance to phenol: Resistance of phenol was analysed by measuring O.D. at 620 nm after incubation at 37 ºC for 24 hrs. Both strains can able to grow at 0.4% phenol. Strains could survive in phenol

Antagonistic activity: This study has shown the survival potential of probiotic bacteria against pathogenic bacteria of normal intestinal microflora. Strains showed a zone of inhibition against four pathogenic strains such as Escherichia coli (ATCC 25922), Salmonella typhi (MTCC733), Klebsiella pneumoniae (ATCC 2146BAA) and Pseudomonas aeruginosa (ATCC PA25619). The presence of a zone of clearance was observed and tabulated (table. 2)

Table 2: Table showing the presence and absence of a zone of inhibition for all four pathogens against LAB cell-free supernatant

Antagonistic Activity Well Diffusion Method									
Strain name	**ATCC 25922**	**MTCC 733**	**ATCC 2146BAA**	**ATCC PA25619**		**Strain name**	**ATCC 25922**	**MTCC 733**	**ATCC 2146BAA**	**ATCC PA25619**
ATCC 8014	+	+	+	+		CU 31*	+	+	+	+
YIT 9029	+	+	+	+		CU 33	+	-	-	+
Media con.	-	-	-	-		CU 40*	+	+	+	+
BM 1	+	+	-	+		CU 41	+	+	-	-
BM 2	-	-	-	-		CU 102	-	-	-	+
BM 3*	+	+	+	+		HB 1	+	+	+	-
BM 4	+	+	+	+		HB 2	+	+	-	+
BM 9	-	+	-	+		HB 3	+	+	-	+
BM 13	+	+	-	-		HB 4	+	+	-	+
BM 16	-	-	-	-		HB 5	-	-	+	+
BM 22*	+	+	+	+		TG 2	+	-	-	-
BM 28	+	+	-	+		TG 8	+	+	-	-
BM 101	-	+	+	-		TG 10	+	-	+	-
CU 1	-	-	-	+		TG 18*	+	+	+	+
CU 2	-	+	-	-		TG 28	-	+	+	+
CU 4	+	+	+	+		TG 100	+	+	+	-
CU 5	+	+	-	-		TM 1	+	+	-	-
CU 8	+	-	-	+		TM 3	+	+	-	-
CU 16	+	+	+	+		TM 4	+	-	+	-
CU 17	+	-	-	+		TM 5	-	-	+	-
CU 18	+	+	+	-		TM 6*	+	+	+	+
CU 25	+	+	+	+		TM 7	+	+	-	+
CU 27*	+	+	+	+		TM 21	+	-	-	+
CU 28	+	-	+	-		TM 37*	+	+	+	+
CU 29*	+	+	+	+						

DPPH Free Radical Activity

Bacterial strains that could survive in the gut were further analysed for antioxidant properties the isolated bacterial strains have the potentiality to scavenge 50% of free radicals with the given concentration of CFS results were interpreted in the following table 3.

Table 3: Absorbance value for antioxidant assay and scavenging rate of each bacterial CFS. Note: Highlighted Bacterial strains denote positive result

Antioxidant Activity using DPPH						
Scavenging rate (%)= [1- (Ab Sample - Ab Blank)/Ab Control] * 100 Blank - 0.046, Control - 0.023						
Stain	Absorbance	Scavenging rate %		Stain	Absorbance	Scavenging rate %
ATCC8014	0.202	81.05%		CU 28	0.142	58.47%
YIT9029	0.169	85.39%		CU 29*	0.145	76.63%
Media Con.	0.163	10.58%		CU 31*	0.158	88.65%
BM 1	0.13	55.83%		CU 33	0.163	65.58%
BM 2	0.148	64.79%		CU 40*	0.158	78.65%
BM 3*	0.171	80.67%		CU 41	0.173	69.45%
BM 4	0.138	70.92%		CU 102	0.15	73.56%
BM 9	0.149	84.17%		HB 1	0.173	79.45%
BM 13	0.141	69.08%		HB 2	0.105	71.17%
BM 16	0.159	58.04%		HB 3	0.156	69.88%
BM 19	0.114	25.64%		HB 4	0.129	76.44%
BM 22*	0.152	77.33%		HB 5	0.144	57.24%
BM 28	0.119	62.58%		TG 2	0.129	56.44%

BM 29	0.136	22.15%		TG 8	0.141	59.08%
BM 39	0.178	46.38%		TG 10	0.167	53.13%
BM 100	0.137	41.53%		TG 18*	0.128	77.06%
BM 101	0.144	57.24%		TG 28	0.146	66.01%
BM 102	0.123	30.12%		TG 100	0.157	59.26%
CU 1	0.141	59.08%		TM 1	0.143	67.85%
CU 2	0.176	77.61%		TM 3	0.161	66.81%
CU 4	0.138	80.92%		TM 4	0.133	53.99%
CU 5	0.125	58.90%		TM 5	0.141	69.08%
CU 8	0.172	70.06%		TM 6*	0.169	81.90%
CU 13	0.117	33.80%		TM 7	0.153	51.72%
CU 16	0.182	63.93%		TM 18	0.145	46.63%
CU 17	0.158	58.65%		TM 21	0.118	63.19%
CU 18	0.162	56.20%		TM 24	0.118	33.19%
CU 25	0.106	70.55%		TM 31	0.133	23.99%
CU 27*	0.069	93.25%		TM 37*	0.147	75.40%

Identification of Lactobacillus by PCR amplification

Among 55 strains, 38 strains were identified to the Lactobacillus genus level by using the specific primer. The primer amplifies 234-233 base pairs from V2.1 to V3 region of 16s rRNA. A single crisp band is observed and those DNA samples which get amplified are said to be Lactobacillus Three Reference Lactobacillus (ATCC 8014-L. plantarum, ATCC 9595- L. rhamnosus and YIT 9029- L. casei) was also amplified along with query samples and three negative reference sample Bacillus cereus, E. coli and P. aeruginosa were also amplified. Electrophoresed gel was documented using a Gel Documentation System and shown below in Figure. 2

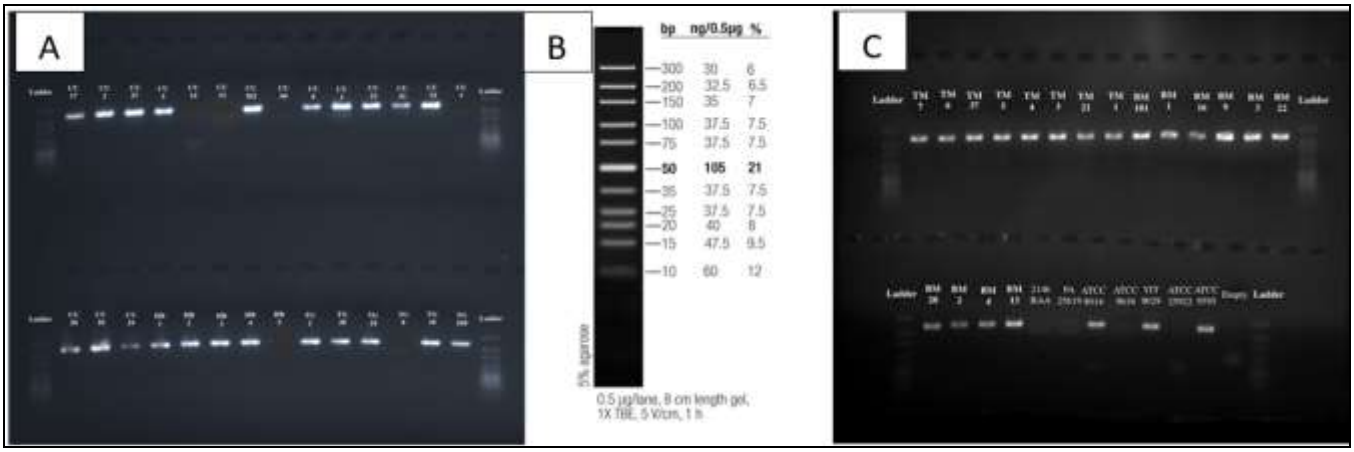

Fig 2: A- Gel image of amplified genomic DNA of isolated strains using specific primer, B- Ultra low range DNA reference Ladder and C- Gel image of amplified genomic DNA of isolated strains and known Lactobacillus sp. using specific primers.

Discussion

The probiotic bacteria such as LAB were Gram-positive, catalase and oxidase negative, non-motile, methyl red test positive and VP, citrate utilization, indole, and urease tests were negative. Out of the 142 strains, 55 strains, have all expected results as putative probiotic bacteria. They all have similar biochemical properties as LAB.

In the current research finding, liquefaction of the gelatin agar was not observed by the any strains which indicate isolates do not produce the gelatinase enzyme. Gelatin is a protein derived from collagen, a material found in the bones, cartilage, and skin of animals that's essential for healthy joints. (De Vuyst *et al*., 2003) [9] reported that L. plantarum strains tested negative for gelatinase enzyme. Safety is one of the suggested features in the FAO/WHO 2001 criteria for the evaluation of probiotics. Probiotic strains are chosen based on the absence of hemolytic and gelatinase activity, which indicates that these bacteria are non-virulent. Zone of hemolysis was absent in both the strain in this research also. Many probiotic Lactobacillus and Enterococcus strains exhibit antibiotic sensitivity to various antibiotics, according to (Yasmin *et al*., 2020) [32]. From the comparison of those results with the current result, probiotic isolated strains are susceptible to routinely used antibiotics like streptomycin, Chloramphenicol, and Rifampin.

One of the main selection criteria for probiotic bacteria is resistance to low pH (C.-Y. Wang *et al*., 2010) [30]. In this investigation, it was discovered that both isolated probiotic strains can live at low pH and 0.3% bile salt concentration. The ability of lactic acid bacteria to resist gastrointestinal stress is dependent on their low pH tolerance (Chou & Weimer, 1999) [4]. For probiotic bacteria to reach the small intestine, they must navigate a variety of challenging situations in the stomach (Prasad *et al*., 1998) [23]. The very important characteristic of LAB is to survive in the small intestine which contains 0.3% w/v bile salt concentration and the recommended stay time is 4 hours (du Toit *et al*., 1998) [11]. Many bacterial strains have bile salt resistance because of the bile salt hydrolase (BSH) enzyme, which hydrolyzes conjugated bile salts and lessens their toxicity (Jack *et al*., 1995) [15].

LAB has been shown to have inhibitory properties, primarily against Gram-positive pathogens and closely related bacteria. LAB strains are usually inactive against

Gram-negative bacteria due to the resistance provided by the outer membrane. In the current study, isolates exhibited inhibition against Gam-positive bacteria but not against Gram-negative bacteria, such as E. coli. These results are similar to (Rijnaarts *et al*., 1993) [25]. This antagonistic activity of isolated bacterial strains might be due to acid production.

The results clearly showed that an increase in NaCl content affected (Ngene *et al*., 2019) [22] the growth of both strains. The LAB isolated from traditional drinking yoghurt was able to grow at a 4% NaCl concentration but not at a 6.5% NaCl concentration. According to, all 55 isolated lactic acid bacteria were able to withstand 2% NaCl.

The bacterial isolates used in the current study demonstrated a high tolerance for phenol concentration (0.4%), indicating that they may withstand the bacteriostatic effects of phenol in the GIT. Phenol is a by-product of the metabolism of aromatic amino acids, which occurs in the gut (Byun *et al*., 2004; Yasmin *et al*., 2020) [2, 32].

The spread plate technique is used for antagonistic activity tests even though agar well diffusion was used in this study. In The present study, 55 isolates against four pathogenic strains such as Escherichia coli (ATCC 25922), Salmonella typhi (MTCC733), Klebsiella pneumonia (ATCC2146BAA) and Pseudomonas aeruginosa (ATCC PA25619) were used in this study. When compared to the results of Gunasena, 2020 isolates from cow milk showed similar activity but isolates from goat milk showed good and better activity against pathogens.

Antioxidant ability is also considered a health-beneficial property (Liu *et al*., 2009) [19]. In the present study, the antioxidant capacity of intact cells was measured using a DPPH assay suggesting that 55 bacteria can resist free radicals, especially hydroxyl radicals. Hydroxyl radicals are free radicals which will have adverse effects on the human body. In the present study, 38 strains have the highest activity has been recorded and it was characterized as Lactobacillus.

Although PCR amplification was done in this study to identify Lactobacillus spp. among the microbial diversity by using specific primers and protocols *framed* by (Byun *et al*., 2004) [2]. From his results and interpretations L. *gasseri*, L. *rhamnosus*, L. *casei*, L. *plantarum*, l. acidophilus and L. *fermentum* were the common strains that can be isolated from dairy samples. Lactobacillus is the most numerically dominant species within the 142 isolates from dairy samples. Here the extracted DNA was amplified using specific primers and electrophoresed in 5% of agarose gel with Thermo Scientific Gene Ruler ULR SM1213 as reference. Among 46 strains, 38 strains were identified to Lactobacillus genus level.

Conclusion

Probiotics are live nonpathogenic microorganisms administered to improve microbial balance, particularly in the gastrointestinal tract and are also regulated as dietary supplements and foods. Probiotics exert their beneficial effects through various mechanisms, including lowering intestinal pH, decreasing colonization and invasion by pathogenic organisms, and modifying the host immune response. The clinical effectiveness of probiotics has been in the treatment of acute diarrhea, most commonly due to rotavirus, and pouchitis. More research is needed to clarify the role of probiotics in preventing antibiotic-associated diarrhea, Clostridium difficile infection, diarrhea, irritable bowel syndrome, ulcerative colitis, Crohn's disease, and vulvovaginal candidiasis. Probiotics have demonstrated efficacy in preventing and treating various medical conditions. The use of probiotics in foods has increased significantly and there are more and more products available with high numbers of viable probiotics. In this present work milk samples from various milk farm shows the enormous number of Lactobacillus spp. presence and this work demonstrates their probiotic character also. These strains can further studied and the compounds produced by these bacteria should be evaluated in future.

Conflict of Interest: Not available
Financial Support: Not available

Reference

1. Bazireh H, Shariati P, Azimzadeh Jamalkandi S, Ahmadi A, Boroumand MA. Isolation of Novel Probiotic *Lactobacillus* and *Enterococcus* Strains From Human Salivary and Fecal Sources. Front Microbiol. 2020;11:597946. https://doi.org/10.3389/fmicb.2020.597946
2. Byun R, Nadkarni MA, Chhour K-L, Martin FE, Jacques NA, Hunter N. Quantitative Analysis of Diverse *Lactobacillus* Species Present in Advanced Dental Caries. J Clin Microbiol. 2004;42(7):3128–3136. https://doi.org/10.1128/JCM.42.7.3128-3136.2004
3. Cerdó T, García-Santos JA, G Bermúdez M, Campoy C. The Role of Probiotics and Prebiotics in the Prevention and Treatment of Obesity. *Nutrients*. 2019;11(3). https://doi.org/10.3390/nu11030635
4. Chou LS, Weimer B. Isolation and characterization of acid- and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. J Dairy Sci. 1999;82(1):23–31. https://doi.org/10.3168/jds.S0022-0302(99)75204-5
5. Corr SC, Hill C, Gahan CGM. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. Adv Food Nutr Res. 2009;56:1–15. https://doi.org/10.1016/S1043-4526(08)00601-3
6. Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol. 2005;3(10):777–788. https://doi.org/10.1038/nrmicro1273
7. Dal Bello F, Walter J, Hammes WP, Hertel C. Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition. Microb Ecol. 2003;45(4):455–463. https://doi.org/10.1007/s00248-003-2001-z
8. Daneshi M, Ehsani MR, Razavi SH, Labbafi M. Effect of refrigerated storage on the probiotic survival and sensory properties of milk/carrot juice mix drink. Electron J Biotechnol. 2013;16(5). https://doi.org/10.2225/vol16-issue5-fulltext-2
9. De Vuyst L, Foulquié Moreno MR, Revets H. Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. Int J Food Microbiol. 2003;84(3):299–318. https://doi.org/10.1016/s0168-1605(02)00425-7
10. Degeest B, De Vuyst L. Indication that the nitrogen source influences both amount and size of exopolysaccharides produced by *Streptococcus*

thermophilus LY03 and modelling of the bacterial growth and exopolysaccharide production in a complex medium. Appl Environ Microbiol. 1999;65(7):2863–2870. https://doi.org/10.1128/AEM.65.7.2863-2870.1999
11. du Toit M, Franz CM, Dicks LM, *et al*. Characterisation and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. Int J Food Microbiol. 1998;40(1–2):93–104. https://doi.org/10.1016/s0168-1605(98)00024-5
12. Dugas B, Mercenier A, Lenoir-Wijnkoop I, *et al*. Immunity and probiotics. Immunol Today. 1999;20(9):387–390. https://doi.org/10.1016/s0167-5699(99)01448-6
13. Fuller R. Probiotics in man and animals. J Appl Bacteriol. 1989;66(5):365–378.
14. Garai-Ibabe G, Dueñas MT, Irastorza A, *et al*. Naturally occurring 2-substituted (1,3)-beta-D-glucan producing *Lactobacillus suebicus* and *Pediococcus parvulus* strains with potential utility in the production of functional foods. Bioresour Technol. 2010;101(23):9254–9263. https://doi.org/10.1016/j.biortech.2010.07.050
15. Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiol Rev. 1995;59(2):171–200. https://doi.org/10.1128/mr.59.2.171-200.1995
16. Kang W, Pan L, Peng C, *et al*. Isolation and characterization of lactic acid bacteria from human milk. J Dairy Sci. 2020;103(11):9980–9991. https://doi.org/10.3168/jds.2020-18704
17. Krasaekoopt W, Bhandari B, Deeth H. Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J. 2003;13(1):3–13. https://doi.org/10.1016/S0958-6946(02)00155-3
18. Lilly DM, Stillwell RH. Probiotics: Growth-promoting factors produced by microorganisms. Science (New York, N.Y.). 1965;147(3659):747–748. https://doi.org/10.1126/science.147.3659.747
19. Liu J, Luo J, Ye H, Sun Y, Lu Z, Zeng X. Production, characterization and antioxidant activities *in vitro* of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. Carbohydr Polym. 2009;78(2):275–281. https://doi.org/10.1016/j.carbpol.2009.03.046
20. Mathew S, Vijay S, Potty VP. Assessment of probiotic potential of lactic acid bacteria isolated from curd and its application using fruit juice. Int. J Curr Microbiol Appl Sci. 2017;6(1):282-289. https://doi.org/10.20546/ijcmas.2017.601.034
21. Motohashi N, Shirataki Y, Kawase M, Tani S, Sakagami H, Satoh K, Kurihara T, Nakashima H, Mucsi I, Varga A, Molnár J. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: a study of kiwifruit extracts. J Ethnopharmacol. 2002;81(3):357-364. https://doi.org/10.1016/s0378-8741(02)00125-3
22. Ngene AC, Onwuakor CE, Aguiyi JC, Ifeanyi VO, Ohaegbu CG, Okwuchukwu CP, Kim EG, Egbere JO. Screening of some lactic acid bacteria isolated from selected Nigerian fermented foods for vitamin production. Adv Microbiol. 2019;9(11):943-955. https://doi.org/10.4236/aim.2019.911060
23. Prasad J, Gill H, Smart J, Gopal PK. Selection and characterisation of Lactobacillus and Bifidobacterium strains for use as probiotics. Int Dairy J. 1998;8(12):993-1002. https://doi.org/10.1016/S0958-6946(99)00024-2
24. Ras M, Lefebvre D, Derlon N, Paul E, Girbal-Neuhauser E. Extracellular polymeric substances diversity of biofilms grown under contrasted environmental conditions. Water Res. 2011;45(4):1529-1538. https://doi.org/10.1016/j.watres.2010.11.021
25. Rijnaarts HH, Norde W, Bouwer EJ, Lyklema J, Zehnder AJ. Bacterial adhesion under static and dynamic conditions. Appl Environ Microbiol. 1993;59(10):3255-3265. https://doi.org/10.1128/aem.59.10.3255-3265.1993
26. Samedi L, Charles AL. Isolation and characterization of potential probiotic lactobacilli from leaves of food plants for possible additives in pellet feeding. Ann Agric Sci. 2019;64(1):55-62. https://doi.org/10.1016/j.aoas.2019.05.004
27. Singh P, Saini P. Food and health potentials of exopolysaccharides derived from lactobacilli. Microbiol Res J Int. 2017;22(2):1-14. https://doi.org/10.9734/MRJI/2017/36935
28. Van Tassell ML, Miller MJ. Lactobacillus adhesion to mucus. Nutrients. 2011;3(5):613-636. https://doi.org/10.3390/nu3050613
29. Wang B, Xu H, Xu F, Zeng Z, Wei H. Efficacy of oral Bifidobacterium bifidum ATCC 29521 on microflora and antioxidant in mice. Can J Microbiol. 2016;62(3):249-262. https://doi.org/10.1139/cjm-2015-0685
30. Wang CY, Lin PR, Ng CC, Shyu YT. Probiotic properties of Lactobacillus strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. Anaerobe. 2010;16(6):578-585. https://doi.org/10.1016/j.anaerobe.2010.10.003
31. Williams NT. Probiotics. Am J Health-Syst Pharm. 2010;67(6):449-458. https://doi.org/10.2146/ajhp090168
32. Yasmin I, Saeed M, Khan WA, Khaliq A, Chughtai MFJ, Iqbal R, Tehseen S, Naz S, Liaqat A, Mehmood T, Ahsan S, Tanweer S. *In vitro* probiotic potential and safety evaluation (hemolytic, cytotoxic activity) of Bifidobacterium strains isolated from raw camel milk. Microorganisms. 2020;8(3). https://doi.org/10.3390/microorganisms8030354

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