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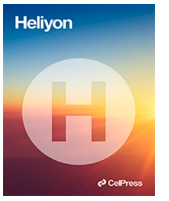

Assessment of probiotic properties of lactic acid bacteria isolated from an artisanal Colombian cheese

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ABSTRACT

Lactic Acid Bacteria play an important role in the milk fermentation processes of traditional cheeses and have become an important target for the development of novel cheese cultures because of their ability to confer health benefits. This study aimed to evaluate the probiotic potential of 12 Lactic Acid Bacteria (LAB) strains previously isolated and molecularly identified from an artisanal Colombian Double-Cream Cheese. Probiotic properties, including safety (hemolysis and sensibility to antibiotics), pH and bile salt tolerance, auto-aggregation, cell surface hydrophobicity, antibacterial activity, and exopolysaccharide production, were examined. None of the strains were hemolytic, and *Pediococcus* (16, 18) and *Lactobacillus* (28, 29) were found to be sensitive to all antibiotics. Moreover, all the strains tolerated pH (3.0, 6.5 and 8.0) and bile salt conditions (0.3, 0.6 and 1.0 % w/v). *Pediococcus pentosaceus* (16), *Leuconostoc citreum* (17), *Pediococcus acidilactici* (18), *Enterococcus faecium* (21,22), *Enterococcus faecalis* (24) and *Limosilactobacillus fermentum* (29) exhibited medium autoaggregation and affinity to chloroform. Six of the strains exhibited a ropy exopolysaccharide phenotype. Antibacterial activity against foodborne pathogens, *Salmonella* Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 19111, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, was found to be strain dependent, with the strains 16, 18, 21, 26, 28 and 29 presenting a higher inhibition (>4 mm) against all of them. According to Principal Component Analysis, *P. pentosaceus* (16), *Leu. mesenteroides* (26), *L. casei* (28), *L. fermentum* (29), and *E. faecium* (21) showed strong probiotic properties. Our findings suggest that five strains out of the 12 sampled strains are potential probiotics that could be used in the processing of traditional dairy products on an industrial scale to improve their quality.

1. Introduction

Fermented foods with probiotics have been proposed as a novel approach for the therapeutic treatment of comorbidities of COVID-

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19 disease (SARS-CoV-2) due to their ability to modulate the immune system and intestinal microbiome and reduce susceptibility to subsequent infections [1–3]. Currently, probiotics have been used to treat or prevent some pathologies such as Crohn's disease, ulcerative colitis, acute respiratory tract infections, necrotizing enterocolitis, food allergies, slow the progression of liver fibrosis, irritable bowel syndrome, type II diabetes, and obesity [4–8]. The benefits of the consumption of probiotics have been found to be directly related to their effect on the gut microbiota, which varies according to genetics, age, ethnicity, and the geographic origin of the host and diet [9]. Also, they may contribute to consumer health by impacting mucosal immune mechanisms and gut microbiota and their influence on other organs of the body through the production of neurotransmitters [10,11].

In traditional cheeses, Lactic Acid Bacteria (LAB) with probiotic properties play an important role when added accidently or intentionally [12]. During milk fermentation, the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Enterococcus* have shown the ability to reduce the concentration of lactose, facilitate the elimination of toxic or antinutritional factors from milk, promote better bioavailability of nutrients, and prevent adverse reactions from their consumption [13]. For LAB to be considered a probiotic, they must meet the requirements established by FAO/WHO which include being safe for consumption, not being hemolytic, resisting the acidic pH of the stomach, bile salts, and gastric juices, being sensitive to antibiotics, having antagonistic activity against pathogens, having autoaggregation, hydrophobicity and adhesion to intestinal epithelium [14]. In addition, new selection criteria include biological activities such as anti-carcinogenic, anti-depressant, anti-obesity, antidiabetic, and hypocholesterolemic activities [15].

In Colombia, various studies have been carried out on probiotics for functional food and animal feed design, including techniques for supplementing plant-based matrices with microorganisms [16–18]. However, commercial strains are often used because of the presence of unknown microbiota in traditional foods [19]. Fresh cheese is the most consumed artisan cheese in Colombia [20] and has a microbiological composition with beneficial properties [21]. Therefore, it is important to study the microbiota of indigenous Colombian foods, such as artisan cheeses, as they could provide new native strains with probiotic properties for developing innovative dairy products with positive effects on human health [22].

Cheese is considered a better carrier of probiotics compared to many dairy products, due to its lower water activity ($aw > 0.90$), low storage temperature (4–8 °C), relatively higher pH compared to traditional dairy products, and textural characteristics (e.g., high fat content, which protects probiotics during gastrointestinal transit) [23]. The aim of this work was to evaluate the probiotic properties of twelve LAB strains previously isolated from a Colombian Double Cream Cheese and select the most promising probiotic strains that could give additional value to the manufacture of traditional dairy products and new, innovative, and precision foods.

2. Material and methods

2.1. Strains and culture conditions

Twelve LAB strains previously isolated and molecularly identified from a Colombian Double Cream Cheese [21] were evaluated for their probiotic properties: *Pediococcus pentosaceus* (16), *Pediococcus acidilactici* (18), *Leuconostoc citreum* (17), *Leuconostoc mesenteroides* subsp. *mesenteroides* (19, 26), *Weissella viridescens* (25), *Lacticaseibacillus casei* (28), *Limosilactobacillus fermentum* (29), *Lactococcus lactis* (27), *Enterococcus faecium* (21, 22) and *Enterococcus faecalis* (24). The strains were used under the permission number 16DE0AC88E5 given by the Autoridad Nacional de Licencias Ambientales (ANLA). As a positive control, *L. rhamnosus* GG ATCC 53103 was used. Bacteria were cultivated in de Man, Rogosa, and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37 °C in an atmosphere with 5 % CO_2 and 95 % air. All the assays were carried out starting from a bacterial solution adjusted to 1.5×10^8 Colony Forming Units (CFU/mL). Briefly, cells were harvested by centrifugation at 3500*g* for 5 min. After washing the pellet, the cell suspension was adjusted in Phosphate Buffered Saline to reach a concentration 0.5 McFarland.

2.2. Biosafety assessment

2.2.1. Hemolytic activity

Hemolytic activity was evaluated using blood agar with 5 % (v/v) human blood. Bacterial overnight cultures in MRS broth were streaked on the surface of the agar, and the plates were incubated at 37 °C for 48 h [24]. Bacteria were classified according to the halo produced around the inoculation line. *Lacticaseibacillus rhamnosus* GG ATCC 53103 and *Staphylococcus aureus* coagulase positive ATCC 25923 were used as negative and positive controls, respectively.

2.2.2. Antibiotic susceptibility assessment

Minimal Inhibitory Concentrations (MICs) of all bacteria to gentamicin, penicillin, chloramphenicol, ampicillin, erythromycin, and vancomycin (Sigma) were determined by the microdilution broth method according to the CLSI (Clinical and Laboratory Standards Institute) and EFSA (European Food Safety Authority) [25,26]. 100 μL of the bacterial inoculum (0.5 McFarland) was added to each well of a 96-well plate containing 100 μL of an increasing concentration of the antibiotics. The microplates were incubated at 37 °C for 24 h, and bacterial MICs were determined as the lowest concentration of an antibiotic at which visible growth was inhibited and compared with the MIC breakpoint values recommended by EFSA [26].

2.3. Screening of probiotic properties

2.3.1. Tolerance to pH and bile salts

Tolerance to pH (3.0, 6.5, and 8.0) and bile salts (0.3, 0.6, and 1.0 % (w/v)) was assessed using the broth microdilution method in

96-well plates, and modified MRS broth solutions were prepared as indicated by Jatmiko [27]. Stock solutions of MRS broth were prepared and adjusted to each of the pH values using HCl and NaOH at 1 mol/L, and the appropriate amount of bovine bile salts was added according to the concentration (Difco Oxgall). Then, 100 μL of each strain (0.5 McFarland) was mixed with the previously prepared MRS broth solutions. The plates were incubated at 37 °C for 24 h in the Multiskan™ GO Thermo spectrophotometer (Thermo Scientific), and the Optical Density (OD) at 600 nm was recorded every hour. To confirm the tolerance to the extreme conditions (pH 3.0 and 1 % (w/v) bile salts), the strains were adjusted to the 0.5 McFarland standard in peptone water (1 % (w/v)), mixed with the previously prepared MRS broth solutions and incubated at 37 °C for 2 h in anaerobic conditions. Serial dilutions were performed, and CFU/mL counts were made by the standard plate count method on MRS agar. Tolerance to these conditions was expressed as Log (CFU/mL) for each strain [28].

2.3.2. Auto-aggregation and cell surface hydrophobicity

The autoaggregation of the LAB strains was assessed according to the method of Armas [29], and the cell surface hydrophobicity was determined using the method described by Lee [30] with small modifications. In both cases, the cells from an overnight culture of each strain were harvested by centrifugation. After washing the pellet, it was resuspended in phosphate-buffered saline (pH 7.2) and the absorbance was adjusted to 0.1 OD_{600}nm (A0, H0). For the auto-aggregation assay, each bacterial suspension was incubated at 37 °C without agitation, and the absorbance of the surface was measured at 2h (A1) at 600 nm using a UV/visible spectrophotometer. Autoaggregation was expressed as %Autoaggregation = (1-(A1)/(A0)) × 100. For hydrophobicity, the bacterial suspensions were mixed with chloroform (a non-polar acid solvent) or ethyl acetate (a non-polar basic solvent) at a 2:1 ratio and vortexed for 1 min. The mixture was incubated at ambient temperature for 1 h until phase separation. Briefly, the aqueous phase was slightly removed to measure its absorbance at 600 nm (H1). The cell surface hydrophobicity was calculated as %H = (1-(H1)/(H0)) × 100.

2.3.3. Exopolysaccharide (EPS) production phenotype

EPS production was evaluated according to the procedure described by Khalil [31]. Briefly, strains were inoculated on the surface of MRS agar supplemented at 2 % (w/v) with sucrose (Merck), and bacterial plates were incubated at 37 °C for 48 h. Subsequently, a smear was made in one of the colonies with an inoculation loop, and the formation of an unbreakable strand indicated a ropy phenotype.

2.3.4. Antibacterial activity against foodborne pathogens

To evaluate the antibacterial activity of LAB against *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Listeria monocytogenes* ATCC 19111, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923, the agar well diffusion method was performed using Mueller Hinton Agar (MHA) or Trypticase Soy Agar (TSA) (Merck) plates [32]. To obtain cell-free supernatant, overnight cultures of LAB until stationary phase were centrifuged at 10000 g for 20 min at 4 °C and sterilized with a filter membrane of 0.22 μm. Briefly, the pathogenic strains were incubated in Brain Heart Infusion (BHI) medium (Merck) at 37 °C for 24 h under aerobic conditions. Thereafter, the cells were harvested by centrifugation, the pellet was washed, resuspended in phosphate-buffered saline (pH 7.2), and the absorbance was adjusted to 0.1 OD_{600}nm. 100 μL of the adjusted suspension of each pathogenic strain was grown over soft MHA plates, except for *L. monocytogenes,* which was inoculated in TSA. Then, wells (5 mm) were made in the agar plates and filled with 50 μL of cell free supernatant of LAB strains. Plates were incubated at 37 °C for 12–24 h. The antibacterial activity scores were assigned according to the diameter of the inhibition zone: Negative (−) for inhibition zones ≤1.0 mm; Intermediate (+) for inhibition zones of 1.1–3.9 mm and High (++) for inhibition zones ≥4.0 mm [33].

2.4. Statistical analysis

The tests were performed in triplicate for each treatment. In all cases, results were expressed as mean ± standard deviation (SD), and significant differences were considered when $p < 0.05$ with a 95 % confidence interval. When the data were non-parametric, the

Table 1
Minimum inhibitory concentration (MIC) (μg/mL).

Strain	Gm	Pe	Vm	*Cm*	Te	Am	Er
16	<4	<1	N.R.	4	4	<1	<0.25
17	16	<1	N.R.	<4	1.5	<0.5	2 R
18	<4	<1	N.R.	<2	4	<1	<0.25
19	32^R	<1	N.R.	16^R	0.19	<0.5	2^R
21	$>64^R$	<1	$>64^R$	16	1.5	<0.5	<1
22	$>64^R$	<1	<2	16	0.125	<0.5	2
24	$>64^R$	<1	<2	16	0.125	<0.5	8^R
25	16	<1	N.R.	8^R	0.25	<0.5	2^R
26	16	<1	N.R.	4	2	1	2^R
27	32	<1	<2	8	0.094	<0.5	2^R
28	8	<1	N.R.	<2	4	<1	<0.25
29	8	<1	N.R.	<2	4	<0.5	0.5

Gm: gentamycin, Pe: penicillin, Vm: vancomycin, *Cm*: chloramphenicol, Te: tetracycline, Am: ampicillin, Er: erythromycin; R: Resistant NR: not required.

Kruskal-Wallis test was performed. For the analysis of the data, a programming routine was carried out using the statistical software SPSS version 25 (IBM® SPSS® Statistics). In addition, a Principal Components Analysis (PCA) using the SPSS Statistics v.24 (IBM) program was performed to determine the most promising probiotic strains.

3. Results

3.1. Biosafety assessment

All of the LAB strains were non-hemolytic. The MIC values for antibiotic susceptibility of the strains to the antibiotics are presented in Table 1. All the strains exhibited MIC values equal to or lower than the MIC breakpoints reported by EFSA [34] for penicillin, tetracycline, and ampicillin, indicating that they were sensitive to these antibiotics. *P. pentosaceus* (*16*), *P. acidilactici* (*18*), *L. casei* (*28*), and *L. fermentum* (*29*) were sensitive to all of the antibiotics tested (Ali et al., 2021). The strains L. *citreum* (*17*), *L. mesenteroides* subsp. *mesenteroides* (*26*), and *L. lactis* (*27*) were only resistant to erythromycin. *L. mesenteroides* subsp. *mesenteroides* (*19*) and *Enterococcus* (*21*, *22* and *24*) presented resistance to several antibiotics.

3.2. 2. Tolerance to pH and bile salts

The results of LAB tolerance at different pH values are presented in Table 2. Vigorous growth was observed in all strains at pH 6.5 and 8.0. *L. casei* (*28*), *L. fermentum* (*29*), *E. faecium* (*21*) and *E. faecalis* (*24*) showed the highest growth of all evaluated pH. At pH 3.0, no significant difference was observed ($p > 0.05$) in strain tolerance, compared with *L. rhamnosus* GG when the microdilution broth method was used. The strains *16*, *18*, *21, 22*, *26*, *28,* and *29* were tolerant to 0.3 % (w/v) and 0.6 % (w/v) of bile salt, showing no significant differences ($p > 0.005$) compared to the control *L. rhamnosus* GG. The growth of all strains was lower at 0.6 % (w/v) biliary salts (Table 3). The growth of strains to pH 3.0 and 1.0 % (w/v) bile salts is presented in Fig. 1. The growth of the strains was higher than 6 Log (CFU/mL) in both conditions, without statistically significant differences when compared to the growth on MRS broth. Additionally, the strains *18, 25,* and *26* presented higher growth at pH 3.0 and the strains *21, 25* and *26* showed a higher growth at 1.0 % (w/v) bile salts. These results confirm the data obtained by the microdilution broth method, affirming the strain's tolerance to gastrointestinal stress conditions.

3.3. Autoaggregation and cell surface hydrophobicity

Table 3 presents the autoaggregation and cell surface hydrophobicity percentages for all the strains. For the aggregation property, the classification proposed by Darmastuti [35] was used, according to which the strains *P. pentosaceus* (*16*), *L. citreum* (*17*),

Table 2
Tolerance of strains to pH and bile salts ($OD_{600\ nm}$ 23h).

Strains	pH				Bile salts (% w/v)			
	MRS	3.0	6.5	8.0	MRS	0.3	0.6	1.0
16	0.605 ± 0.00 abcdef	0.008 ± 0.00[a]	0.525 ± 0.03 afg	0.390 ± 0.15[a]	0.507 ± 0.031 abcde	0.342 ± 0.051 abcd	0.236 ± 0.016 abcde	0.213 ± 0.016 abcd
17	0.409 ± 0.13 cdef	0.002 ± 0.00[a]	0.580 ± 0.01 abcdefg	0.544 ± 0.19[a]	0.550 ± 0.035 bcde	0.180 ± 0.043 de	0.149 ± 0.052 cde	0.144 ± 0.057 bcd
18	0.635 ± 0.01 abcde	0.004 ± 0.00[a]	0.587 ± 0.01 abcdef	0.397 ± 0.15[a]	1.137 ± 0.063 abc	0.360 ± 0.007 abc	0.331 ± 0.039 abc	0.255 ± 0.035 ab
19	0.206 ± 0.03[f]	0.003 ± 0.00[a]	0.557 ± 0.02 bcdefg	0.527 ± 0.27[a]	0.688 ± 0.185[e]	0.154 ± 0.018[e]	0.092 ± 0.009[e]	0.076 ± 0.017[d]
21	1.032 ± 0.00 ab	0.054 ± 0,00[a]	1064 ± 0,01 abcde	0,866 ± 0,30[a]	0.678 ± 0.466 abcde	0.337 ± 0.046 abcd	0.238 ± 0.105 abcde	0.180 ± 0.041 abcd
22	0,714 ± 0,18 abcde	0,003 ± 0,00[a]	0,644 ± 0,25 cdfg	0,599 ± 0,29[a]	0.762 ± 0.410 abcde	0.307 ± 0.069 abcde	0.242 ± 0.118 abcde	0.189 ± 0.063 abcd
24	0.687 ± 0.32 abcdef	0.024 ± 0.04[a]	0.742 ± 0.31 bcdefg	0.588 ± 0.37[a]	0.438 ± 0.028 de	0.203 ± 0.040 cde	0.146 ± 0.058 cde	0.125 ± 0.051 cd
25	0.387 ± 0.03 def	0.004 ± 0.00[a]	0.537 ± 0.04 dfg	0.498 ± 0.16[a]	0.516 ± 0.078 cde	0.154 ± 0.038[e]	0.136 ± 0.054 de	0.135 ± 0.050 bcd
26	0.484 ± 0.03 bcdef	0.000 ± 0.00[a]	0.601 ± 0.19 dfg	0.335 ± 0.09[a]	0.546 ± 0.006 abcd	0.383 ± 0.039 ab	0.256 ± 0.046 abcd	0.223 ± 0.064 abc
27	0.324 ± 0.10 ef	0.003 ± 0.00[a]	0.551 ± 0.01 defg	0.240 ± 0.14[a]	0.576 ± 0.091 abcde	0.241 ± 0.096 bcde	0.196 ± 0.065 bcde	0.177 ± 0.055 abcd
28	1.024 ± 0.05[a]	0.024 ± 0.04[a]	1.069 ± 0.01 abe	0.811 ± 0.26[a]	1.122 ± 0.005[a]	0.324 ± 0.047 abcde	0.363 ± 0.039 ab	0.264 ± 0.006[a]
29	0.963 ± 0.14 abc	0.010 ± 0.01[a]	1.074 ± 0.01 ab	0.863 ± 0.30[a]	1.131 ± 0.015[a]	0.326 ± 0.053 abcd	0.390 ± 0.030[a]	0.275 ± 0.009[a]
Control Rha	1.072 ± 0.04[a]	0.161 ± 0.28[a]	1.209 ± 0.03[a]	0.858 ± 0.44[a]	1.169 ± 0.050[a]	0.657 ± 0.035[a]	0.435 ± 0.036[a]	0.292 ± 0.049[a]

Means in the same column without a common superscript letter differ significantly ($P < 0.05$). Rha, *L. rhamnosus* GG.

Table 3
Autoaggregation and hydrophobicity percentages.

Strain	% Autoaggregation		% Hydrophobicity			
			Ethyl acetate		Chloroform	
16	21.49 ± 4.46	abcd	31.89 ± 3.79	abcde	57.81 ± 3.80	abc
17	23.77 ± 3.49	ab	46.48 ± 0.86	a	53.98 ± 1.52	abcd
18	35.76 ± 3.37	a	24.44 ± 2.37	bcde	18.59 ± 2.34	cd
19	19.27 ± 2.45	bcde	18.90 ± 2.91	cde	66.85 ± 3.62	ab
21	20.32 ± 4.59	bcde	15.99 ± 1.23	de	44.93 ± 5.15	abcd
22	20.14 ± 1.80	abcde	30.50 ± 1.44	abcde	17.66 ± 4.23	cd
24	21.27 ± 2.42	abc	18.91 ± 0.12	cde	13.74 ± 3.87	d
25	14.09 ± 3.57	e	41.97 ± 2.19	ab	30.71 ± 0.26	abcd
26	23.30 ± 7.25	abcd	35.99 ± 4.57	abc	69.74 ± 11.28	ab
27	15.32 ± 3.37	cde	36.25 ± 0.38	abc	22.30 ± 1.35	bcd
28	15.77 ± 3.82	cde	28.28 ± 3.00	abcde	42.57 ± 4.75	abcd
29	21.82 ± 2.41	abc	14.03 ± 3.98	e	87.55 ± 0.43	a
ATCC 53103	15.91 ± 1.78	de	33.31 ± 0.44	abcd	87.20 ± 2.55	a

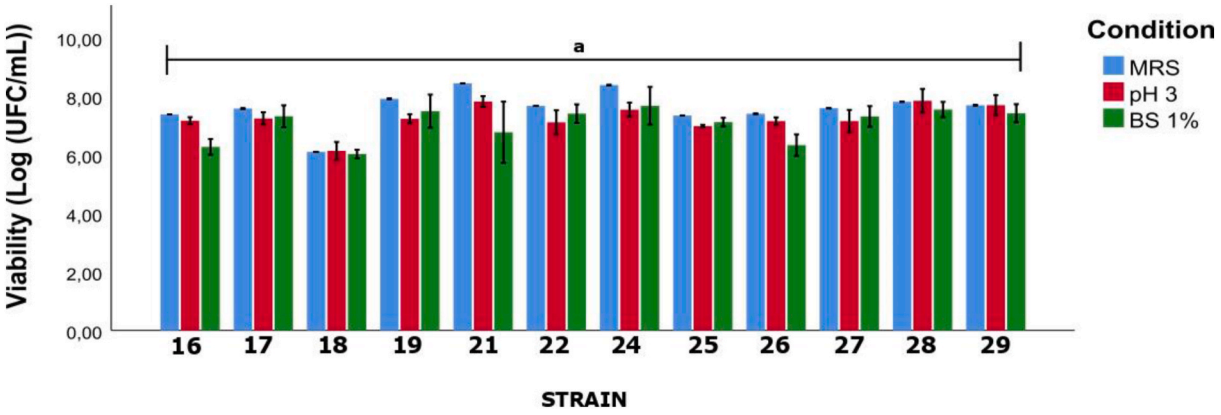

Fig. 1. Viability of LAB strains to pH and bile salts conditions.

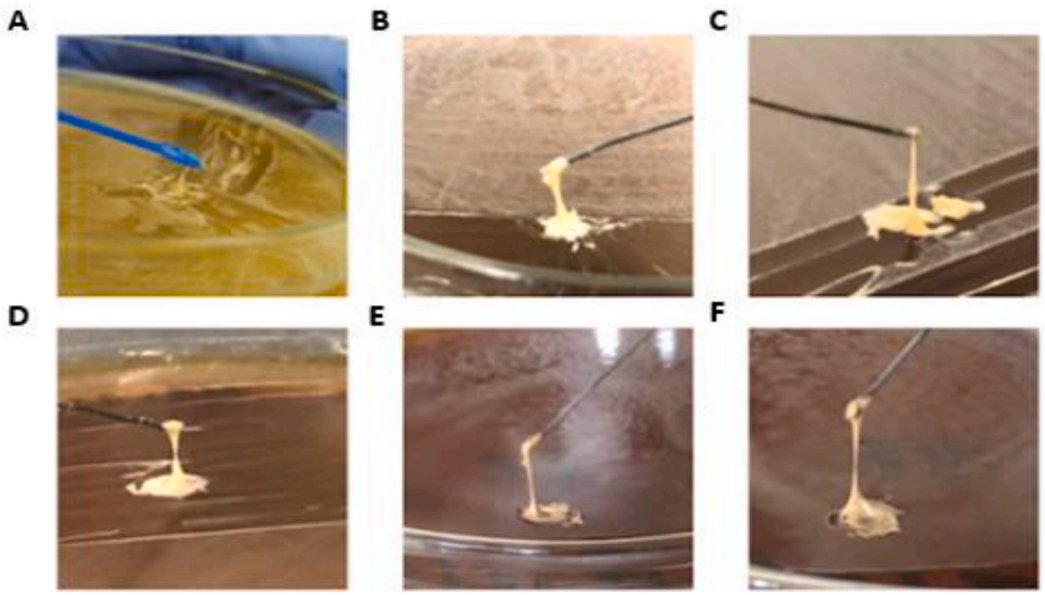

Fig. 2. EPS producing LAB. A. *P. pentosaceus* (*16*), B. *P. acidilactici* (*18*), C. *E. faecium* (*21*), D. *W. viridescens* (*25*), E. *L. casei* (*28*) and F. *L. fermentum* (*29*).

P. acidilactici (*18*), *E. faecium* (*22*), *E. faecalis* (*24*), *L. mesenteroides* (*26*), and *L. fermentum* (*29*) presented medium autoaggregation (20–70 %), and the other strains were classified with low autoaggregation (<20 %). Strains were classified as having low (0–30 %), medium (31–60 %) or high (>60 %) hydrophobicity. For ethyl acetate, the strains *16, 17, 25, 26* and *27* were classified in the medium hydrophobicity range without statistical differences ($p > 0.05$) with the strains *24* and *28*; the remaining strains were classified as having low hydrophobicity. For chloroform, 41.6 % of strains were classified as low hydrophobic, 33.3 % as medium, and 25 % as high. Nevertheless, there was no difference ($p > 0.05$) in the hydrophobicity percentage for the strains *16, 17, 19, 21, 25, 26, 28,* and *29*.

3.4. Exopolysaccharide production phenotype

Fig. 2 (A-F) illustrates the production of EPS by LAB strains. *P. pentosaceus* (*16*), *P. acidilactici* (*18*), *E. faecium* (*21*), *W. viridescens* (*25*), *L. casei* (*28*) and *L. fermentum* (*29*) exhibited the phenotype "ropy", which was observed as a thin filament when a colony was picked up with a bacteriological loop.

3.5. Antibacterial activity against foodborne pathogens

The antibacterial activity results are provided in Fig. 3 (A-D). All LAB strains showed antibacterial activity against gram-negative pathogens. *P. pentosaceus* (*16*), *E. faecium* (*21*), *L. mesenteroides* subsp. *mesenteroides* (*26*) and *L. casei* (*28*), were classified as having a high inhibitory capacity (4.0–4.25 mm) against *E. coli* without statistical differences ($p > 0.05$). Antibacterial activity against Gram-positive pathogens was variable, most notably for *L. monocytogenes*, in which 50 % of the strains presented inhibition, with inhibition zones ranging from 3 to 4 mm, while 10 strains inhibited *S. aureus. P. pentosaceus* showed the highest antibacterial activity against all pathogens studied (>4 mm) but there was no significant difference ($p > 0.05$) with the strains *18, 21, 26, 28,* and *29*.

3.6. PCA analysis

The results on the integration of probiotic properties as assessed with a PCA, including the tests of antibacterial activity, pH, and bile salt tolerance, autoaggregation, and hydrophobicity are presented in Fig. 4. This analysis showed two components, PC1 and PC2, explaining the greatest amount of variance for the probiotic properties data, with a final contribution of 44.07 % and 23.64 %, respectively, totaling 67.61 % of the variation. The PCA showed that antibacterial activity against all pathogens and autoaggregation were correlated with PC1; 1.0 % (w/v) bile salt and pH 3.0 tolerance, and hydrophobicity with chloroform were correlated with PC2 (Table 4). This suggests that the variables that were correlated to PC1 and PC2 contribute to the selection of the most suitable strains with probiotic properties. According to this, the strains that were grouped in the first quadrant (*16, 21, 26, 28* and *29*) were the probiotic candidates with the most potential.

4. Discussion

In the selection of microorganisms with probiotic properties, a sequence of multiple tests is required to progressively reduce the number of probiotic candidates, according to the qualities exhibited, the industrial requirements, and the beneficial effects. The strains

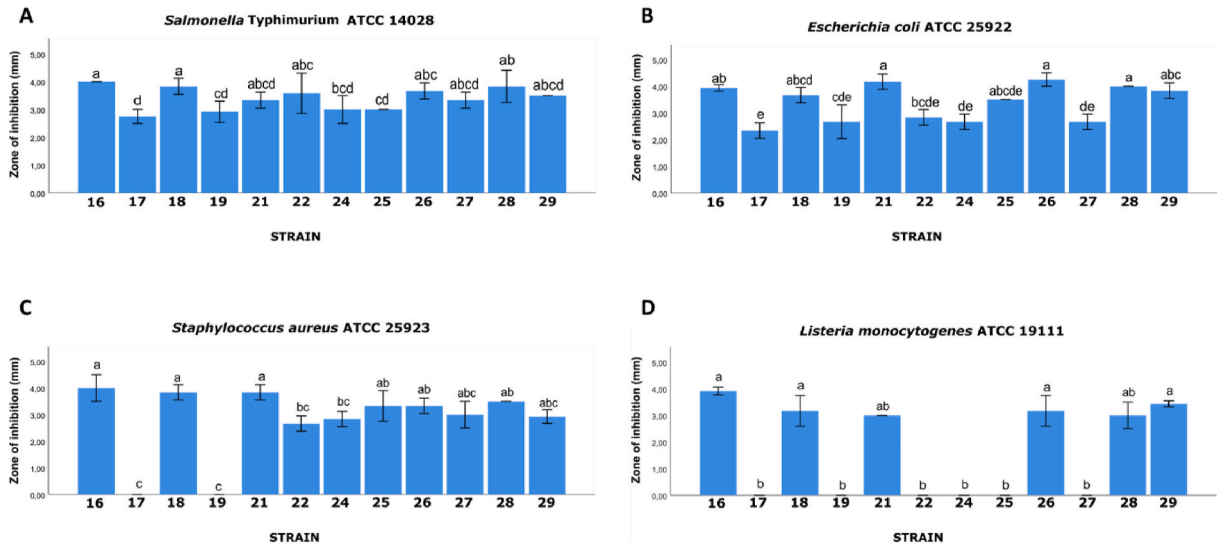

Fig. 3. Antibacterial activity of LAB strains against A. *Salmonella* Typhimurium ATCC 14028, B. *Escherichia coli* ATCC 25922, C. *Staphylococcus aureus* ATCC 25923 and D. *Listeria monocytogenes* ATCC 19111.

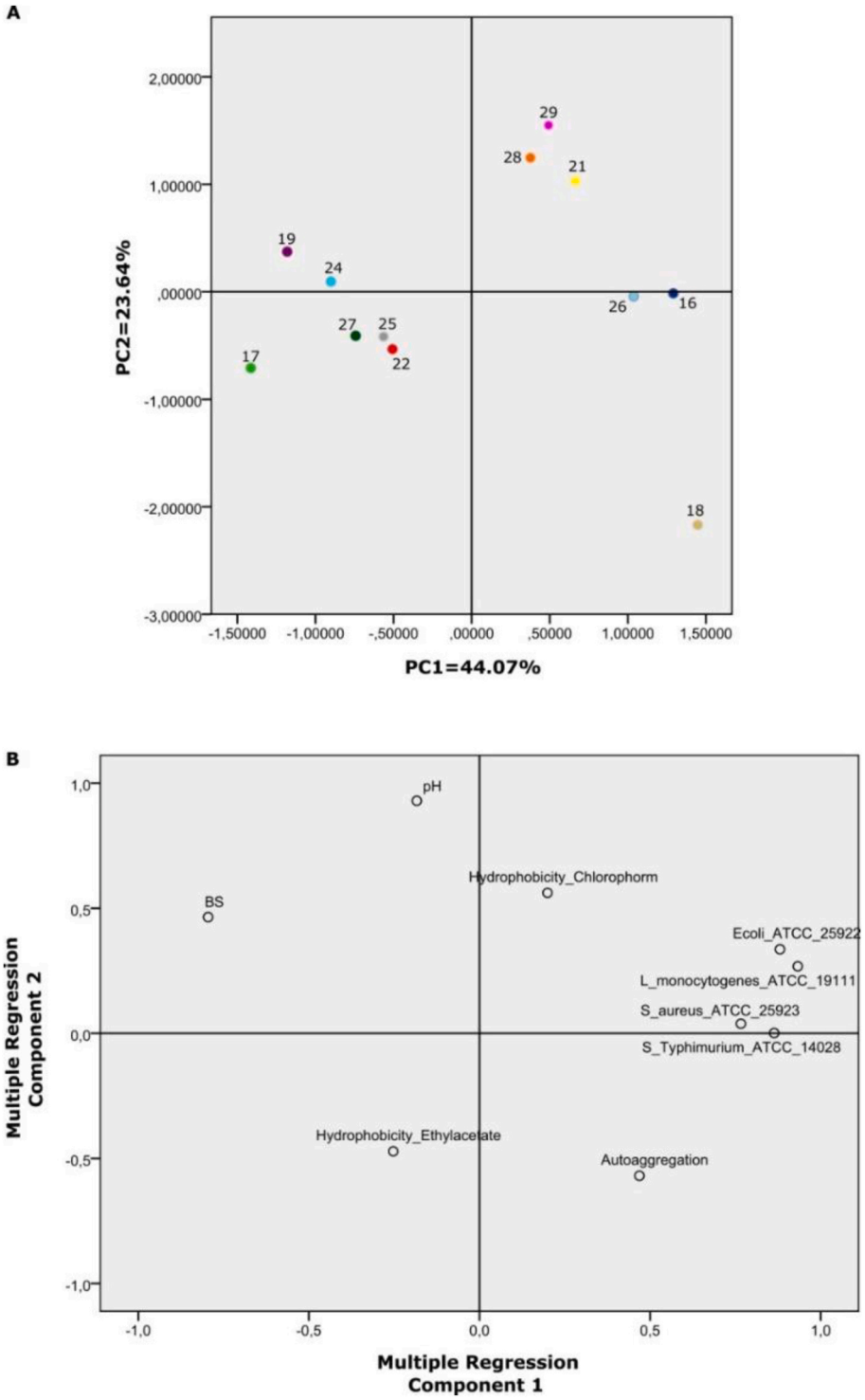

Fig. 4. Principal Component Analysis (PCA) based on the probiotic properties antibacterial activity, pH, and bile salt tolerance, autoaggregation and hydrophobicity. A. Strain distribution, B. Distribution of probiotic properties.

evaluated must comply with the *in vitro* evaluation guidelines proposed by the FAO/WHO [14,15]. One of the first probiotic tests is the safety evaluation, which guarantees no risk to human health. The studied strains did not have hemolysin or toxic by-products that could lyse red blood cells. These results agree with previous reports carried out for the genera *Pediococcus* [36], *Leuconostoc* [37], *Enterococcus* [38], *Weissella* [39], *Lactococcus* [40], and *Lactobacillus* [41], which is a requirement to classify bacteria as having

Table 4
Principal component coefficient matrix.

Probiotic properties	PC1	PC2
Antibacterial activity against *E. coli*	0.222	0.158
Antibacterial activity against *S.* Typhimurium	0.218	0.001
Antibacterial activity against *S. aureus*	0.193	0.018
Antibacterial activity against *L. monocytogenes*	0.235	0.126
Autoaggregation	0.118	−0.268
Hidrophobicity with ethyl acetate	−0.064	−0.222
Hidrophobicity with chloroform	0.050	0.264
Tolerance to pH 3.0	−0.046	0.437
Tolerance to bile salts 1.0 % (w/v)	−0.200	0.218

Qualified Presumption of Safety (QPS) according to EFSA [42].

Another criterion that should be considered in the evaluation of a probiotic is sensitivity to antibiotics. In this study, all the strains belonging to the genera *Pediococcus*, *Limosilactobacillus,* and *Lacticaseibacillus* were sensitive to the antibiotics tested (Table 2), as stated by the EFSA [42], indicating that these genera do not present atypical resistance to clinically relevant antimicrobials. Likewise, other studies have reported the same sensitivity profile for these genera [43–45]. Erythromycin resistance in the strains *Leuconostoc* (*17, 19* and *26*), *Weissella viridescens,* and *Lactococcus* has been associated with the gene *erm(B)* [46–48] and in the case of *Weissella viridescens,* the gene *catA* has been associated with chloramphenicol resistance [49]. On the other hand, for some strains of *Enterococcus* there have been reports that identify them as multiresistant [38,50]. Most LAB are recognized as Generally Recognized As Safe (GRAS) or Qualified Presumption of Safety (QPS), however, there is a possibility that these strains could become reservoirs for resistance genes [51,52]. The evaluation of sensitivity to antibiotics of each strain was phenotypically done. The microdilution broth method is one of the most used phenotypic methods to detect antibiotic resistance. Therefore, the type of resistance that a strain may have (intrinsic or acquired) is unknown and must be verified by genotypic methods before ruling out potential candidate strains for probiotics [53].

The ability to survive the conditions of the gastrointestinal tract is one of the most important functional features to consider in the selection of a probiotic [54]. In this study, the pH of 6.5, 3.0, and 8.0 corresponding to the oral cavity, stomach, and large intestine, respectively, and bile salts in the small intestine were evaluated (0.3 % and 0.6 % (w/v)) [55], conditions that were tolerated by all strains. Several researchers have reported tolerance to these pH conditions for these species [44,56,57]. The different methods each strain employs to tolerate pH and bile salts, which are unique to each strain, may be the cause of the variance in the survival of the strains in the environment of the gastrointestinal system [38]. For example, *Lactobacillus* presents mechanisms for resistance to acidity such as proton pumps, the ability to alkalinize the external environment, modification of the lipid content of the membrane, and the formation of biofilms, which facilitate auto-aggregation and protection from extreme pH conditions [58,59]. The tolerance of probiotics to bile salts is related to the enzyme Bile Salt Hydrolase (SBH), the active efflux of salts and bile acids, and the alteration of the membrane and cell wall [60,61].

Autoaggregation is a desired characteristic for probiotics as it ensures that they arrive at a high density in the intestine, protecting them from stress conditions [62] to enable their interaction with the host, contribute to the adhesion capacity and colonization of the GIT [63] and exert beneficial effects such as the inhibition of potential pathogens [33]. According to Cisneros [33], autoaggregation percentages equal to or lower than 10 % correspond to strains that are unable to autoaggregate, while strains with percentages higher than 10 % can naturally autoaggregate. In this study, 50 % of the strains presented autoaggregation values between 20 % and 35 %, as reported by several authors for the same species [64,65]. Different molecules associated with the property of auto-aggregation have been described, such as the aggregation promoting fact-related surface protein and exopolysaccharides in *Pediococcus* [66], the Promoting Factors of Aggregation (APF) in *Lactobacillus* [67], the clumping properties of *Leuconostoc* [68] and the enterococcal surface protein, aggregation substance, and collagen-binding protein for *Enterococcus* [69].

Hydrophobicity in probiotics has been related to the interaction between the bacterial surface and the epithelial cells of the intestine. It depends on non-polar molecules such as glycoproteins and polysaccharides present in the membranes and the cell wall [70]. This property is an indirect estimate of the successful adhesion and colonization of probiotics [71]. Therefore, its evaluation allows to characterize the capacity of the surface of the strains to interact with the mucosa and the cells of the intestine, but it is not a prerequisite for a strong adhesion [72]. The strains *19, 26,* and *29* showed a higher affinity for the non-polar acid solvent (Chloroform) and a lower affinity for the ethyl acetate (non-polar basic solvent), coinciding with the reports of other authors [73,74], which indicates that these strains are electron donors because chloroform has the characteristics of an electron acceptor [75].

Visual screening of colonies in a solid culture medium is one of the most frequently used methods for the detection of the exopolysaccharide production phenotype. EPS production depends on the carbon and nitrogen sources, with sucrose being the sugar that promotes growth in screening EPS-producing colonies [76]. In this study, the strains of the genera *Pediococcus*, *Limosilactobacillus, Lacticaseibacillus*, *E. faecium,* and *W. viridescens* presented a ropy phenotype, indicating that they can produce exopolysaccharides. Other authors have reported that these species produce ropy colonies on sucrose-containing media [77,78]. There are no reports of EPS production by *W. viridescens*, but other species have been considered producers, including *W. confusa*, *W. cibaria,* and *W. hellenica* [79, 80], which means that the production of EPS is a strain-specific characteristic and not of an entire bacterial genus. Probiotics produce EPS on the cell envelope as capsules or paracrystalline layers to protect against phagocytosis, fag attack, desiccation, and osmotic stress, and have therapeutic applications with antimicrobial, immunomodulatory, anti-inflammatory, antioxidant, antitumor, antiviral, and cholesterol-lowering properties [81].

Antibacterial activity is an essential factor in selecting probiotic bacteria due to their activity against foodborne pathogens. The results revealed that the cell-free culture supernatant (CFS) from strains 16, 18, 21, 26, 28 and 29 showed an inhibitory effect against all pathogens. Their activity against Gram-negative indicator bacteria was higher than that against Gram-positive bacteria, providing evidence that *P. pentosaceus* (16) had a higher inhibition (>4.0 mm) against all evaluated pathogens. These results coincide with reports made by other authors in these species [77,82]. This inhibitory activity of LAB has been attributed to its ability to produce different metabolites such as bacteriocin, organic acids (lactic, acetic, and propionic), ethanol, diacetyl and hydrogen peroxide [83].

5. Conclusions

Overall, *Pediococcus*, *Limosilactobacillus* and *Lacticaseibacillus* strains exhibited strong probiotic properties, such as being safe, having good tolerance to pH and bile conditions, autoaggregation, and hydrophobicity, showing a broad spectrum of antibacterial activities against foodborne pathogens such as *L. monocytogenes* and *S.* Typhimurium, and having an EPS ropy phenotype, for which they can be proposed as potential probiotics for studies in development on an industrial scale and their future application to elaborate functional food. Although, strains *L. mesenteroides* (19) and *E. faecium* (21) presented positive results in most of the probiotic tests carried out, they were resistant to a few clinically effective antibiotics; therefore, it is necessary to carry out more safety studies for these strains for their potential use as probiotics. The test carried out in this study constitute a preliminary evaluation of the possible probiotic use of these strains for the formulation of functional foods. However, technological tests such as studies on cell lines and cytotoxicity, as well as viability tests on food, need to be performed.

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Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Samantha Roldán Pérez: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Sara Lucía Gómez Rodríguez:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **José Uriel Sepúlveda:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Orlando Simón Ruiz Villadiego:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **María Elena Márquez Fernández:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Olga Inés Montoya Campuzano:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Mónica María Durango Zuleta:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B. Bottari, V. Castellone, E. Neviani, "Probiotics and Covid-19," 72 (3) (2020) 293–299, https://doi.org/10.1080/09637486.2020.1807475, 1807475.
[2] F.C. Mgbodile, T.N.T. Nwagu, Probiotic therapy, African fermented foods and food-derived bioactive peptides in the management of SARS-CoV-2 cases and other viral infections, Biotechnology Reports 38 (Jun. 2023), e00795, https://doi.org/10.1016/J.BTRE.2023.E00795.
[3] D. Xavier-Santos, et al., Evidences and perspectives of the use of probiotics, prebiotics, synbiotics, and postbiotics as adjuvants for prevention and treatment of COVID-19: a bibliometric analysis and systematic review, Trends Food Sci. Technol. 120 (Feb. 2022) 174–192, https://doi.org/10.1016/J.TIFS.2021.12.033.
[4] T. Asai, et al., Encouraging tactics with genetically modified probiotics to improve immunity for the prevention of immune-related diseases including cardiometabolic disorders, Biomolecules 13 (2023), https://doi.org/10.3390/BIOM13010010. *Page* 10, vol. 13, no. 1, p. 10, Dec. 2022.
[5] A. Rousseaux, C. Brosseau, M. Bodinier, Immunomodulation of B Lymphocytes by prebiotics, probiotics and synbiotics: application in pathologies, Nutrients 2023 15 (2) (Jan. 2023) 269, https://doi.org/10.3390/NU15020269, 15, Page 269.
[6] S.M. Won, et al., Gut lactobacillus and probiotics lactobacillus lactis/rhamnosis ameliorate liver fibrosis in prevention and treatment, J. Microbiol. 61 (2) (Feb. 2023) 245–257, https://doi.org/10.1007/S12275-023-00014-Y/METRICS.
[7] Y.Y. Huang, et al., Advances in the study of probiotics for immunomodulation and intervention in food allergy, Molecules 2023 28 (3) (Jan. 2023) 1242, https://doi.org/10.3390/MOLECULES28031242, 28, Page 1242.
[8] G. Vakadaris, et al., The role of probiotics in inducing and maintaining remission in Crohn's disease and ulcerative colitis: a systematic review of the literature, Biomedicines 11 (2) (Feb. 2023) 494, https://doi.org/10.3390/BIOMEDICINES11020494/S1.
[9] J.S. Escobar, B. Klotz, B.E. Valdes, G.M. Agudelo, The gut microbiota of Colombians differs from that of Americans, Europeans and Asians, BMC Microbiol. 14 (1) (2015) 311, https://doi.org/10.1186/s12866-014-0311-6. Dec.
[10] T.C. Judkins, D.L. Archer, D.C. Kramer, R.J. Solch, Probiotics, nutrition, and the small intestine, Curr. Gastroenterol. Rep. 22 (1) (2020), https://doi.org/10.1007/S11894-019-0740-3. Jan.

[11] World Gastroenterology Organisation, Probiotics and Prebiotics, ISSN, Feb. 2023, pp. 1539–2031, https://doi.org/10.1097/MCG.0b013e31816fc2f6. "note": "PMID.

[12] M.C. Coelho, F.X. Malcata, C.C.G. Silva, "Lactic acid bacteria in raw-milk cheeses: from starter cultures to probiotic functions," foods, Multidisciplinary Digital Publishing Institute 11 (15) (2022), https://doi.org/10.3390/foods11152276, 2276, Jul. 29.

[13] Md A. Ali, et al., Functional dairy products as a source of bioactive peptides and probiotics: current trends and future prospectives, J. Food Sci. Technol. 59 (4) (2022) 1263–1279, https://doi.org/10.1007/s13197-021-05091-8.

[14] FAO/WHO, Probiotics in Food Health and Nutritional Properties and Guidelines for Evaluation, Roma, 2006.

[15] G.V. de Melo Pereira, B. de Oliveira Coelho, A.I. Magalhães Júnior, V. Thomaz-Soccol, C.R. Soccol, How to select a probiotic? A review and update of methods and criteria, Biotechnol. Adv. 36 (8) (Dec. 2018) 2060–2076, https://doi.org/10.1016/j.biotechadv.2018.09.003.

[16] C.A. Bernal Castro, C. Díaz-Moreno, C. Gutiérrez-Cortés, C.A. Bernal Castro, C. Díaz-Moreno, C. Gutiérrez-Cortés, Probióticos y prebióticos en matrices de origen vegetal: avances en el desarrollo de bebidas de frutas, Rev. Chil. Nutr. 44 (4) (2017) 383–392, https://doi.org/10.4067/S0717-75182017000400383.

[17] N. De Araújo-Uribe, O.S. Ruiz-Villadiego, O.I. Montoya-Campuzano, L.A. Gutiérrez-Ramírez, Viability of probiotic bacteria bacillus polymyxa, bacillus megaterium and lactobacillus delbruekii subsp. Bulgaricus microencapsulated under the spray-drying technique, DYNA (Colombia) 85 (204) (2018) 272–276, https://doi.org/10.15446/dyna.v85n204.61644.

[18] P.A. García-Rincón, J.M. Núñez-Ramírez, A.F. Bahamón-Monje, Physicochemical and sensory characteristics of fermented almonds of national cacao (Theobroma cacao L.) with addition of probiotics in the amazonic research center, Cimaz Macagual (Caquetá, Colombia), Ingeniería y Competitividad 23 (2) (2021), https://doi.org/10.25100/iyc.23i2.10885.

[19] L.A. Silva Rubio, Invenciones relacionadas con alimentos funcionales con probióticos a nivel internacional, Bogotá, 2014.

[20] F.C. Herrera, M.L. García-López, J.A. Santos, Short communication: characterization of methicillin-resistant Staphylococcus aureus isolated from raw milk fresh cheese in Colombia, J. Dairy Sci. 99 (10) (Oct. 2016) 7872–7876, https://doi.org/10.3168/jds.2016-11322.

[21] M.M. Durango-Zuleta, M. Fuentes-Vanegas, J.U. Sepúlveda-Valencia, C.X. Moreno Herrera, Isolation, identification, and antimicrobial activity of lactic acid bacteria associated with two traditional Colombian types of cheese: quesillo and double-cream cheese, LWT 171 (Dec. 2022), 114119, https://doi.org/10.1016/J.LWT.2022.114119.

[22] C.L. Ramos, R.F. Schwan, Technological and nutritional aspects of indigenous Latin America fermented foods, Curr. Opin. Food Sci. 13 (2017) 97–102, https://doi.org/10.1016/j.cofs.2017.07.001.

[23] F.R.L. Rolim, O.C. Freitas Neto, M.E.G. Oliveira, C.J.B. Oliveira, R.C.R.E. Queiroga, Cheeses as food matrixes for probiotics: in vitro and in vivo tests, Trends Food Sci. Technol. 100 (2020) 138–154, https://doi.org/10.1016/j.tifs.2020.04.008.

[24] S.N. Casarotti, et al., In vitro assessment of safety and probiotic potential characteristics of Lactobacillus strains isolated from water buffalo mozzarella cheese, Ann. Microbiol. 67 (4) (Apr. 2017) 289–301, https://doi.org/10.1007/s13213-017-1258-2.

[25] Clinical and Laboratory Standars Institute (CLSI), Performance Standards for Antimicrobial M100-S23, 29th ed., Pensilvania, 2019 https://doi.org/10.1126/science.273.5271.122.

[26] G. Rychen, et al., Guidance on the characterisation of microorganisms used as feed additives or as production organisms, EFSA J. 16 (3) (2018), https://doi.org/10.2903/J.EFSA.2018.5206. Mar.

[27] Y.D. Jatmiko, G.S. Howarth, M.D. Barton, Assessment of probiotic properties of lactic acid bacteria isolated from Indonesian naturally fermented milk, AIP Conf. Proc. (1) (2017), 50008, https://doi.org/10.1063/1.5012732, 1908.

[28] L. Stasiak-Różańska, A. Berthold-Pluta, A.S. Pluta, K. Dasiewicz, M. Garbowska, Effect of simulated gastrointestinal tract conditions on survivability of probiotic bacteria present in commercial preparations, Int. J. Environ. Res. Publ. Health 18 (3) (Feb. 2021) 1–17, https://doi.org/10.3390/IJERPH18031108.

[29] F. Armas, C. Camperio, C. Marianelli, "In Vitro Assessment of the probiotic potential of Lactococcus lactis LMG 7930 against ruminant mastitis-causing pathogens,", PLoS One 12 (1) (Jan. 2017), e0169543 https://doi.org/10.1371/journal.pone.0169543.

[30] S. Lee, et al., Probiotic characteristics of *Bacillus* strains isolated from Korean traditional soy sauce, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 79 (Jun. 2017) 518–524, https://doi.org/10.1016/J.LWT.2016.08.040.

[31] E.S. Khalil, M.Y. Manap, S. Mustafa, M. Amid, A.M. Alhelli, A. Aljoubori, Probiotic characteristics of exopolysaccharides-producing Lactobacillus isolated from some traditional Malaysian fermented foods, Journal of Foods 16 (1) (Jan. 2018) 287–298, https://doi.org/10.1080/19476337.2017.1401007.

[32] M.F. Vanegas, A. Londoño Zapata, M. Durango Zuleta, M. Gutiérrez Buriticá, S. Ochoa Agudelo, J. Sepúlveda Valencia, Capacidad Antimicrobiana de Bacterias Ácido Lácticas autóctonas aisladas de queso doble crema y quesillo colombiano, Biotecnoloía en el Sector Agropecuario y Agroindustrial 15 (1) (2017) 45, https://doi.org/10.18684/BSAA(15)45-55.

[33] L. Cisneros, et al., Lactic acid bacteria biofilms and their ability to mitigate Escherichia coli O157:H7 surface colonization, Lett. Appl. Microbiol. 73 (2) (Aug. 2021) 247–256, https://doi.org/10.1111/LAM.13509.

[34] EFSA, Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance, EFSA J. 10 (6) (2012), https://doi.org/10.2903/j.efsa.2012.2740. Jun.

[35] A. Darmastuti, P.N. Hasan, R. Wikandari, T. Utami, E.S. Rahayu, D.A. Suroto, Adhesion properties of lactobacillus plantarum dad-13 and lactobacillus plantarum mut-7 on sprague dawley rat intestine, Microorganisms 9 (11) (2021), https://doi.org/10.3390/microorganisms9112336.

[36] A. Vasiee, F. Falah, B.A. Behbahani, F. Tabatabaee-yazdi, 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) (2020) 471–479, https://doi.org/10.1016/j.jbiosc.2020.07.002.

[37] Md Z. Islam, Md E. Uddin, Md T. Rahman, M.A. Islam, Md Harun-ur-Rashid, Isolation and characterization of dominant lactic acid bacteria from raw goat milk: assessment of probiotic potential and technological properties, Small Rumin. Res. 205 (2021), 106532, https://doi.org/10.1016/j.smallrumres.2021.106532.

[38] Y. Nami, R. Vaseghi Bakhshayesh, H. Mohammadzadeh Jalaly, H. Lotfi, S. Eslami, M.A. Hejazi, Probiotic properties of Enterococcus isolated from artisanal dairy products, Front. Microbiol. 10 (2019).

[39] M.G. Sica, Bacterias lácticas del estuario de Bahía Blanca : evaluación de sus propiedades probióticas para su potential uso en el cultivo de trucha arcoíris (*Oncorhynchus mykiss*), Universidad Nacional del Sur Bahía Blanca, 2013.

[40] T. Kim, et al., Safety evaluation of Lactococcus lactis IDCC 2301 isolated from homemade cheese, Food Sci. Nutr. 10 (1) (Jan. 2022) 67–74, https://doi.org/10.1002/fsn3.2648.

[41] A. Pino, E. Bartolo, C. Caggia, A. Cianci, C.L. Randazzo, Detection of vaginal lactobacilli as probiotic candidates, Sci. Rep. 9 (1) (2019) 1–10, https://doi.org/10.1038/s41598-019-40304-3. Dec.

[42] EFSA BIOHAZ Panel, Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA, EFSA J. 15 (3) (2017), https://doi.org/10.2903/J.EFSA.2017.4664. Mar.

[43] K.K. Bhukya, B. Bhukya, Unraveling the probiotic efficiency of bacterium Pediococcus pentosaceus OBK05 isolated from buttermilk: an in vitro study for cholesterol assimilation potential and antibiotic resistance status, PLoS One 16 (11 November) (2021) 1–20, https://doi.org/10.1371/journal.pone.0259702.

[44] S. Divyashree, P.G. Anjali, R. Somashekaraiah, M.Y. Sreenivasa, Probiotic properties of Lactobacillus casei – MYSRD 108 and Lactobacillus plantarum-MYSRD 71 with potential antimicrobial activity against Salmonella paratyphi, Biotechnology Reports 32 (Dec. 2021), e00672, https://doi.org/10.1016/j.btre.2021.e00672.

[45] K. Marchwińska, D. Gwiazdowska, Isolation and Probiotic Potential of Lactic Acid Bacteria from Swine Feces for Feed Additive Composition, vol. 204, 2022, p. 61, https://doi.org/10.1007/s00203-021-02700-0.

[46] C. Devirgiliis, S. Barile, A. Caravelli, D. Coppola, G. Perozzi, Identification of tetracycline- and erythromycin-resistant Gram-positive cocci within the fermenting microflora of an Italian dairy food product, J. Appl. Microbiol. 109 (1) (2010) 313–323, https://doi.org/10.1111/J.1365-2672.2010.04661.X. Jul.

[47] K. El Issaoui, et al., Molecular identification and antibiotic resistance of bacteriocinogenic lactic acid bacteria isolated from table olives, Arch. Microbiol. 203 (2) (Mar. 2021) 597–607, https://doi.org/10.1007/s00203-020-02053-0.

[48] A.B. Flórez, et al., Antibiotic susceptibility profiles of dairy Leuconostoc, analysis of the genetic basis of atypical resistances and transfer of genes in vitro and in a food matrix, PLoS One 11 (1) (2016), e0145203, https://doi.org/10.1371/journal.pone.0145203.

[49] N. DE Parma Augusto Castilho, BACTERIOCINOGENIC POTENTIAL OF LACTIC ACID BACTERIA ISOLATES FROM ARTISANAL FERMENTED MEAT PRODUCTS, Universidade Federal de Viçosa, Minas Gerais, 2018.

[50] L.C.S. Nascimento, S.N. Casarotti, S.D. Todorov, A.L.B. Penna, Probiotic potential and safety of enterococci strains 69 (3) (2019) 241–252.

[51] I. Stefańska, E. Kwiecień, K. Jóźwiak-Piasecka, M. Garbowska, M. Binek, M. Rzewuska, Antimicrobial susceptibility of lactic acid bacteria strains of potential use as feed additives - the basic safety and usefulness criterion, Front. Vet. Sci. 8 (2021) 720, https://doi.org/10.3389/FVETS.2021.687071/BIBTEX. Jul.

[52] F. Ben Taheur, et al., Anti-bacterial and anti-biofilm activity of probiotic bacteria against oral pathogens, Microb. Pathog. 97 (2016) 213–220, https://doi.org/10.1016/j.micpath.2016.06.018. Aug.

[53] A. Fessard, F. Remize, Why are Weissella spp. not used as commercial starter cultures for food fermentation? Fermentation 3 (38) (2017) 17–18, https://doi.org/10.3390/fermentation3030038ï.

[54] M.L. Fernándes, L.M. Perin, S.D. Todorov, L.A. Nero, E.R. De Alencar, M. De Aguiar Ferreira, In vitro evaluation of the safety and probiotic and technological potential of pediococcus pentosaceus isolated from sheep milk, Semina Ciências Agrárias 39 (1) (Jan. 2018) 113–132, https://doi.org/10.5433/1679-0359.2018v39n1p113.

[55] P.L. Hu, Y.H. Yuan, T.L. Yue, C.F. Guo, Bile acid patterns in commercially available oxgall powders used for the evaluation of the bile tolerance ability of potential probiotics, PLoS One 13 (3) (2018) 1–11, https://doi.org/10.1371/journal.pone.0192964.

[56] X. Zhang, et al., Probiotic characteristics of Lactobacillus strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens, Saudi J. Biol. Sci. 27 (12) (Dec. 2020) 3505–3513, https://doi.org/10.1016/J.SJBS.2020.10.022.

[57] R. Rai, J.P. Tamang, In vitro and genetic screening of probiotic properties of lactic acid bacteria isolated from naturally fermented cow-milk and yak-milk products of Sikkim, India, World Journal of Microbiology and Biotechnology 2021 38:2 38 (2) (Jan. 2022) 1–20, https://doi.org/10.1007/S11274-021-03215-Y.

[58] N. Guan, L. Liu, Microbial response to acid stress: mechanisms and applications, Appl. Microbiol. Biotechnol. 104 (1) (2020) 51–65, https://doi.org/10.1007/s00253-019-10226-1.

[59] J. Wang, et al., Screening for potential probiotic from spontaneously fermented non-dairy foods based on in vitro probiotic and safety properties, Ann. Microbiol. 68 (12) (Dec. 2018) 803–813, https://doi.org/10.1007/s13213-018-1386-3.

[60] M.H. Foley, et al., Lactobacillus bile salt hydrolase substrate specificity governs bacterial fitness and host colonization, Proc. Natl. Acad. Sci. U.S.A. 118 (6) (Feb. 2021), https://doi.org/10.1073/pnas.2017709118.

[61] Š. Horáčková, M. Plocková, K. Demnerová, Importance of microbial defence systems to bile salts and mechanisms of serum cholesterol reduction, Biotechnol. Adv. 36 (3) (2018) 682–690, https://doi.org/10.1016/j.biotechadv.2017.12.005.

[62] T. Trunk, H.S. Khalil, J.C. Leo, Bacterial autoaggregation, AIMS Microbiology 4 (1) (2018) 140–164, https://doi.org/10.3934/microbiol.2018.1.140.

[63] S. Byakika, I.M. Mukisa, Y.B. Byaruhanga, C. Muyanja, A review of criteria and methods for evaluating the probiotic potential of microorganisms, Food Rev. Int. 35 (5) (2019) 427–466, https://doi.org/10.1080/87559129.2019.1584815. Jul.

[64] Y.R. Song, C.M. Lee, S.H. Lee, S.H. Baik, Evaluation of probiotic properties of pediococcus acidilactici m76 producing functional exopolysaccharides and its lactic acid fermentation of black raspberry extract, Microorganisms 9 (7) (2021), https://doi.org/10.3390/microorganisms9071364.

[65] T.M.R. De Albuquerque, E.F. Garcia, A. De Oliveira Araújo, M. Magnani, M. Saarela, E.L. De Souza, "In vitro characterization of lactobacillus strains isolated from fruit processing by-products as potential probiotics,", Probiotics and Antimicrobial Proteins 2017 10:4 10 (4) (2017) 704–716, https://doi.org/10.1007/S12602-017-9318-2. Aug.

[66] V. Vidhyasagar, K. Jeevaratnam, Evaluation of Pediococcus pentosaceus strains isolated from Idly batter for probiotic properties in vitro, J. Funct.Foods 5 (1) (Jan. 2013) 235–243, https://doi.org/10.1016/J.JFF.2012.10.012.

[67] T. Teame, et al., Paraprobiotics and postbiotics of probiotic lactobacilli, their positive effects on the host and action mechanisms: a review, Front. Nutr. 7 (Oct. 2020) 191, https://doi.org/10.3389/FNUT.2020.570344/BIBTEX.

[68] M. Nikolic, B. Jovcic, M. Kojic, L. Topisirovic, Surface properties of Lactobacillus and Leuconostoc isolates from homemade cheeses showing auto-aggregation ability, Eur. Food Res. Technol. 231 (6) (2010) 925–931, https://doi.org/10.1007/s00217-010-1344-1.

[69] D. Stępień-Pyśniak, T. Hauschild, U. Kosikowska, M. Dec, R. Urban-Chmiel, Biofilm formation capacity and presence of virulence factors among commensal Enterococcus spp. from wild birds, Scientific Reports 2019 9:1 9 (1) (2019) 1–7, https://doi.org/10.1038/s41598-019-47602-w. Aug.

[70] S. Abbasiliasi, et al., In vitro assessment of Pediococcus acidilactici Kp10 for its potential use in the food industry, BMC Microbiol. 17 (1) (May 2017) 1–11, https://doi.org/10.1186/S12866-017-1000-Z/TABLES/5.

[71] F. Falah, et al., Evaluation of adherence and anti-infective properties of probiotic Lactobacillus fermentum strain 4-17 against Escherichia coli causing urinary tract infection in humans, Microb. Pathog. 131 (Jun. 2019) 246–253, https://doi.org/10.1016/j.micpath.2019.04.006.

[72] B.M.S. de Souza, T.F. Borgonovi, S.N. Casarotti, S.D. Todorov, A.L.B. Penna, Lactobacillus casei and lactobacillus fermentum strains isolated from mozzarella cheese: probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions, Probiotics and antimicrobial proteins 11 (2) (2019) 382–396, https://doi.org/10.1007/s12602-018-9406-y. Jun.

[73] M.Ž. Grujović, K.G. Mladenović, D.D. Nikodijević, L.R. Čomić, Autochthonous lactic acid bacteria-presentation of potential probiotics application, Biotechnol. Lett. 41 (11) (2019) 1319–1331, https://doi.org/10.1007/s10529-019-02729-8. Nov.

[74] C. Luan, et al., Antibacterial and anti-biofilm activities of probiotic Lactobacillus curvatus BSF206 and Pediococcus pentosaceus AC1-2 against Streptococcus mutans, Microb. Pathog. 164 (2022), 105446, https://doi.org/10.1016/j.micpath.2022.105446.

[75] K.M. Kim, et al., Probiotic properties and immune-stimulating effect of the Jeju lava seawater mineral-coated probiotics, LWT 126 (2020), 109299, https://doi.org/10.1016/j.lwt.2020.109299.

[76] V. Milanovic, et al., Selection of cereal-sourced lactic acid bacteria as candidate starters for the baking industry, PLoS One 15 (7 July) (2020) 1–21, https://doi.org/10.1371/journal.pone.0236190.

[77] A.T. Adesulu-Dahunsi, A.I. Sanni, K. Jeyaram, Diversity and technological characterization of Pediococcus pentosaceus strains isolated from Nigerian traditional fermented foods, LWT 140 (2021), 110697, https://doi.org/10.1016/j.lwt.2020.110697.

[78] S.H. Jung, D.K. Hong, S.J. Bang, K. Heo, J.J. Sim, J.L. Lee, The functional properties of lactobacillus casei hy2782 are affected by the fermentation time, Appl. Sci. 11 (6) (2021), https://doi.org/10.3390/app11062481.

[79] D. Kavitake, P.B. Devi, P.H. Shetty, Overview of exopolysaccharides produced by Weissella genus – a review, Int. J. Biol. Macromol. 164 (2020) 2964–2973, https://doi.org/10.1016/j.ijbiomac.2020.08.185.

[80] J.F. Sánchez, Caracterización molecular de bacterias ácido lácticas aisladas de frutos procedentes de la Región Loreto, Universidad Nacional Mayor de San Marcos, Lima, 2019.

[81] J. Angelin, M. Kavitha, Exopolysaccharides from probiotic bacteria and their health potential, Int. J. Biol. Macromol. 162 (2020) 853–865, https://doi.org/10.1016/j.ijbiomac.2020.06.190. January.

[82] H. Barzegar, B. Alizadeh Behbahani, F. Falah, Safety, probiotic properties, antimicrobial activity, and technological performance of Lactobacillus strains isolated from Iranian raw milk cheeses, Food Sci. Nutr. 9 (8) (2021), https://doi.org/10.1002/FSN3.2365. Aug.

[83] N. Vieco-Saiz, et al., Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production, Front. Microbiol. 10 (2019) 1–17, https://doi.org/10.3389/fmicb.2019.00057. February.