BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Probiotic characterization of lactic acid bacteria from artisanal goat cheese for functional dairy product development

Caracterização probiótica de bactérias láticas de queijo de leite de cabra artesanal para desenvolvimento de produtos lácteos funcionais

Abraham Alejandro Sesín[1], Juan José Carol Paz[1], Ana Estela Ledesma[1,2], María Pía Taranto[3], Ana Yanina Bustos[1,4,5]*

[1]Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL-UNSE-CONICET), Santiago del Estero - Argentina
[2]Universidad Nacional de Santiago del Estero, Facultad de Ciencias Exactas y Tecnologías, Departamento Académico de Química, Santiago del Estero - Argentina
[3]Centro de Referencia de Lactobacilos (CERELA-CONICET), San Miguel de Tucumán - Argentina
[4]Universidad Nacional de Santiago del Estero, Facultad de Agronomía y Agroindustrias, Santiago del Estero - Argentina
[5]Universidad Nacional de Santiago del Estero, Facultad de Humanidades, Ciencias Sociales y de la Salud, Santiago del Estero - Argentina

***Corresponding Author:** Ana Yanina Bustos, Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL-UNSE-CONICET), Ruta N 9, km 1125, Villa El Zanjon, CEP: 4206, Santiago del Estero/Santiago del Estero - Argentina, e-mail: abustos@uspt.edu.ar

Cite as: Sesín, A. A., Paz, J. J. C., Ledesma, A. E., Taranto, M. P., & Bustos, A. Y. (2023). Probiotic characterization of lactic acid bacteria from artisanal goat cheese for functional dairy products development. *Brazilian Journal of Food Technology*, *26*, e2023024. https://doi.org/10.1590/1981-6723.02423

Abstract

The increasing interest in functional foods has encouraged the search for new lactic acid bacteria (LAB) with singular characteristics, including technological and probiotic properties, present in natural sources. The present research aimed to isolate and *in vitro* characterize the probiotic potential of LAB isolated from artisanal goat cheese. In addition, the acidifying and proteolytic capacity of the strains were evaluated in order to develop a functional dairy product. Fifty strains, presumptively identified as LAB, were isolated from artisanal goat cheese. From them, seventeen strains were selected for identification through Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry (MS) and 16S gene sequence. None of the tested strains showed hemolytic ability, while all strains showed at least 76% of survival to low pH and conjugated bile salts. Hydrophobicity and auto-aggregation values varied among LAB with ranges between 15.5 ± 1.8 to 84.7 ± 3.2, and 4.72% to 59.2%, respectively. Selected LAB were capable of adhering to intestinal mucus in a range of 5.08 to 6.90 Log CFU/mL. Remarkably, eight strains showed high bile salt hydrolase activity. Autochthons strains were able to grow and acidify milk after 24 h. Besides, proteolytic activity showed a range between 10 and 151 mmol/L amino acids. PCA analysis and technological properties allow us to propose *Lactiplantibacillus plantarum* CB5, CB8 and *Lentilactobacillus parabuchneri* CB12 strains as potential probiotic starter cultures. The isolation of new LAB strains with demonstrated

functional properties is of interest to academic institutions and the food industry as it allows the design of indigenous dairy cultures with added probiotic and technological properties.

Keywords: Probiotic; Lactic acid bacteria; Bile salt hydrolase; Functional dairy food.

Resumo

O crescente interesse por alimentos funcionais tem incentivado a busca por novas bactérias láticas com características singulares, incluindo propriedades tecnológicas e probióticas, presentes em fontes naturais. A presente pesquisa teve como objetivo isolar *in vitro* e caracterizar o potencial probiótico de BAL isoladas de queijo artesanal de leite de cabra. Além disso, as capacidades acidificante e proteolítica das cepas foram avaliadas a fim de desenvolver um produto lácteo funcional. Cinquenta cepas, presumivelmente identificadas como BAL, foram isoladas de queijo de leite de cabra artesanal. Destas, 17 linhagens foram selecionadas para identificação por Espectrometria de Massa MALDI-TOF e sequência do gene 16S. Nenhuma das cepas testadas apresentou capacidade hemolítica, enquanto todas as cepas apresentaram pelo menos 76% de sobrevivência a pH baixo e sais biliares conjugados. Os valores de hidrofobicidade e autoagregação variaram entre LAB com faixas de 15,5 ± 1,8 a 84,7 ± 3,2, e 4,72% a 59,2%, respectivamente. Todas as bactérias láticas selecionadas foram capazes de aderir ao muco intestinal na faixa de 5,08 a 6,90 Log UFC/mL. Notavelmente, oito cepas mostraram alta atividade de hidrolase de sais biliares. Cepas autóctones foram capazes de crescer e acidificar o leite após 24 h. Além disso, verificou-se atividade proteolítica mostrando uma faixa entre 10 e 151 mmol/L de aminoácidos. A análise de PCA e as propriedades tecnológicas permitem propor cepas de *Lactiplantibacillus plantarum* CB5 e CB8, e *Lentilactobacillus parabuchneri* CB12 como culturas iniciais probióticas. O isolamento de novas cepas de bactérias láticas com propriedades funcionais demonstradas é de interesse para instituições acadêmicas e para a indústria alimentícia, pois permite o desenho de culturas lácteas nativas com potenciais propriedades probióticas e tecnológicas agregadas.

Palavras-chave: Probiótico; Bactérias láticas; Hidrolase de sais biliares; Alimentos lácteos funcionais.

Highlights

- LAB with singular technological and probiotic properties were isolated from goat cheese
- Strains presented bile salt hydrolase activity and good surface properties
- Some strains showed good development, acidifying and proteolytic capacity in milk

1 Introduction

Fermented foods, especially artisanal cheeses, are well-known sources of lactic acid bacteria (LAB) with singular characteristics, including technological and probiotic properties (Kamimura et al., 2019; Margalho et al., 2020). In particular, in northern Argentina, the production of cheese made from goat's milk is of economic and social importance, as this region is the second largest goat producer in South America. In general, artisanal goat cheeses are freshly made without the addition of any selected starter culture; thus, fermentation is spontaneously obtained by LAB activity (Taboada et al., 2014).

Among the commonly isolated strains, the former genus *Lactobacillus* stands out as it has a long history of safe use. On the other hand, genera such as *Enterococcus* are considered autochthonous microbiota of cheeses but have not yet obtained GRAS (generally recognized as safe) status (Kamimura et al., 2019; Samedi & Charles, 2019; Margalho et al., 2020).

For several years, the literature has described innumerable benefits attributed to probiotic bacteria including the reduction of serum cholesterol levels. This effect is often associated with the bile salt hydrolase (BSH) activity of some bacteria (Bustos et al., 2018; Hernández-Gómez et al., 2021). Therefore, there is continued

interest in the identification of probiotic strains that produce BSH since, as is well-known, high cholesterol levels are associated with the development of cardiovascular disease, which is the leading cause of death in adults between 35 and 70 years of age (Gheziel et al., 2019). In addition, some authors suggest that LAB strains originally isolated from dairy products are probably the most suitable candidates for inclusion in various types of foods since they are well adapted to adverse conditions. This would provide adaptive advantages over probiotic strains isolated from other sources (Turchi et al., 2013; Jeronymo-Ceneviva et al., 2014). Therefore, the isolation and characterization of new LAB strains with potential probiotic and technological properties are of interest to both academic institutions and industry. However, probiotics must meet specific criteria such as the ability to resist harsh conditions of the gastrointestinal tract (GIT) mainly the acid environment of the stomach and bile salt (BS) presence in the gut (Bustos et al., 2015; Carasi et al., 2015), in besides to other many criteria to achieve probiotic status. Cell surface characteristics that allow the adhesion to the intestinal mucosa are another major selection criterion for a potentially successful probiotic (Muñoz-Provencio et al., 2009). In addition, the strains must be safe; they must not present virulence factors or hemolytic activity, among others. These criteria should first be evaluated exhaustively *in vitro* and then *in vivo* tests. Finally, the use of probiotics in humans will require *in vivo* human trials.

Fermented dairy products are the main carrier of probiotics due to the buffering capacity of milk that allows the growth and survival of bacteria during fermentation and storage (Luz et al., 2021). However, many LAB do not have considerable activity growth, acidification, and proteolytic capacity to achieve adequate milk fermentation (Uroić et al., 2014). Therefore, these technological properties should be tested in the selection of LAB to be administered in dairy products.

The present study aimed to isolate LAB from artisanal goat cheeses and study their probiotic potential by evaluating hemolytic activity, tolerance to low pH and bile salts, cell surface characteristics, adhesion properties, and BSH activity. In addition, the potential of the strains for the development of fermented milk products was evaluated by measuring their growth capability, acidification and proteolytic activity in milk.

2 Materials and methods

2.1 Samples

Samples of fresh artisanal goat milk cheese were collected at farmhouses from Santiago del Estero, Argentina, chilled at 4 °C, and transported immediately to the laboratory to be analyzed. Artisanal goat's milk cheeses were made with natural rennet and, without added salt, a characteristic of regional artisanal cheese making (Taboada et al., 2014).

2.2 Isolation of LAB

Representative samples of 10 g cheese were homogenized in 90 mL of sterile 0.8% (w/v) sodium chloride solution in a Stomacher 4000 (A. J. Seward Ltd., London, UK). Decimal dilutions of cheese homogenates were processed by the pour-plate method using MRS agar for LAB isolation. Plates were incubated under microaerophilic conditions at 37 °C for 48-72 h. After that, 10 representative colonies were randomly selected from the last dilution at which growth occurred. The purity of the isolates was checked by streaking and sub-culturing in MRS broth as well as MRS agar, followed by microscopic examinations. Isolates that were Gram-positive, catalase-negative, non-spore forming, non-motile were selected and presumptively considered as LAB. The short-term conservation of the pure isolates was done in MRS broth at 4 °C and cultures were renewed every month while stock cultures were kept frozen (–20 °C) in MRS broth with 20% v/v glycerol.

2.3 Bacterial identification by MALDI-TOF Mass Spectrometry and 16S gene sequence

The identification of isolated strains from goat cheese was performed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) in the Microbiology Sector of the Central

Laboratory of the German Hospital, Buenos Aires, Argentina. For this purpose, the method of direct extraction of colonies on MRS plates was employed, as previously described by Barberis et al. (2014). MALDI-TOF plates were inoculated with one colony of each freshly grown pure strain and covered with 1 mL of 70% formic acid (Sigma-Aldrich). Each spot was allowed to dry and subsequently covered with 1 mL of matrix (α-cyano-4-hydroxycinnamic acid).

Mass spectra were acquired using the MALDI-TOF MS spectrometer in a linear positive mode (Microflex, Bruker Daltonics). The bacterial test standard (BTS, Bruker) was used for instrument calibration. Mass spectra were analyzed in a m/z range of 2,000 to 20,000. The MALDI Biotyper library version 3.0 and MALDI Biotyper software version 3.1 were used for bacterial identification. The cut-off scores for identification proposed by Barberis et al. (2014) were employed. Scores values ≥ 1.5 were required for genus level, ≥ 1.7 for species level while values < 1.5 were not considered to give rise to a reliable identification. A minimum difference of 10% between the top and next closest score was required for a different genus or species.

The genetic identification of the selected strain was determined based on sequencing of the variable (V1) region of the 16S rDNA. The oligonucleotides for PCR reactions were: PLB16, 5′-AGAGTTTGATCCTGGCTCAG-3′, and MLB16, 5′-GGCTGCTGGCACGTAGTTAG-3′. PCR amplification consisted of 30 cycles for 30 s at 94 °C, 30 s at 50 °C, and 1 min at 72 °C. Final PCR products were purified using a commercial kit (AccuPrep® PCR Purification Kit; Bioneer Corporation, Genbiotech, Buenos Aires, Argentina) and subjected to sequencing (Sequencing Service, Science and Technology Center, CONICET, Tucumán, Argentina). The resulting sequences were analysed online using the NCBI BLAST algorithms (Zhang et al., 2000).

2.4 Safety of LAB probiotic strains: hemolytic activity assay

Hemolytic activity of LAB isolates was determined according to Uroić et al. (2014). Overnight cultures of LAB isolates grown in MRS broth were streaked onto MRS agar plates containing 5% (v/v) of blood from healthy donors and then incubated 48 h at 37 °C. Hemolytic activities of the strains were recorded by the presence of beta (β) hemolysis (indicated by a clear, colourless/lightened yellow zone surrounding the colonies depicting total lysis of red blood cells, alpha (α) hemolysis (indicated by a small zone of greenish to brownish discoloration of the media) and gamma (ϒ) hemolysis (with no change observed in the media). Non-hemolytic strains were considered for further testing.

2.5 Acid stress survival

Overnight cultures for each LAB strain were inoculated into fresh MRS broth acidified up to a pH of 2.5 with 2 N HCl according to the technique described in Castorena-Alba et al. (2018) with modifications. The average initial viable cell count was about 8 Log CFU/mL. Cultures were incubated at 37 °C. The A_{560nm} were measured at 0, 1.5, and 3 h. The results were expressed as survival percentages, according to the following Equation 1:

$$Survival\ (\%) = \frac{Abs_{T_0}}{Abs_{T_X}} \times 100 \quad (1)$$

Where AbsT0 is the absorbance of the samples at the beginning of the assay and AbsTx is the absorbance of each strain at different times.

2.6 Bile salt stress survival

To assess the BS tolerance, overnight LAB cultures were inoculated into fresh MRS broth with and without 5 mM taurodeoxycholic acid (TDCA) or deoxycholic acid (DCA), to give 7 log CFU/mL. The cultures were

incubated at 37 °C for 24 hours and the cells' survival percentage was determined by A_{560nm} measurement, using the following Equation 2:

$$Survival\ (\%) = \frac{Abs_{T_0}}{Abs_{T_X}} \times 100 \quad (2)$$

Where AbsBS is the absorbance of the samples with TDCA or DCA and AbsC is the absorbance of the control without bile salts.

2.7 Screening of bile salt deconjugation

To screen the ability of the strains to deconjugate BS, plates were prepared by adding 5 mM of sodium salts (Sigma Aldrich, Buenos Aires Argentina) of TDCA to MRS agar. The strains were streaked on the media and the plates were incubated at 37 °C for 72 h. The presence of precipitated BS around colonies (opaque halo) was considered a positive result. Further, BSH activity was confirmed, quantifying BS hydrolysis by High Performance Liquid Chromatography (HPLC), according to the technique developed by our working group with some modifications (Bustos et al., 2018). Non-proliferating cells were prepared from overnight cultures of each strain. For this purpose, cells were centrifuged at 10000 × *g* for 10 min, washed with sterile saline solution, and resuspended in 0.1 M acetate buffer pH 5.2 (optimum pH of most BSH enzymes) with the addition of 5 mM TDCA. Non-proliferating cells were incubated at 37 °C for 2 h and a 250 µL aliquot was taken at the beginning and after 2 h of incubation. The aliquots were centrifuged and supernatant samples were prepared for HPLC analysis by adding 2.5 µL of 6 M HCl and 250 µL of methanol. Samples were mixed in a vortex for 10 min, centrifuged at 10000 × *g* for 20 min at 10 °C and the supernatants were filtered through a 0.22 (PVDF membrane; Merck Millipore). Analyses were performed on a RP C18 column (250 x 4.6 mm, 5 µm particles, Grace, Research AG S.A., USA). The solvents used were 0.05 M sodium acetate buffer adjusted to pH 4.3 with *O*-phosphoric acid and filtered through a 0.22 µm filter (Solvent A) and HPLC-grade methanol (Solvent B). An isocratic elution was applied, consisting of 30% of Solvent A and 70% of Solvent B at 1 mL/min. Standards for calibration containing different concentrations of TDCA were treated as above. The reference strain *Lactiplantibacillus reuteri* CRL 1098 was included as positive control (Bustos et al., 2018). Results were expressed as a percentage of BS hydrolysis, considering 5 mM of TDCA as 100%.

2.8 Hydrophobicity assay

The ability of the organisms to adhere to hydrocarbons as a measure of their hydrophobicity was determined according to the method of Pringsulaka et al. (2015) with some modifications. Cultures of the strains were harvested in the stationary phase by centrifugation at 12000 x *g* for 5 min at 5 °C, washed twice with 50 mM K_2HPO_4 (pH 6.5) buffer, and finally resuspended in the same buffer. The cell suspension was adjusted to an A_{560nm} value of approximately 1 and 3 mL of the bacterial suspensions that were put in contact with 3 mL of chloroform and vortexed for 120 s. The two phases were allowed to separate for 1 h at room temperature. The aqueous phase was carefully removed and the A_{560nm} was measured.

The decrease in the absorbance of the aqueous phase was taken as a measure of the cell surface hydrophobicity (H%), which was calculated with the Equation 3:

$$H\% = [(A_0 - A)/A_0] \times 100 \quad (3)$$

Where A0 and A are the absorbance before and after extraction with chloroform, respectively.

Isolates with a H% greater than 70% was classified as highly hydrophobic, while H% values between 50 and 70% were classified as moderate, and H% values lower than 50% were classified as low hydrophobic according to Pringsulaka et al. (2015).

2.9 Auto-aggregation assay

The method of Polak-Berecka et al. (2014) with some modifications was used in determining the auto-aggregation ability of LAB strains. Stationary phase LAB strains were harvested and washed as described in the previous section. Bacterial suspensions of each strain were prepared and adjusted to an A_{560nm} value of 0.9 in sterile saline solution. Initial absorbance was recorded after vortexing each cell suspension for 10 s, and then these cell suspensions were incubated for 2 h at 37 °C. The A_{560nm} of the supernatant after 1, 2, 3 and 24 h of incubation was measured. The results were expressed as a percentage of auto-aggregation according to the following Equation 4:

$$\%Auto - aggregation = [(A_0 - A)/A_0] \times 100 \quad (4)$$

Where A0 and A are the absorbance before and after incubation at different times.

2.10 Adhesion to intestinal mucus assay

Mucus extraction was based on the experimental protocol described by Carasi et al. (2014) with some modifications. Entire GIT of healthy Balb/c male mouse (6-8 weeks old) were gently donated by the biotherium of the Multidisciplinary Institute of Health, Technology and Development (IMSaTeD, CONICET-UNSE). The procedure was performed strictly in accordance with International Directives. All animals were kept under standard conditions and were fed with a standard diet. Gut contents were removed aseptically, and gut segments were opened, repeatedly washed with PBS and then, mucus was isolated from walls by gentle scraping into PBS. Epithelial cells and large cellular components were removed by centrifugation at 11500 × *g* for 15 min. The protein concentration of mucus preparations was determined by the Bradford method. The extract concentration was fixed in 0.5 g/L.

The plates for binding assays were prepared by adding 100 µL of the mucus solution in each well of 96-well polystyrene microplates and were incubated for 1 h at 37 °C and then overnight at 4 °C. Then, a second incubation was performed for 2 h at 37 °C with 150 µL of the same solution in order to minimize the number of empty binding sites in the polystyrene microtitre plates. Finally, the wells were washed twice with 200 µL PBS. It was washed twice with 200 µL of PBS.

Bacterial preparation was performed according to Carasi et al. (2014). Isolated LAB strains were grown for 18 h at 37 °C in MRS-broth. Aliquots of 1 mL were sampled and centrifuged at 10000 × *g* at 10 °C for 5 min and the pellets were washed twice with sterile PBS and resuspended in the same to give 9 Log CFU/mL. One hundred microlitres of the bacterial suspension were added to each well. The plates were incubated for 2 h at 37 °C. The wells were washed 6 times with 200 µL of sterile PBS to remove unbound bacteria and treated with 200 µL of a 5 mL/L Triton X-100 solution for 30 min at 37 °C, to desorb the bound bacteria. One hundred microlitres of the content of each well were removed, diluted in PBS and plated on MRS agar plates. The concentration of Triton X-100 and the temperature of contact used were tested on all strains in order to determine the influence on bacterial viability.

2.11 Preparation of fermented milk: growth and acidifying capacity

Each LAB strain was cultivated in MRS broth and incubated for 24 h at 37 °C. The cultures were centrifuged at 5000 × *g* for 15 min and were washed twice with sterile saline solution. The bacterial suspensions were inoculated at 7 Log CFU/mL into skim milk (reconstituted at 10% w/v).

Growth, titratable acidity, and pH were determined after 24 h of incubation at 37 °C. Growth evaluation was performed by plate count on MRS agar under microaerophilic conditions. The pH was measured using a pH meter (pH 209; Hanna Instruments, Póvoa de Varzim, Portugal). Acidifying activity was measured according to Taboada et al. (2014). For measurement of titratable acidity, 10 mL of fermented milk was transferred to an erlenmeyer flask and drops of 0.1% w/v solution of phenolphthalein were added. Content

was titrated with 0.11 N NaOH (Dornic solution) to appearance of the faint pink color that will not get lost for over 2 minutes. The results were expressed in grams of lactic acid/liter of milk, considering that 1 mL of Dornic solution is equivalent to 0.1 g of lactic acid per liter.

2.12 Proteolytic activity in milk

The proteolytic activity of whole cells in milk was determined by using the *O*-phthaldialdehyde (OPA) spectrophotometric assay. The test is based on the reaction of the free α-amino groups released by hydrolysis of the casein (after a 24 h period of incubation of the strains in the milk) with OPA, in the presence of β-mercaptoethanol, to form a complex, which strongly absorbs at 340 nm. The results were calculated from a calibration curve obtained from the dilution of glycine in distilled water and were expressed in mmol of glycine/liter of milk.

2.13 Statistical analysis

Results are means of at least three independent experiments. Single factor analysis of variance (ANOVA) was performed on the mean of the data. The standard deviation (SD) of the data was also presented. Tukey's test was used to assess significant differences between the means of each strain in the statistical analysis of all trials. Student's t-test was used to evaluate the differences between treatments in resistance to acidity (1.5 h and 3 h) and bile salts (TDCA and DCA). The statistical software PAST version 3.16 was used, and a significance level of 5% ($p < 0.05$) was adopted.

3 Results and discussion

3.1 Isolation of LAB

The cheeses used for sampling were handmade, with the objective of isolating LAB present during the natural fermentation and not by the addition of starter cultures. Microbial counts of 6.87 ± 0.19 Log CFU/mL on average were obtained from the processed cheese samples. From these, 50 presumptive LAB strains were isolated based on their typical morphological, appearance of the colonies (small pinpointed and creamy white colonies), Gram-positive, catalase negative and non-motile, coccus and rod-shaped (Reuben et al., 2019). Of the isolated strains, 64% were coccus while the remaining 36% corresponded to bacilli of different sizes. Based on purity criteria and taking into account that former *Lactobacillus* are generally recognized as safe (GRAS), unlike the genera *Enterococcus*, *Staphylococcus*, among others (Samedi & Charles, 2019), 17 strains with rod-shaped were selected. They are named CB (for CIBAAL Institute) followed by a cardinal number and identified by MALDI-TOF MS. According to Barberis et al. (2014) a cut-off score ≥ 1.5 was employed for genus identification level, ≥ 1.7 for species-level, while a score < 1.5 was considered as resulting in no reliable identification. As shown in Table 1, CB 12 and 14 strains were identified as *Lentilactobacillus (L.) parabuchneri* (former *Lactobacillus parabuchneri*), CB6 was identified as *Leuconostoc mesenteroides* while CB18 could not be successfully identified. The remaining strains were identified as *Lactiplantibacillus (L.) plantarum/paraplantarum/pentosus* as neither MALDI-TOF nor partial sequencing of the 16S rDNA gene can distinguish between these closely related strains. In addition, the identification of the two strains *Lentilactobacillus parabuchneri* CB12 and 14 was confirmed by the 16S rRNA sequence using the online tool BLAST. Strains CB3, 11, 13 and 17 were identified by 16S rRNA sequence as *L. plantarum*. The non-starter LAB microbiota of cheese is dominated by mesophilic bacilli. Although *Lacticaseibacillus (L.) casei*, *L. paracasei*, *L. rhamnosus*, and *L. plantarum* are the strains most commonly encountered during cheese ripening. Besides, *L. parabuchneri* as well as *Levilactobacillus* (*L.) brevis*, and *L. fermentum*, may be present, at the final stages of ripening. Remarkably, the higher incidence of *L. plantarum* stands out in these matrices due to its ubiquitous character, indicating that they are present

in the environment, dairy and non-dairy products, fermented vegetables and human intestines (Margalho et al., 2020). On the other hand, in cheese, *L. parabuchneri* is usually responsible for histamine formation, but also could have a desirable impact on eye formation in cheese (Fröhlich-Wyder et al., 2013).

Table 1. Identification of lactic acid bacterial (LAB) isolates using MALDITOF MS and 16S rRNA gene sequencing and their accession numbers from GenBank.

Strains	Organism*	GenBank accession number¥	% of identity	e-value
CB1	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB2	*Lactiplantibacillus plantarum/paraplantarum/pentosus*	OQ107531	100	0.0
CB3	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB4	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB5	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB6	*Leuconostoc mesenteroides*			
CB8	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB9	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB10	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB11	*Lactiplantibacillus plantarum/paraplantarum/pentosus*	OQ107532	100	0.0
CB12	*Lentilactobacillus parabuchneri*	OQ107533	100	0.0
CB13	*Lactiplantibacillus plantarum/paraplantarum/pentosus*	OQ107534	100	0.0
CB14	*Lentilactobacillus parabuchneri*	OQ107535	99.84	0.0
CB15	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB16	*Lactiplantibacillus plantarum/paraplantarum/pentosus*			
CB17	*Lactiplantibacillus plantarum/paraplantarum/pentosus*	OQ107536	100	0.0
CB18	*Unidentified*			

*Strains identified by MALDI-TOF MS. A cut-off scores ≥ 1.5 was employed for genus identification level, and ≥ 1.7 for species-level. ¥ Strains identification confirmed by the 16S rRNA sequence using the online tool BLAST.

3.2 Hemolytic ability

None of the 17 LAB isolates tested showed α- or β-hemolytic ability due to the absence of a clear ring around the colonies caused by the destruction of red blood cells (data not shown). The absence of hemolytic activity is considered a safety prerequisite in the selection of probiotic strains, so all our isolates were selected for subsequent assays.

3.3 Survival to gastrointestinal conditions

The results of LAB isolates survival in MRS at pH = 2.0 are presented in Figure 1. After 1.5 h exposure to the acidic environment, 15 of the 17 strains showed survival percentage greater than 75%. Among them, CB2, CB5, CB11, CB12, CB13, CB14 and CB16, maintained 100% survival up to 3 h of incubation, while no statistically significant differences ($p > 0.05$) were observed between the two incubation times for any of the strains evaluated. Our finding corresponds with previous studies who reported moderate to good survival to acidic environment of LAB strains isolated from different sources (Turchi et al., 2013; Barberis et al., 2014; Yerlikaya, 2019; Margalho et al., 2020).

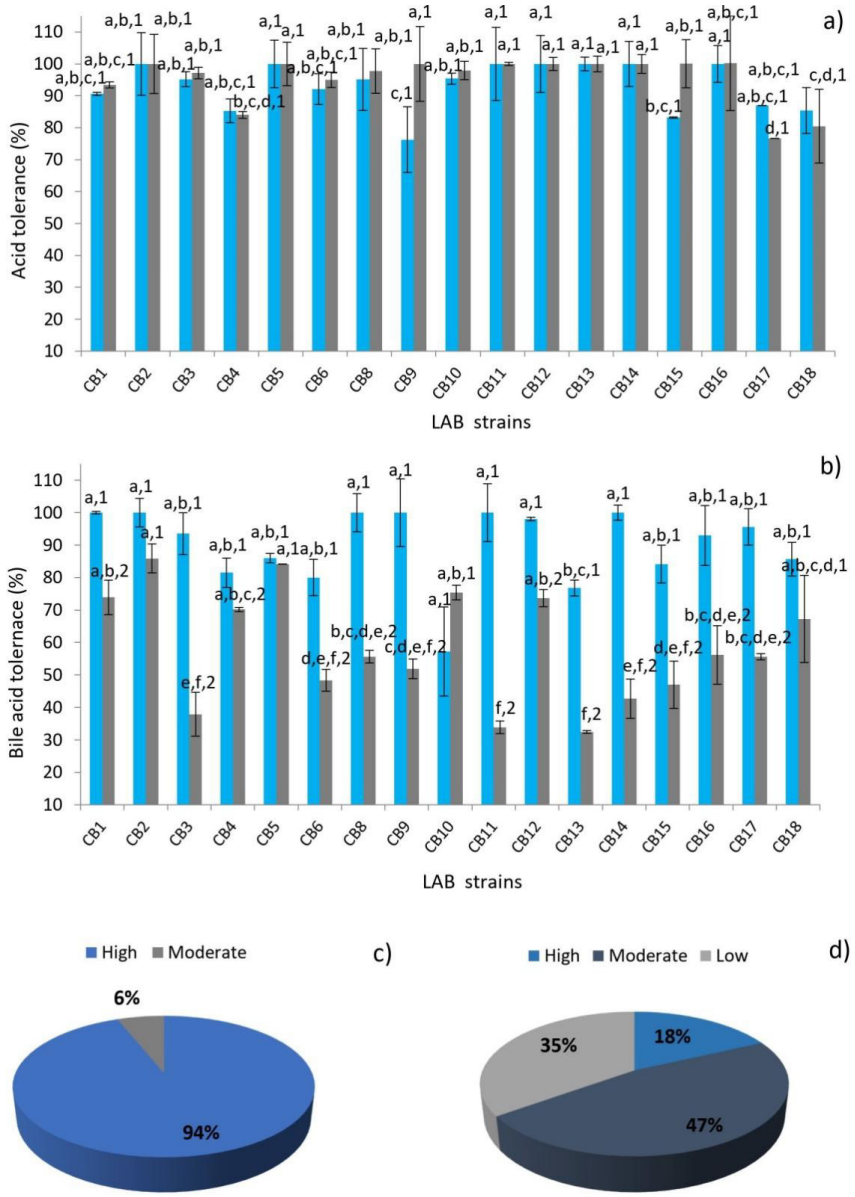

Figure 1. Percentage survival of LAB at acid and bile salts stress. (a) LAB survival after 1.5 h (light blue bars) and 3 h (grey bars) at pH 2.5. (b) LAB survival to TDCA (light blue bars) or DCA (grey bars). (c) Strains percentage with moderate or high tolerance to TDCA. (d) Strains percentage with low, moderate or high tolerance to DCA. The data are the means of triplicate experiments, and error bars indicate SD. Identical letters indicate that there are not significantly differences between strains in the same treatment while identical numbers indicate that there are no significant differences between treatments (1.5 h and 3 h or TDCA and DCA) for each strain.

On the other hand, the tolerance to 5 mM TDCA after 24 h of exposure was between 57 and 100% (Figure 2). BS concentrations were chosen considering physiological conditions. According to the Margalho et al. (2020) classification, 94% of our isolates showed high resistance to TDCA (above 75%) and the remaining 6% showed moderate resistance (between 50 and 75%) (Figure 2b). Remarkably, CB1, 2, 8, 9, 11 and 14 were not affected by the presence of the conjugated BS.

On the contrary, in the presence of DCA, survival rates were significantly ($p < 0.05$) lower than with TDCA. In fact, only 18% of the strains showed high tolerance to DCA, while 47% and 35% showed moderate and low resistance, respectively (Figure 2c). From the evaluated strains, only CB2, CB5 and CB10 showed survival above 75%, while CB10 showed the highest survival (84.2%). Besides, as reported by other authors (Turchi et al., 2013; Bustos et al., 2015; Castorena-Alba et al., 2018; Margalho et al., 2020), we found that

the response to BS depends on the strain as well as on the type of BS used. In fact, isolated LAB strains showed remarkable resistance to TDCA, while DCA was the most harmful BA, results that are consistent with previously published data (Yerlikaya, 2019).

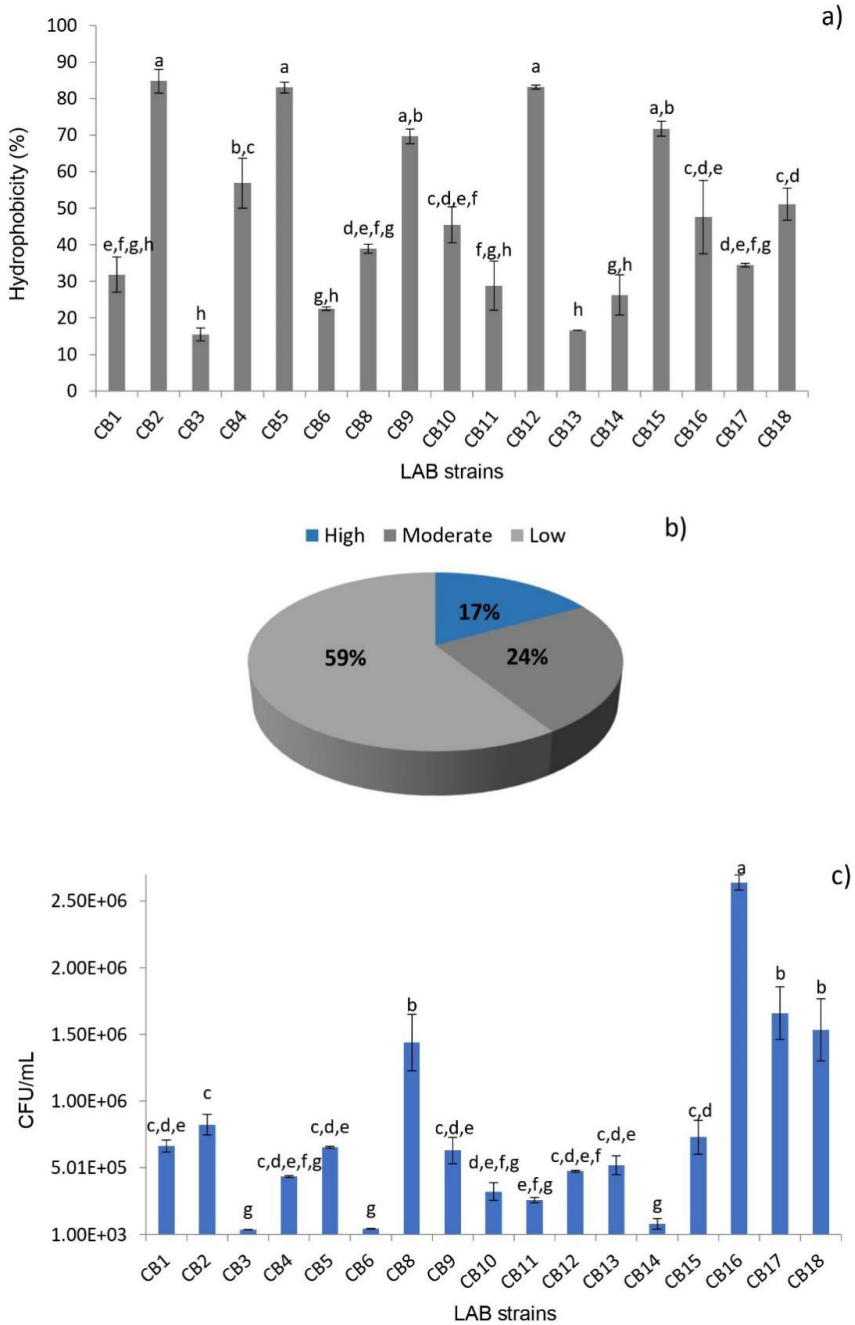

Figure 2. (a) Surface hydrophobicity (%). (b) Strains percentage with low, moderate or high hydrophobicity degree. Identical letters indicate that there are no significant differences between strains. (c) Adhesion of LAB to colon mucus extract. The data are the means of triplicate experiments, and error bars indicate SD. Identical letters indicate that there are no significant differences between strains.

In order to provide beneficial health effects for the host, probiotic bacteria must survive through the GIT, tolerating acid and BS presence, and then adhere and colonize the gut. The pH of the gastric juice is around 2.0 to 3.5 and a residence time is between 90 and 120 min. These harsh conditions cause the death of most exogenous microorganisms when ingested. In addition, once in the intestine, microorganisms must remain longer and face the antimicrobial effect of BS, mainly related to its detergent action that alters the bacterial

cell membrane, damages DNA and causes protein misfolding (Hernández-Gómez et al., 2021). Our results showed that most of the studied strains would be able to *in vitro* withstand the harsh conditions of GIT to exert beneficial effects. Besides, some authors related the tolerance of LAB (mostly *Enterococcus* and *Lactobacillus* genus) to the presence of BSH enzymes.

3.4 Detection of bile salt hydrolase activity

The ability of microorganisms to hydrolyze BS was screened in isolated LAB by the presence of precipitated free BS around colonies growing in MRS with TDCA. The activity was presumptively detected in eight strains from 17 tested (Table 2). Then, the hydrolysis of the conjugated BS in non-proliferating cells was quantified employing HPLC. The evaluated strains showed a high degree of hydrolysis against TDCA, ranged between 74.35 ± 0.92% and 98.41 ± 2.25%, values similar and even higher to those observed in CRL 1098 strain, included as reference.

Table 2. Bile salt hydrolase activity of LAB isolates. Results are expressed as percentage of hydrolysis of TDCA for the different strains.

Strains	Screening of BSH activity	BSH activity quantification
CB1	-	ND
CB2	+	91.60 ± 6.35
CB3	-	ND
CB4	-	ND
CB5	-	ND
CB6	+	96.44 ± 2.41
CB8	-	ND
CB9	-	ND
CB10	-	ND
CB11	+	97.64 ± 3.34
CB12	+	98.41 ± 2.25
CB13	+	87.51 ± 4.10
CB14	+	91.49 ± 4.43
CB15	+	74.35 ± 0.92
CB16	-	ND
CB17	-	ND
CB18	+	81.29 ± 0.34
CRL 1098	+	75 ± 0.99

-: Absence of halo; +: Presence of halo; ND: Not determined.

Our results are in line with those reported by Alameri et al. (2022) and Yerlikaya (2019) among others, who reported a high prevalence of BSH activity in *Lactobacillus* and *Enterococcus* isolated from different sources. In the gut, the BSH enzyme catalyses the hydrolysis of conjugated BS and converts them into free BS plus the amino acids glycine or taurine (Alameri et al., 2022). Free BS is more readily secreted in the feces, leading to the hepatic synthesis of new BS from cholesterol (Bustos et al., 2018). Thus, BSH activity is implicated in lowering total serum cholesterol levels and modulating the ratio of high-density lipoproteins to low-density lipoproteins and consequently reducing cases of hypertension and cardiovascular diseases (Samedi & Charles, 2019). For this reason, BSH activity is an essential biomarker for probiotic selection. In addition, BSH activity

has been proposed as a mechanism of bacterial detoxification, as less soluble BS precipitates, reducing its detergent and antimicrobial properties. However, since some LAB isolates that do not have BSH can withstand the presence of BS, other tolerance mechanisms are involved (Bustos et al., 2018).

3.5 Cell surface hydrophobicity

Cell surface hydrophobicity is another important requirement when selecting potential probiotic candidates, as this characteristic is related to the ability of bacteria to adhere to enterocytes (Jeronymo-Ceneviva et al., 2014). A hydrocarbon adhesion test is extensively employed to assess the hydrophobicity of bacterial cell surfaces. Adherence of the isolated LAB strains to chloroform is shown in Figure 2a. The hydrophobicity values ranged from 15.5 ± 1.8% to 84.7 ± 3.2%. Seventeen percent of the strains showed high hydrophobicity, 24% showed moderate hydrophobicity, and the remaining exhibited low hydrophobicity, with values below 50% (Figure 2b). The most hydrophobic strains were CB2, CB5, and CB12, while the least hydrophobic were CB3 and CB13.

Our results showed higher hydrophobicity values than those reported by Uroić et al. (2014) who found that the affinity to chloroform ranged in 18% to 63% for LAB isolated from artisanal fresh soft and white pickled cheeses. However, the authors could find better affinity to chloroform than hexane, results that could be related with the basic character of LAB cell surfaces. Margalho et al. (2020) reported a similar range of hexane adhesion (from 5 to 64.3%) for strains isolated from Brazilian artisanal cheeses.

While the occurrence of surface (glycol-)proteinaceous compounds at the surface of cells lead to higher hydrophobicity, improving the first contact between microorganisms and host cells, the existence of polysaccharides enhances affinity with hydrophilic surfaces (Carasi et al., 2014). Besides, the higher hydrophobicity values found in this work might be associated with all stresses faced during the processing of traditionally fermented cheeses, likely resulting in the selection of well-adapted species with remarkable properties, such as probiotic potential (Zhang et al., 2015). Remarkably, previous reports have shown a correlation between LAB hydrophobicity and adhesion to intestinal mucosal and epithelial cells (Zhang et al., 2015).

3.6 Adhesion of LAB strains to intestinal mucus

The ability of LAB to adhere to mucus extracted from mouse gut is shown in Figure 2c. Our results showed that all strains were able to interact with the substrate, exhibiting adhesion capacities ranging from 5.05 to 6.90 Log CFU/mL. Although adhesion is a strain-specific property, we found small variations in the response. Our findings are in agreement with those reported by MacKenzie et al. (2010) and Carasi et al. (2014).

The mucus layer attached to the gastrointestinal epithelium is a continuous gel matrix composed mainly of complex glycoproteins (mucins) and glycolipids that act as a barrier to protect the host from harmful antigens and promote luminal motility. The adhesion to the intestine allows, on the one hand, probiotic bacteria to interfere with the binding of pathogens and, on the other hand, the interaction with immune cells (Carasi et al., 2015). A variety of molecular mechanisms has been reported to mediate microbial adhesion to the host, either through fimbriae, exopolysaccharides (EPS) or probiotic surface proteins. In this sense, Pérez et al. (1998) suggested that highly and poorly adhesive strains employed different mechanisms to adhere to mucus. Additionally, some researchers have reported a correlation between hydrophobicity and adhesion (Barberis et al., 2014; Polak-Berecka et al., 2014; Margalho et al., 2020). Our results showed correlation between hydrophobicity and adhesion only for some strains, as the hydrophobicity profiles are highly varied, suggesting that these strains display different molecular mechanisms of adhesiveness.

3.7 Auto-aggregation ability

Figure 3 shows the results of the self-aggregation capacity at different incubation times of the tested LAB strains. Considering all the evaluated times, the auto-aggregation ranged from 4.72 to 59.2%, while the maximum values were reached for strain CB13. For some strains (CB3, CB4 CB5, CB14, CB15, CB16 and CB17), a stepwise increase of auto-aggregation with incubation time was observed. On the other hand, CB10 showed the opposite phenomenon, a stepwise decrease over time. CB1, CB6 and CB9 strains showed a different behavior, peaking at 3 h of incubation and then decreasing, while CB2, CB8, CB11, CB12 and CB13 showed two peaks.

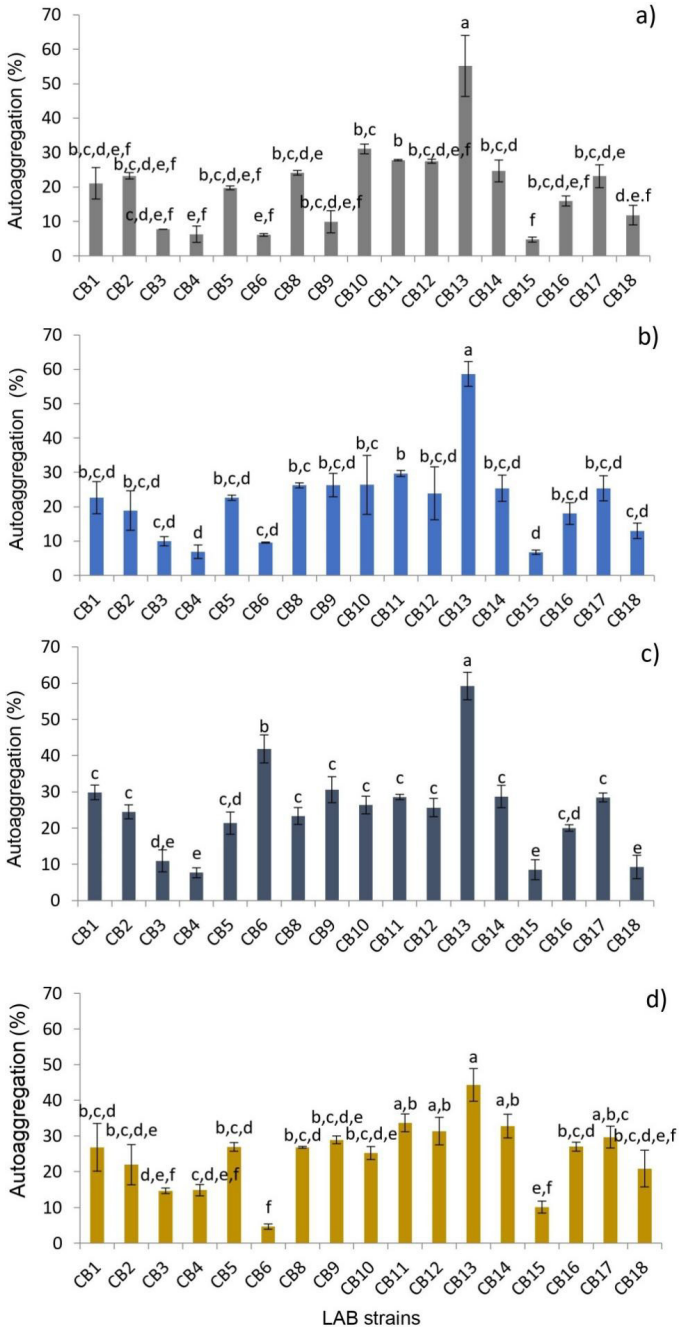

Figure 3. Auto-aggregation assays (%) after 1 h (a), 2 h (b), 3 h (c), and 24 h (d) incubation. The data are the means of triplicate experiments, and error bars indicate SD. Identical letters represent that there are no significant differences between strains.

Auto-aggregation is a strain-specific property. Reported values vary widely among authors. In agreement with our results, Luz et al. (2021) showed an auto-aggregation range of 9-31% for LAB isolated from breast

milk. In addition, the reference probiotic *L. rhamnosus* GG showed an auto-aggregation rate of 25%. Surprisingly, Margalho et al. (2020) reported that most of their tested strains showed high (> 70%) or moderate (20-70%) auto-aggregation values. Auto-aggregation is the interconnection of cells through chemical–physical interactions and surface structures. The aggregating bacteria may achieve an adequate mass to form biofilms or adhere to the mucosal surfaces of the host and exert their functions. In fact, due to this ability, LAB can prevent the adhesion of pathogenic bacteria to the intestinal mucosa by creating a barrier (Fröhlich-Wyder et al., 2013).

3.8 Principal component analysis

Two principal component analysis (PCA) were performed to evaluate the probiotic potential of the strains (N = 17). The percentage distribution of the explained variances in 11 dimensions is presented in Supplementary material Table S1. The cumulative probability column indicates that the three principal components explain 67% of the total variability, where principal component one (PC1) contributes 38%, PC2 15%, and PC3 contributes 14% (Supplementary material Table S2). PC1 is defined by the positive contribution of acid resistance at 1.5 and 3 h, auto-aggregation and HSB activity, while PC2 has a strong positive contribution by DCA resistance, hydrophobicity and mucus adhesion, and to a lesser extent by acid resistance at 1.5 h and auto-aggregation (1, 2 and 24 h).

In Figure 4 generated from PCA, LAB strains (dots on the plane) are dispersed in different quadrants (A, B, C and D) according to their potential probiotic properties. The distances between the strains represent a measure of the observed differences. The variables evaluated (acid stress, TDCA and DCA resistance, BSH activity, hydrophobicity, auto-aggregation, and adhesion to intestinal mucus) are represented in vectors, whose direction and length indicate their relationship to the PC1 and PC2 (X and Y axis). From this, two distinct strain clouds can be observed: cloud 1, which is outside the vectors (CB3, 4, 6, 9, 15, and 18), and cloud 2, which is between the vectors (CB1, 2, 5, 8, 10, 11, 12, 14, 16 and 17), while *L. plantarum* CB13 appears away from the central data set.

According to the PCA, the strains located in cloud 1 and *L. plantarum* CB13, do not have the most relevant probiotic characteristics since they are distant from the variables that most contribute to the explained variation. Those strains that are located close to the variables with the highest contribution to PC2, such as *L. plantarum* CB2, 5, 16 and 17 are defined as probiotics with moderate potential. Finally, *L. plantarum* CB8, *L. plantarum* CB10, *L. plantarum* CB11, *L. parabuchneri* CB12 and *L. parabuchneri* CB14, which are closer to the variables that contribute most to PC1, are considered the best candidates (Bhushan et al., 2021).

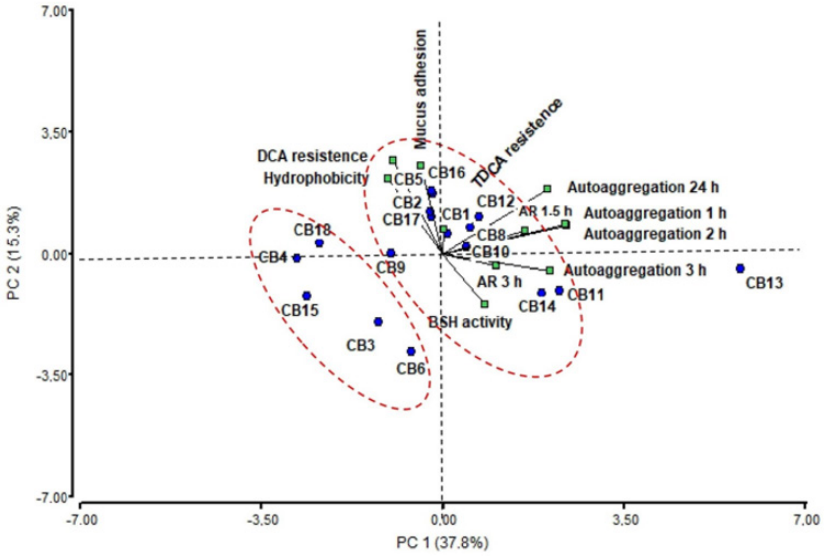

Figure 4. Result from the principal components analysis (PCA) to group LAB (n = 17) isolates from artisanal goat cheeses. Analysis was performed based on probiotic properties such as acid resistance (AR), TDCA and DCA resistance, bile salt hydrolase (BSH) activity, and cell surface properties (hydrophobicity, auto-aggregation, and adhesion to intestinal mucus).

In this work, it was observed that the best probiotic strains would be defined mostly by their auto-aggregation capacity since it was the variable that most contributed in PC1, followed by acid tolerance. PCA also provides information on inter-variable relationships based on the closeness of the vectors along the plane. When the angle of the vectors is small, it means that these variables are closely related to each other. Thus, a strong link between acid resistance at 3 h with auto-aggregation at 3 h was observed. *L. plantarum* CB11 and *L. parabuchneri* CB14 strains, which are located in close proximity to these vectors, are characterized by high survival as self-aggregation. Another example is the close link between ADC resistance and mucus adhesion. Strains such as *L. plantarum* CB2 and *L. plantarum* CB17 are better characterized by these variables.

3.9 Growth, acidifying capacity, and proteolytic activity of BAL in milk

Fermented dairy products have been recognized as the best vehicles for probiotic delivery (Luz et al., 2021). However, many LAB do not have strong acidifying or proteolytic activity to achieve proper fermentation of milk (Herreros et al., 2003). Therefore, we evaluated the ability of the LAB strains isolated to grow in milk as a previous step in the design of a probiotic food.

Table 3 shows the growth of potentially probiotic strains in sterile skim milk after 24 h of incubation. Strains were inoculated at a concentration of 6 Log CFU/mL. Our results showed that all strains evaluated were able to grow in milk, in a range from 2.18 to 2.95 Log CFU/mL. Strain CB4 showed the highest growth, reaching 8.95 Log CFU/mL while CB1 was the strain that showed the lowest growth, reaching 8.18 Log CFU/mL.

On the other hand, acidification capacity is one of the main sought-after technological properties of LAB involved in food processing. The decrease of pH by the production of lactic and acetic acids mostly favors food preservation, inhibiting the development of certain contaminating microorganisms that often act as pathogens and spoilage agents, and negatively impact the organoleptic characteristics of foods (Atanasova et al., 2014).

Table 3. Growth, acidification and proteolytic activity of LAB isolates in milk.

Strains	Growth (log UFC/mL)	pH	Titratable acidity (g lactic acid/100 mL)	Proteolytic activity (at 24 h) (mmol glycine/mL)
CB1	8.18 ± 0.20^{d}	$5.63 \pm 0.15^{b,c}$	$2.35 \pm 0.07^{e,f}$	$68.90 \pm 7.73^{e,f}$
CB2	$8.59 \pm 0.08^{a,b,c,d}$	6.08 ± 0.13^{a}	$2.50 \pm 0.42^{e,f}$	$124.00 \pm 22.00^{a,b,c}$
CB3	$8.25 \pm 0.19^{c,d}$	$5.79 \pm 0.11^{a,b}$	$2.15 \pm 0.07^{f,g}$	$144.40 \pm 9.00^{a,b}$
CB4	8.95 ± 0.09^{a}	$5.15 \pm 0.08^{d,e}$	$2.70 \pm 0.00^{d,e,f}$	$54.75 \pm 0.27^{f,g}$
CB5	$8.87 \pm 0.11^{a,b}$	4.56 ± 0.10^{f}	3.60 ± 0.14^{b}	$46.89 \pm 5.17^{f,g}$
CB6	$8.53 \pm 0.08^{a,b,c,d}$	$5.11 \pm 0.07^{d,e}$	3.70 ± 0.14^{b}	$33.17 \pm 1.20^{g,h}$
CB8	$8.53 \pm 0.12^{a,b,c,d}$	$5.09 \pm 0.08^{d,e}$	4.95 ± 0.21^{a}	$34.90 \pm 0.53^{g,h}$
CB9	$8.79 \pm 0.03^{a,b}$	$4.74 \pm 0.19^{e,f}$	$3.00 \pm 0.28^{b,c,d,e}$	$144.93 \pm 8.61^{a,b}$
CB10	$8.81 \pm 0.07^{a,b}$	$5.13 \pm 0.05^{d,e}$	$2.35 \pm 0.07^{e,f}$	$108.43 \pm 25.04^{c,d}$
CB11	$8.38 \pm 0.06^{b,c,d}$	$5.81 \pm 0.10^{a,b}$	$3.55 \pm 0.07^{b,c}$	$101.50 \pm 10.00^{c,d,e}$
CB12	$8.78 \pm 0.09^{a,b}$	$4.92 \pm 0.12^{d,e}$	$2.80 \pm 0.00^{d,e,f}$	$89.92 \pm 13.05^{d,e}$
CB13	$8.78 \pm 0.20^{a,b}$	$5.02 \pm 0.09^{d,e}$	5.00 ± 0.28^{a}	$121.49 \pm 0.40^{a,b,c,d}$
CB14	$8.68 \pm 0.06^{a,b,c,d}$	$6.05 \pm 0.12^{a,b}$	1.45 ± 0.07^{g}	151.32 ± 11.47^{a}
CB15	$8.38 \pm 0.09^{b,c,d}$	$5.68 \pm 0.05^{a,b,c}$	$3.25 \pm 0.21^{b,c,d}$	10.07 ± 0.80^{h}
CB16	$8.38 \pm 0.20^{b,c,d}$	$5.65 \pm 0.12^{a,b,c}$	$2.85 \pm 0.07^{c,d,e,f}$	9.59 ± 0.73^{h}
CB17	$8.58 \pm 0.12^{a,b,c,d}$	$5.31 \pm 0.09^{c,d}$	$3.00 \pm 0.00^{b,c,d,e}$	$117.22 \pm 1.33^{b,c,d}$
CB18	$8.75 \pm 0.14^{a,b,c}$	5.18 ± 0.05^{d}	$2.90 \pm 0.14^{b,c,d,e}$	$92.45 \pm 0.90^{d,e}$

Identical letters in the superscript indicate that there are no significant differences.

As shown in Table 3, at 24 h of fermentation, the pH values and titratable acidity were in a range of 4.56 to 6.08 and 1.45 to 4.95 g/L lactic acid, respectively. CB13 strain showed the highest acidifying capacity while CB3 train showed the lowest acidifying capacity. The pH values recorded at 24 hours of incubation were higher than values reported by Herreros et al. (2003) for LAB strains isolated from Spanish goats' milk cheese.

The strains with high acidifying activity play an important role in the initial milk coagulation process, so they can be used as starter culture while non-starter LAB are selected on the basis of their health benefits contributing to the final characteristics of products (Turchi et al., 2013; Gheziel et al., 2019; Margalho et al., 2020). Remarkably, our results showed that *L. plantarum* CB5, CB8, and *L. parabuchneri* CB12 showed remarkable probiotic properties and are also able to develop and acidify milk, which would its use as probiotic starter cultures.

Finally, the proteolytic activity of the isolated LAB strains significantly varied among the strains (Table 2) showing a range between 10 and 151 mmol/L amino acids. CB3, CB9, and CB14 strains were the strains that showed the highest release of amino groups giving values up to 140 mmol/L at 24 h. In this sense, the authors reported highly variable values of proteolytic activity in LAB. Herreros et al. (2003) found values between 0.04 and 2.84 mm/L for LAB isolated from Armada cheese while Atanasova et al. (2014) reported proteolytic activity in a range of 10 and 20 mmol/L for LAB grown in goat milk.

The proteolytic system allows LAB to utilize milk protein as a source of nitrogen and essential amino acids and this plays an essential role in the production of different types of metabolites in fermented food products. In fact, because of proteolysis, peptides with biological activity such as antihypertensives, antimicrobials, immunomodulators, among others, are released. Therefore, the use of proteolytic LAB is considered a novel strategy for the design of functional foods enriched in biopeptides (Liu et al., 2010).

4 Conclusions

The increasing demand for products with health benefits beyond their nutritional properties encourages the isolation and characterization of strains with probiotic potential for use in the design of functional foods. In this sense, artisanal goat-made cheeses are generally well-known natural sources of LAB with singular characteristics, including technological and probiotic properties.

In the present work, in seventeen autochthonous strains the absence of hemolytic activity, low pH tolerance, BS resistance, BSH activity, hydrophobicity, auto-aggregation, and mucus adherence, were evaluated. The outcome of these parameters was used as input data for PCA to select the most promising isolates. Finally, isolated strains were inoculated in milk samples and growth, as well as acidifying capacity, and proteolytic activity were measured. Our evaluations allowed us to propose the strains *L. plantarum* CB5, CB8 and *L. parabuchneri* CB12 for the design of functional dairy products, due to their potential *in vitro* probiotic properties and their excellent capacity for development in milk. However, *in vivo* evaluations in animal models and human studies will be necessary for these strains to achieve the probiotic status. The isolation of new LAB strains with proven functional properties is of interest to academic institutions, the food industry, and indirectly lead to the improvement of the rural economy, as it would allow the design of autochthonous dairy cultures with added probiotic and technological properties.

Acknowledgements

The authors acknowledge the financial support from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (Project No. 11220200102036CO, and 28720210100456CO), Agencia Nacional de Promoción Científica y Tecnológica (Project No. 2019-01323), and Consejo de Investigaciones Científicas y Técnicas (CITyC-UNSE, Project 23/c147 and 23/A297).

The authors also acknowledge to Dr. Lucía Mendoza (CERELA-CONICET, Tucumán Argentina) for her valuable collaboration in the molecular identification of the strains and Dr. Liliana Fernendez Canigia (Hospital Aleman, Buenos Aires Argentina) for the identification of strains by MALDI-TOF.

References

Alameri, F., Tarique, M., Osaili, T., Obaid, R., Abdalla, A., Masad, R., Al-Sbiei, A., Fernandez-Cabezudo, M., Liu, S.-Q., Al-Ramadi, B., & Ayyash, M. (2022). Lactic acid bacteria isolated from fresh vegetable products: Potential probiotic and posbiotic characteristics including immunomodulatory effects. *Microorganisms*, *10*(2), 389. PMid:35208844. http://dx.doi.org/10.3390/microorganisms10020389

Atanasova, J., Moncheva, P., & Ivanova, I. (2014). Proteolytic and antimicrobial activity of lactic acid bacteria grown in goat milk. *Biotechnology, Biotechnological Equipment*, *28*(6), 1073-1078. PMid:26019593. http://dx.doi.org/10.1080/13102818.2014.971487

Barberis, C., Almuzara, M., Join-Lambert, O., Ramírez, M. S., Famiglietti, A., & Vay, C. (2014). Comparison of the bruker MALDI-TOF mass spectrometry system and conventional phenotypic methods for identification of Gram-positive rods. *PLoS One*, *9*(9), e106303. PMid:25184254. http://dx.doi.org/10.1371/journal.pone.0106303

Bhushan, B., Sakhare, S. M., Narayan, K. S., Kumari, M., Mishra, V., & Dicks, M. T. (2021). Characterization of riboflavin-producing strains of *Lactobacillus plantarum* as potential probiotic candidate through in vitro assessment and principal component analysis. *Probiotics and Antimicrobial Proteins*, *13*(2), 453-467. PMid:32808141. http://dx.doi.org/10.1007/s12602-020-09696-x

Bustos, A. Y., Font de Valdez, G. M., Fadda, S. G., & Taranto, M. P. (2018). New insights into bacterial bile resistance mechanisms: The role of bile salt hydrolase and its impact on human health. *Food Research International*, *112*, 250-262. PMid:30131136. http://dx.doi.org/10.1016/j.foodres.2018.06.035

Bustos, A. Y., Font de Valdez, G., Raya, R., Almeida, A. M., Fadda, S., & Taranto, M. P. (2015). Proteomic analysis of the probiotic *Lactobacillus reuteri* CRL1098 reveals novel tolerance biomarkers to bile acid-induced stress. *Food Research International*, *77*, 599-607. http://dx.doi.org/10.1016/j.foodres.2015.10.001

Carasi, P., Ambrosis, N. M., Antoni, G. L., Bressollier, P., Urdaci, M. C., & Serradell, M. A. (2014). Adhesion properties of potentially probiotic *Lactobacillus kefiri* to gastrointestinal mucus. *The Journal of Dairy Research*, *81*(1), 16-23. PMid:24168928. http://dx.doi.org/10.1017/S0022029913000526

Carasi, P., Racedo, S. M., Jacquot, C., Romanin, D. E., Serradell, M. A., & Urdaci, M. C. (2015). Impact of kefir derived *Lactobacillus kefiri* on the mucosal immune response and gut microbiota. *Journal of Immunology Research*, *2015*, 361604. PMid:25811034. http://dx.doi.org/10.1155/2015/361604

Castorena-Alba, M. M., Vázquez-Rodríguez, J. A., López-Cabanillas, M., & González-Martínez, B. E. (2018). Cholesterol assimilation, acid and bile survival of probiotic bacteria isolated from food and reference strains. *CyTA - Journal of Food*, *16*(1), 36-41. http://dx.doi.org/10.1080/19476337.2017.1335347

Fröhlich-Wyder, M.-T., Guggisberg, D., Badertscher, R., Wechsler, D., Wittwer, A., & Irmler, S. (2013). The effect of *Lactobacillus buchneri* and *Lactobacillus parabuchneri* on the eye formation of semi-hard cheese. *International Dairy Journal*, *33*(2), 120-128. http://dx.doi.org/10.1016/j.idairyj.2013.03.004

Gheziel, C., Russo, P., Arena, M. P., Spano, G., Ouzari, H. I., Kheroua, O., Saidi, D., Fiocco, D., Kaddouri, H., & Capozzi, V. (2019). Evaluating the probiotic potential of *Lactobacillus plantarum* strains from Algerian infant feces: Towards the design of probiotic starter cultures tailored for developing countries. *Probiotics and Antimicrobial Proteins*, *11*(1), 113-123. PMid:29460213. http://dx.doi.org/10.1007/s12602-018-9396-9

Hernández-Gómez, J. G., López-Bonilla, A., Trejo-Tapia, G., Ávila-Reyes, S. V., Jiménez-Aparicio, A. R., & Hernández-Sánchez, H. (2021). *In vitro* bile salt hydrolase (BSH) activity screening of different probiotic microorganisms. *Foods*, *10*(3), 674. PMid:33810002. http://dx.doi.org/10.3390/foods10030674

Herreros, M. A., Fresno, J. M., González Prieto, M. J., & Tornadijo, M. E. (2003). Technological characterization of lactic acid bacteria isolated from Armada cheese. *International Dairy Journal*, *13*(6), 469-479. http://dx.doi.org/10.1016/S0958-6946(03)00054-2

Jeronymo-Ceneviva, A. B., Paula, A. T., Silva, L. F., Todorov, S. D., Franco, B. D. G. M., & Penna, A. L. B. (2014). Probiotic properties of lactic acid bacteria isolated from water-buffalo mozzarella cheese. *Probiotics and Antimicrobial Proteins*, *6*(3-4), 141-156. PMid:25117002. http://dx.doi.org/10.1007/s12602-014-9166-2

Kamimura, B. A., Magnani, M., Luciano, W. A., Campagnollo, F. B., Pimentel, T. C., Alvarenga, V. O., Pelegrino, B. O., Cruz, A. G., & Sant'Ana, A. S. (2019). Brazilian artisanal cheeses: An overview of their characteristics, main types and regulatory aspects. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1636-1657. PMid:33336917. http://dx.doi.org/10.1111/1541-4337.12486

Liu, M., Bayjanov, J. R., Renckens, B., Nauta, A., & Siezen, R. J. (2010). The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genomics*, *11*(1), 36. PMid:20078865. http://dx.doi.org/10.1186/1471-2164-11-36

Luz, C., Calpe, J., Quiles, J. M., Torrijos, R., Vento, M., Gormaz, M., Mañes, J., & Meca, G. (2021). Probiotic characterization of *Lactobacillus* strains isolated from breast milk and employment for the elaboration of a fermented milk product. *Journal of Functional Foods*, *84*, 104599. http://dx.doi.org/10.1016/j.jff.2021.104599

MacKenzie, D. A., Jeffers, F., Parker, M. L., Vibert-Vallet, A., Bongaerts, R. J., Roos, S., Walter, J., & Juge, N. (2010). Strain-specific diversity of mucus-binding proteins in the adhesion and aggregation properties of *Lactobacillus reuteri. Microbiology*, *156*(11), 3368-3378. PMid:20847011. http://dx.doi.org/10.1099/mic.0.043265-0

Margalho, L. P., Feliciano, M. D., Silva, C. E., Abreu, J. S., Piran, M. V., & Sant'Ana, A. S. (2020). Bazilian artisanal cheeses are rich and diverse sources of nonstarter lactic acid bacteria regarding technological, biopreservative, and safety properties: Insights through multivariate analysis. *Journal of Dairy Science*, *103*(9), 7908-7926. PMid:32684468. http://dx.doi.org/10.3168/jds.2020-18194

Muñoz-Provencio, D., Llopis, M., Antolin, M., Torres, I., Guarner, F., Pérez-Martínez, G., & Monedero, V. (2009). Adhesion properties of *Lactobacillus casei* strains to resected intestinal fragments and components of the extracellular matrix. *Archives of Microbiology*, *191*(2), 153-161. PMid:18974977. http://dx.doi.org/10.1007/s00203-008-0436-9

Pérez, P. F., Minnaard, Y., Disalvo, E. A., & Antoni, G. L. (1998). Surface properties of bifidobacterial strains of human origin. *Applied and Environmental Microbiology*, *64*(1), 21-26. PMid:9435057. http://dx.doi.org/10.1128/AEM.64.1.21-26.1998

Polak-Berecka, M., Waśko, A., Paduch, R., Skrzypek, T., & Sroka-Bartnicka, A. (2014). The effect of cell surface components on adhesion ability of *Lactobacillus rhamnosus. Antonie van Leeuwenhoek*, *106*(4), 751-762. PMid:25090959. http://dx.doi.org/10.1007/s10482-014-0245-x

Pringsulaka, O., Rueangyotchanthana, K., Suwannasai, N., Watanapokasin, R., Amnueysit, P., Sunthornthummas, S., Sukkhum, S., Sarawaneeyaruk, S., & Rangsiruji, A. (2015). *In vitro* screening of lactic acid bacteria for multi-strain probiotics. *Livestock Science*, *174*, 66-73. http://dx.doi.org/10.1016/j.livsci.2015.01.016

Reuben, R. C., Roy, P. C., Sarkar, S. L., Alam, R. U., & Jahid, I. K. (2019). Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. *BMC Microbiology*, *19*(1), 253. PMid:31718570. http://dx.doi.org/10.1186/s12866-019-1626-0

Samedi, L., & Charles, A. L. (2019). Evaluation of technological and probiotic abilities of local lactic acid bacteria. *Journal of Applied & Environmental Microbiology*, *7*(1), 9-19. http://dx.doi.org/10.12691/jaem-7-1-3

Taboada, N., Núñez, M., Medina, R., & López Alzogaray, M. S. (2014). Characterization and technological properties of lactic acid bacteria isolated from traditional Argentinean goat's milk products. *Food Biotechnology*, *28*(2), 123-141. http://dx.doi.org/10.1080/08905436.2014.895944

Turchi, B., Mancini, S., Fratini, F., Pedonese, F., Nuvoloni, R., Bertelloni, F., Ebani, V. V., & Cerri, D. (2013). Preliminary evaluation of probiotic potential of *Lactobacillus plantarum* strains isolated from Italian food products. *World Journal of Microbiology & Biotechnology*, *29*(10), 1913-1922. PMid:23613101. http://dx.doi.org/10.1007/s11274-013-1356-7

Uroić, K., Nikolić, M., Kos, B., Pavunc, A. L., Beganović, J., Lukić, J., Jovčić, B., Filipić, B., Miljković, M., Golić, N., Topisirović, L., Čadež, N., Raspor, P., & Šušković, J. (2014). Probiotic properties of lactic acid bacteria isolated from Croatian fresh soft cheese and Serbian white pickled cheese. *Food Technology and Biotechnology*, *52*, 232-241. Retrieved in 2023, September 22, from https://hrcak.srce.hr/122348

Yerlikaya, O. (2019). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. *lactis* strains isolated from raw milk and kefir grains. *Journal of Dairy Science*, *102*(1), 124-134. PMid:30391179. http://dx.doi.org/10.3168/jds.2018-14983

Zhang, B., Zuo, F., Yu, R., Zeng, Z., Ma, H., & Chen, S. (2015). Comparative genome-based identification of a cell wall-anchored protein from *Lactobacillus plantarum* increases adhesion of *Lactococcus lactis* to human epithelial cells. *Scientific Reports*, *5*(1), 14109. PMid:26370773. http://dx.doi.org/10.1038/srep14109

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, *7*(1-2), 203-214. PMid:10890397. http://dx.doi.org/10.1089/10665270050081478

Funding: Ministerio de Ciencia, Tecnología e Innovación/Agencia Nacional de Promoción Científica y Tecnológica (2019-01323). Consejo de Investigaciones Científicas y Técnicas (23/A297 - 23/c147). Consejo Nacional de Investigaciones Científicas y Tecnológicas (11220200102036CO - 28720210100456CO).

Received: Mar. 19, 2023; **Accepted:** Sept. 22, 2023

Associate Editor: Airton Vialta.

Supplementary Material

Supplementary material accompanies this paper.

Table S1. Eigenvalue output by the statistical program for the eleven dimensions.

Table S2. Eigenvectors output by the statistical program for the eleven variables.

This material is available as part of the online article from https://doi.org/10.1590/1981-6723.02423