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Investigation of some probiotic and technological properties of lactic acid bacteria strains isolated from artisanal sheep milk cheese and their growth in goat milk

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Short version of title (running head)

Probiotic and technological potential of LAB strains

Abstract

The objective of this study was to assess the probiotic and technological properties of lactic acid bacteria (LAB) isolated from artisanal sheep milk cheeses for potential use in dairy products made from goat milk. Strains studied were *Lacticaseibacillus paracasei* LC6, *Lactiplantibacillus plantarum* LP48, *Lactococcus lactis subsp. hordniae* LH69 and *Leuconostoc mesenteroides* LM99. The criteria studied were fermentative capacity, carbohydrate fermentation profile, β-galactosidase (lactase) activity, auto-aggregation capacity, hydrophobicity, antimicrobial activity, resistance to simulated gastric secretion, bile resistance, and resistance to different pH and salts concentrations, as well as growth and viability in goat milk. In general, the strains studied showed characteristics compatible with potential probiotic effects. However, according to criteria studied, *L. plantarum* LP498 best met the conditions; it was compatible with probiotic potential under all the conditions considered and showed noteworthy differences compared to the other micro-organisms studied, particularly in its capacity for self-aggregation and pH resistance. Therefore, *L. plantarum* LP48 could be adequate for the manufacture of artisanal goat milk products.

1. Introduction

In recent years, the general opinion is that consumer habits have shifted towards foods featuring functional benefits that promote well-being. Although the supply of these products available on the market has increased, it is still insufficient (Egbuna and Dable Tupas, 2020). Dairy products are a natural source of health-promoting lactic acid bacteria

(LAB) and the technological capability is available to incorporate probiotic microorganisms into these substrates (Amadoro et al., 2018), making them one of the most promising matrices for the development of functional products. Importantly, the use of probiotic in dairy products has a long history and is considered safe (Carchi Carbo and Vargas Chavarría, 2016). Certain species of LAB (*Lacticaseibacillus*, *Lactiplantibacillus*, *Lactococcus* and *Leuconostoc*) are used to improve consumer health, by enhancing the intestinal microbiota and preventing pathogen colonisation (Corzo, 2015). Probiotic LAB are also used as biopreservatives in food products because of their antibacterial properties (Hossain et al., 2021), and are also an important source of antioxidants, thereby helping to reduce the impact of oxidative reactions in the context of many chronic diseases (Ngamsomchat et al., 2022).

Developing a probiotic food involves meeting specific requirements, including LAB selection and identification and ensuring their survival in the digestive system in order for them to exert the desired effects (Dlamini et al., 2019). In this setting, the relative scarcity of probiotic products based on goat milk makes this a potentially underexploited market (Ranadheera et al., 2019). Goat milk is suitable for dairy-intolerant individuals and boasts various health benefits that dairy milk does not, and it is recommended for children, older adults, and high-performance diets. Indeed, in addition to its lower allergenic potential (Nayik et al., 2021), the size of its fat globules, which are quickly broken down in the presence of bile acids, makes goat milk more digestible (Zhang et al., 2024). Goat milk is also considered “heart-healthy” because it contains short and medium chain fatty acids (Mollica et al., 2021) that reduce cholesterol and do not contribute to intestinal mucosa inflammation, which can lead to discomfort or the absorption of immunogenic substances.

Compared to dairy milk, goat milk is richer in calcium, potassium, copper, selenium, magnesium, phosphorus, chlorine, manganese, and vitamin A, while it contains less sodium, iron, zinc, and molybdenum (Biadała and Konieczny, 2018).

Given all the above, goat milk could be used in the production of a variety of products and as a carrier for functional components such as probiotic bacteria (Verruck et al., 2019). Therefore, identifying micro-organisms with probiotic properties that are well adapted to this matrix is of great interest. The selection of a probiotic strain must consider criteria related to gastrointestinal tract survival, intestinal cell adherence, the exclusion of pathogens, immune system stimulation, and resistance to environmental factors, among others. Thus, the objective of this study was to assess the technological and functional capabilities of native LAB obtained from artisanal cheeses for potential use in goat milk-based dairy products.

2. Materials and Methods

2.1. Lactic acid bacteria selection and growth conditions

The LAB selected were isolated from artisanal cheeses made with sheep milk obtained in the city of Córdoba, Spain, and were identified by 16S rRNA next generation sequencing in the Central Research Support Service of the University of Córdoba, Spain, as *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *Lactococcus lactis* subsp. *hordniae*, and *Leuconostoc mesenteroides* (Ruiz et al., 2023). To conduct the different analyses described in this work, the *L. paracasei* and *L. plantarum* strains were cultured in MRS medium (Britania, Buenos Aires, Argentina) at 37°C for 48 hours under microaerobic conditions (H_2:CO_2:O_2=85:10:5), while the *L. lactis* subsp. *hordniae* LH69

and *L. mesenteroides* LM99 strains were recovered in M17 medium (Biokar, Beauvais, France) at 37°C for 48 hours under aerobic conditions.

2.2. Fermentative capacity

The homofermentative or heterofermentative capacity was determined using LAB counting plates (Petrifilm, 3M®, Two Harbors, Minnesota, USA; Medina and Jordano, 2019). Heterofermentative LAB appear as red colonies and are associated with entrapped gas, while red colonies without gas are considered homofermentative.

2.3. Carbohydrate fermentation profile

The carbohydrate fermentation profile was determined using the API50CHL® enzymatic gallery and API50CH® medium (BioMérieux, Inc., Marcy-l'Étoile, Lyon, France; Rafii and Khare, 2014). The enzymatic gallery is a standardised system consisting of 50 biochemical tests for the study of carbohydrate metabolism in isolated microorganisms. Analysis was performed following the protocol specified by the manufacturer, using the first microtube without a substrate as a negative control. The rest of the microtubes contained dehydrated carbohydrate substrates and their derivatives (heterosides, polyalcohols, and uronic acids). Each LAB culture, with a concentration equivalent to tube 2 according to the McFarland Standards, was introduced into 5 mL of API50CH® medium (BioMérieux). The panels were dispensed with 10 mL of sterile water and the microtubes were filled with 100 µL of the bacterial suspensions and were then sealed with Vaseline and incubated at 37°C for 48 hours. The fermentation produces organic acids, especially lactic acid, which lowers the pH and causes the bromocresol purple to turn yellow. The

results obtained were tabulated and species designations were identified using ABIS software (https://www.tgw1916.net/bacteria_abis.html).

2.4. β-galactosidase (lactase) activity

The enzymatic determination was carried out based on Rodríguez Olivenza (2015), using o-nitrophenyl-β-D-galactopyranoside (ONPG; Merck KGaA, Darmstadt, Germany) as a lactose analogue. When in contact with β-galactosidase, ONPG is hydrolysed and produces a compound (o-nitrophenol) that has an intense yellow colour which turns the medium yellow for positive tests and remains colourless if negative. In these experiments, *Escherichia coli* was used as a positive control and *Salmonella* spp. as a negative control. Enzymatic activity was quantified by measuring the absorbance at an optical density (OD) of 420 nm (OD420) in a spectrophotometer (Ultrospec III, Pharmacia LKB, Uppsala, Sweden).

2.5. Auto-aggregation

Auto-aggregation was tested according to Khalil et al. (2018). Bacterial cultures were washed twice with phosphate-buffered saline (PBS, pH 7.2), were resuspended in PBS, gently homogenised, and then incubated at 37°C for 5 hours. Aliquots (1 mL) of each sample were taken at 0 and 5 hours and the absorbance at OD_{660} was determined in a spectrophotometer. The percentage of auto-aggregation was calculated using the equation $(A_0-A_5/A_0)\times100$, where A_5 represented the absorbance after 5 hours of incubation and A0 represented the baseline absorbance. The results characterised the strains as having a good (greater than 40%), moderate (10% to 40%), or weak (less than 10%) auto-aggregative capacity.

2.6. Hydrophobicity study

Hydrophobicity was measured as a primary indicator of the potential adhesion of strains to mucosal surfaces using a method based on the microbial tendency to adhere to the surface of liquid hydrocarbons after a brief period of contact. The LAB were centrifuged for 10 minutes at 4000 g and each pellet was then suspended in 2 mL PBS until the OD_{600} = 1. The suspension was then placed in a 10mm diameter cylindrical cuvette with 0.4 mL of xylene as an organic solvent (Farid et al., 2021). The initial OD_{600} (OD_i) was determined with a spectrophotometer. The phases were then homogenised for 120 seconds and allowed to rest at room temperature for one hour and then the final OD_{600} (OD_f) was measured. The percentage of hydrophobicity was calculated with the formula $(OD_i - OD_f) \times 100 / OD_i$, (Khalil et al., 2018) to classify the strains as highly hydrophobic (≥ 50%), moderately hydrophobic (20 to 50%), or hydrophilic (≤ 20%) (Merino, 2019).

2.7. Antimicrobial activity

Antimicrobial activity was evaluated using the agar diffusion test, with *Salmonella enterica* serovar Typhimurium (hereinafter *Salmonella Typhimurium*), Shiga toxin-producing *E. coli* (STEC), and *Staphylococcus aureus* used as foodborne pathogens. Briefly, each LAB was spiked onto MRS agar (Britania) and M17 agar (Biokar) plates prepared for *L. paracasei* LC6 and *L. plantarum* LP48, *L. lactis* subsp. *hordniae* LH69, and *L. mesenteroides* LM99, respectively. After incubation for 48 hours at 37°C under microaerobic (for *L. paracasei* LC6 and *L. plantarum* LP48) and aerobic conditions (for *L. lactis* subsp. *hordniae* LH69 and *L. mesenteroides* LM99), the plates were exposed to

chloroform vapours for 2 hours to inactivate the bacteria that had grown. The plates were then covered with a layer of soft trypticase soy agar (TSA, Britania; 0.75% agar) containing the pathogenic bacteria. The observation of translucent circular areas around the colonies was considered indicative of inhibition of the growth of the pathogenic bacteria because of the action of the LAB. The presence of inhibition zones greater than 1 mm was considered a positive result (Ruiz et al., 2017).

2.8. Resistance to simulated gastric secretion

A 'gastric-like' solution (GS) was prepared based on the study by (Villarreal, 2002). 1 mL of culture from each strain was centrifuged and washed in duplicate to obtain two pellets per strain for each repetition. The GS was added to one of them, while a buffer was added to the other as a control (C). Decimal solutions were made from the GS and C pellets and were incubated at 37°C for 3 hours, before sowing them on plates with MRS agar (Britania) and M17 agar (Biokar) followed by incubation at 37°C for 72 hours. Viability loss was evaluated and was inversely proportional to gastric resistance. The results were expressed as viable counts (log_{10} colony-forming units (CFU/mL) of LAB strains under optimal (C) and gastric (GS) growth conditions.

2.9. Bile resistance analysis

LAB resistance to bile was studied using an agar diffusion test to detect the occurrence of agents that inhibit the growth of a microorganism immersed in an agarised patina (Villarreal, 2002). Tubes were prepared with 20 mL of MRS agar (Britania; for *L. paracasei* LC6 and *L. plantarum* LP48) and M17 agar (Biokar; for *L. lactis* subsp. *hordniae* LH69 and *L. mesenteroides* LM99). Aliquots of 200 µL of overnight LAB broth cultures were

inoculated and homogenised in the corresponding agar, previously conditioned at 45°C. The medium was poured into sterile Petri dishes and once solidified, 10mm diameter wells were made. Aliquots of 180 µL each of 0.15%, 0.3%, 0.6%, and 1.0% sterile ox bile solution (Britania) were placed in the corresponding wells and were allowed to diffuse at room temperature. The plates were incubated at 37°C for 24 hours under the conditions previously described for each microorganism. The halos subsequently observed on the plates were classified as total inhibition (a completely translucent area caused by the absence of growth), partial inhibition (area with less intense turbidity than the rest of the patina because of weak growth), and non-inhibition (the absence of a halo).

2.10. Resistance to pH changes and salts

A qualitative method was used to determine if the pH of the medium inhibited LAB development. The MRS broths (Britania) were prepared at different pHs (4.5, 5.0, and 5.5) by adding lactic acid and a control without lactic acid was maintained at the standard pH of the medium 6.4 ± 0.2 and each strain was incubated in these conditions at 37°C for 24 hours (Sánchez and Tromps, 2014). The same procedure was used to evaluate resistance to salts by using MRS broth (Britania) supplemented with either sodium chloride (NaCl) or and potassium chloride (KCl) at 1%, 2%, and 3% (w/v). Resistance or sensitivity to different media conditions was visualised in terms of its effect on turbidity and was confirmed by the plate count (CFU/mL).

2.11. LAB growth and viability in goat milk

LAB strains were activated for 48 hours under the conditions previously described for each microorganism and were later grown in their respective media. The LAB were then washed twice with 0.85% physiological solution (Biopack, CABA, Argentina) and centrifuged at 5000 g (PeetLab LC-404R, Anhui, China). The pellet obtained was resuspended in 0.85% physiological solution (Biopack) and the bacterial concentration was adjusted to OD_{600} = 0.5. The goat milk was reconstituted with potable water as indicated on the product packaging for consumption. The product was then subjected to flowing steam for 30 min to eliminate any potential microorganisms that could contaminate it. An aliquot of 10 µL of each bacterial suspension was then inoculated into 50 mL of whole goat milk reconstituted. The tubes were incubated in aerobic conditions at 37°C for 5 days. At 24 hours and 5 days, aliquots of each treatment were taken, and different dilutions were plated on specific media to quantify the LAB growth and viability.

2.12. Statistical analysis

The statistical analysis (ANOVA and Tukey tests) was carried out using Infostat software (version 2014, Grupo Infostat, FCA, Universidad Nacional de Córdoba, Argentina; http://www.infostat.com.ar). A significance level of $p < 0.05$ was set to determine significant differences.

3. Results

L. paracasei subsp. *paracasei* LC6, *L. plantarum* LP48, and *L. mesenteroides* LM99 showed heterofermentative capacity, while *L. lactis* subsp. *hordniae* LL69 showed homofermentative capacity. The carbohydrate fermentation profiles of the LAB studied

are shown in table 1. The β-galactosidase assay using ONPG discs showed positive results for the *L. paracasei* LC6 and *L. plantarum* LP48 strains, and negative results for the *L. lactis* subsp. *hordniae* LL69 and *L. mesenteroides* LM99 strains. In terms of auto-aggregation, the results showed values of 4.20%, 3.77%, and 5.02% for *L. paracasei* LC6, *L. lactis* subsp. *hordniae* LL69, and *L. mesenteroides* LM99, respectively, thereby categorising them as weakly auto-aggregative. In terms of hydrophobicity, *L. plantarum* LP48 showed a value of 17.17%; *L. paracasei* LC6, a value of 67.92%; and *L. lactis* subsp. *hordniae* LL69 and *L. mesenteroides* LM99 showed values of 3.44% and 4.54%, respectively. The results obtained for antimicrobial activity against pathogenic bacteria (STEC, *S. Typhimurium*, and *S. aureus*) are shown in table 2 while table 3 shows results for resistance to simulated GS.

The bile salts resistance study showed that LAB can survive in concentrations of 0.15%, with their responses differing with changing bile salt concentrations. In the optimal growth medium with 0.3% ox bile salts, the *L. paracasei* LC6 strain showed partial inhibition, while the other LAB strains tested survived. At a concentration of 0.6%, *L. paracasei* LC6 and *L. plantarum* LP48 were partially inhibited, while *L. lactis* subsp. *hordniae* LL69 and *L. mesenteroides* LM99 showed complete inhibition. Finally, in the medium with the highest concentration of bile salts (1%), all four strains were inhibited. The results of LAB resistance with different salts are shown in table 4, while the results of their resistance to different pH values are shown in table 5.

Regarding the ability of LAB to grow in a goat dairy model, after 24 hours of incubation, growth showed average counts of 6.74 $\log_{10}$ CFU/mL, 6.04 $\log_{10}$ CFU/mL, 6.86 $\log_{10}$ CFU/mL, and 6.28 $\log_{10}$ CFU/mL for *L. paracasei* LC6, *L. plantarum* LP48, *L. lactis* subsp.

hordniae LL69, and *L. mesenteroides* LM99, respectively. After 5 days, the counts showed a significant ($p < 0.05$) increase, with 7.24 log_{10} CFU/mL, 7.28 log_{10} CFU/mL, 7.21 log_{10} CFU/mL, and 7.28 log_{10} CFU/mL noted for *L. paracasei* LC6, *L. plantarum* LP48, *L. lactis* subsp. *hordniae* LL69, and *L. mesenteroides* LM99, respectively.

4. Discussion

4.1. Fermentative capacity

Our results for fermentative capacity are consistent with the categorisation of sugar fermentation established in Bergey's Manual: A, for obligatory homofermentative; B, for facultative heterofermentative; and C, for obligatory heterofermentative. This classification places the bacterial species *L. paracasei* subsp. *paracasei*, *L. plantarum* (Vos et al., 2011), and *L. mesenteroides* (Soetaert et al., 1995) in Category B, and *L. lactis* subsp. *hordniae* LH69 in Category A (Mataragas, 2020).

4.2. Carbohydrate fermentation profiles

The substrates could be metabolised through different biochemical routes: assimilation when LAB grows in the microtube with a substrate as the only carbon source; oxidation because of aerobic production of acid detected by the pH indicator present in the microtube; and/or fermentation as the result of anaerobic production of acid detected by the pH indicator present in the microtube. After entering the API50CHL® results into ABIS software and considering the most significant sugar profiles, we observed that the four microorganisms studied fermented both glucose and fructose. For fructose, all the samples were positive after 72 hours, or from the beginning in the case of *L. lactis* subsp.

hordniae LL69 and *L. mesenteroides* LM99. This behaviour is valued in potential probiotic microorganisms given the interest in fructooligosaccharides as prebiotics (Cunningham et al., 2021; Farias et al., 2019). Interestingly, similar behaviour was observed for galactose, with galactooligosaccharides also representing interesting prebiotics. Finally, only *L. paracasei* LP6 did not ferment lactose.

4.3. Auto-aggregation

The auto-aggregation tests evaluated the physical interaction between bacteria and the substrate, resulting in them forming clusters that settled to the bottom in a static suspension. Bacterial clusters can be quantified by monitoring the spontaneous sedimentation of an immobile bacterial suspension by measuring the change in OD_{600} (Merino, 2019). Bacteria with the ability to auto-aggregate show a decrease in turbidity or a drop in OD_{600} over time. Determination of the auto-aggregation capacity and surface hydrophobicity can be used as a preliminary criterion in the search for bacteria capable of adhering to surfaces (Li et al., 2015).

Bandyopadhyay et al. (2022) showed that auto-aggregation ranges from 15% to 25% over 24 hours for the strain *Lactococcus lactis* subsp. *lactis*, which concurred with the findings of 18.3% by Cunha et al. (2021), with all these values being higher than those found in this current study. However, *L. plantarum* LP48 showed a value of 25.53%, which categorises it as having a moderate auto-aggregative capacity. This capacity to auto-aggregate is essential to achieve high cell density in the gastrointestinal tract to ensure adherence to intestinal epithelial cells and thereby avert potential colonisation by pathogenic microorganisms (Rodríguez-Sánchez et al., 2021). Thus, in this sense, *L.*

plantarum LP48 was more suitable for use as a probiotic than the other microorganisms we studied.

4.4. Hydrophobicity study

With a hydrophobicity value of 17.17%, the *L. plantarum* LP48 strain was classified as hydrophilic. Similarly, other researchers have shown that *L. plantarum* 83114 and 8327 were hydrophilic in xylene since the surface hydrophobicity measured did not exceed 20% (Merino, 2019). These results also agree with previous studies indicating that *L. plantarum* has a hydrophilic surface (Golowczyc et al., 2008; Ramos et al., 2013). Indeed, numerous publications have indicated a possible positive correlation between auto-aggregative capacity and surface hydrophobicity in *L. plantarum* strains (Nikolic et al., 2010; Chen et al., 2010; Merino, 2019). In our study, both auto-aggregation and hydrophobicity for *L. plantarum* showed similar values (25.53% and 17.17%, respectively).

Moreover, in agreement with the results reported by Golbaghi et al. (2022), Qureshi et al. (2020), and Romero-Luna et al. (2020), who demonstrated that strains of *L. paracasei* presented good hydrophobicity (57.82%, 84.76%, and 97–99%, respectively), our results for *L. paracasei* LC6 also classified it as being hydrophobic. We also showed that *L. lactis* subsp. *hordniae* LL69 and *L. mesenteroides* LM99 had hydrophilic capacity with values of 3.44% and 4.54%, respectively. In this respect, a study by Rai and Tamang (2022), showed that species of *L. lactis* subsp. *hordniae* contained genes associated with hydrophobicity (mapA, mub1, msa, and apf). In turn, these authors also indicated that some, but not all of the strains of *L. mesenteroides* they studied lacked these genes.

4.5. Antimicrobial activity

In terms of the power of LAB to inhibit pathogenic bacteria, the agar diffusion test results indicated that they had a total inhibitory capacity against STEC, *S. Typhimurium*, and *S. aureus*. For STEC, the LAB showed a mean inhibition zone diameter of 15.75 mm, for S. *Typhimurium* it was 15.42 mm, and for *S. aureus* it was 12.58 mm. No differences in inhibitory capacity against the pathogens studied were seen ($p = 0.2949$). These results are consistent with previous research on the antibacterial capacity of LAB (Ibrahim et al., 2021) and in particular, *L. plantarum* isolates (Ngamsomchat et al., 2022). However, it is necessary to individually study each bacterial strain to determine the production capacity of the antibacterial metabolite and its effect on different pathogens associated with foodborne diseases.

4.6. Resistance to simulated gastric secretion

Potential probiotic candidates must be able to survive gastric conditions. In this sense, acidity is considered an important destructive factor that affects the viability of LAB. Given that the pH of intestinal gastric juice is 2.0 to 3.0, this could significantly hinder the survival of ingested microorganisms (Bernatek et al., 2022). Our results showed that all four LAB strains exhibited a high survival rate in the presence of GS. Although there were significant differences ($p < 0.05$) between the counts in the GS and C conditions over time ($p = 0.003$), there were no significant differences in the counts between the groups for each LAB at 3 hours ($p = 0.06$). This was consistent with the results reported by Tulumoglu et al. (2013) and Wang et al. (2010) who observed high survivability by

Lactobacillus strains isolated from the faeces of breast-fed infants and children. Furthermore, researchers have demonstrated that when *L. plantarum* strains were incubated for 3 hours under simulated gastric conditions, absorbance values increased, demonstrating their ability to grow under these conditions (Missaoui et al., 2019; Rodríguez-Sánchez et al., 2021).

4.7. Resistance to bile salts

In addition to having the ability to resist gastric conditions, potential probiotic candidates must survive the bile salts present in the duodenum in order to deliver health benefits to the host (Anandharaj et al., 2015). It has been shown that the average concentration of bile salts is 0.3% and that intestinal retention of food lasts between 4 and 6 hours (Khalil et al., 2018). In the case of the LAB strains studied in this work, our results coincided with those of Khalil et al. (2018) who analysed the influence of 0.3% bile salts on bacterial growth, showing a survival rate of 98.15%. Previous studies by Pan et al. (2014) and Tulumoglu et al. (2013) also observed a reduction from an initial count of 8.54 to 6.09 log10CFU/g. Furthermore, a study by Romero-Luna et al. (2020) showed that all the isolates, including a strain identified as *L. paracasei*, were able to tolerate the bile salt stress test. The survival of LAB cells in the presence of bile salts is strain-specific, with extreme variability in resistance found within the same species (Khalil et al., 2018). Thus, different concentrations of bile (considered within the normal physiological range in humans) were used to simulate its effect on the viability of LAB after passing through the stressful environment of the stomach. *L. paracasei* LC6 and *L. plantarum* LP48 showed a good survival capacity under these conditions.

4.8. Resistance to pH changes and salts

As shown in table 4, the *L. paracasei* LC6 strain showed similar behaviour in both the NaCl and KCl conditions. Growth was observed in the medium with 1% added salt, while the addition of 2% salt produced doubtful or weak growth, and no growth was observed with 3% salt. The *L. plantarum* LP48, *L. lactis* subsp. *hordniae* LL69, and *L. mesenteroides* LM99 strains showed resistance to both types of salt at all three concentrations, resulting in growth in all the tubes. In turn, the resistance results for LAB at different pH conditions, using pH 6.4 as a control, are shown in table 5. *L. paracasei* LC6 showed weak growth at pH 4.5 and average growth at pH 5.0 and 5.5. *L. lactis* subsp. *hordniae* LL69 and *L. mesenteroides* LM99 showed weak growth at pH 4.5, 5.0, and 5.5. However, the strain that performed best was *L. plantarum* LP48, which was resistant to all three pH conditions studied. Many previous studies have also shown that *L. plantarum* can survive under extremely low pH conditions and with different percentages of salts (Nguyen et al., 2021), making *L. plantarum* LP48 the best candidate we studied in this current work.

4.9. LAB growth in a goat dairy model

On the basis of the results obtained, goat milk would be a suitable matrix for the development and maintenance of the LAB we studied. In addition to its processing potential, this would allow the use of goat milk for the formulation of numerous common dairy ingredients and in the development of new dairy products (Nayik et al., 2021). Furthermore, Ngamsomchat et al. (2022) reported that *L. plantarum* demonstrated high

tolerance to acid, bile, and salts, antibacterial activity against foodborne pathogens, and the highest percentage of adhesion to Caco-2 cells, thereby indicating its suitability, with a good survival rate, for the production of goat cheese. Moreover, *L. plantarum* was previously found to contribute positive properties to functional skimmed fresh cheese (de Oliveira et al., 2023).

5. Conclusion

The probiotic and technological potential of LAB depends on strain. The results obtained in this current work show, in general, that the strains we studied showed characteristics compatible with potential probiotic effects. However, *L. plantarum* LP498 adapted the best to the criteria we studied; it exhibited probiotic potential and was differentiated in respect to self-aggregation and growth in a wide pH range. Therefore, it could be adequate for the manufacture of artisanal goat milk products, although further studies to test its adherence to the intestinal mucosa will still need to be conducted in future work.

Author contributions

M.J.R. designed the research and the methodology and did the writing - original draft and the writing - revision and editing of the manuscript. L.M.M.C. worked on data curation, formal analysis, visualization and manuscript supervision. M.I.P. worked on methodology and the writing of the manuscript. S.E. performed the statistical analysis of the results. M.F.V. performed laboratorial analysis. L.S.F. revised and edited of the manuscript. A.I.E. conducted fundraising and project administration, contributed research, resources, supervision, and writing, reviewing, and editing the manuscript.

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Conflicts of Interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The data will be available in the CONICET Institutional Digital Repository (https://ri.conicet.gov.ar/), upon reasonable request.

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Table 1: Carbohydrate fermentation profile of LAB in the API50CHL® enzymatic gallery

		L. paracasei LC6			*L. plantarum* LP48			*L. hordniae* LL69			*L. mesenteroides* LM99		
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
0	CONTROL	-	-	-	-	-	-	-	-	-	-	-	-
1	**GLY**cerol	-	-	-	-	-	-	-	-	-	-	-	-
2	**ERY**throl	-	-	-	-	-	-	-	-	-	-	-	-
3	**D-ARA**binose	-	-	-	-	-	-	-	-	-	-	-	-
4	**L-ARA**binose	-	-	-	-	-	-	-	-	-	-	-	-
5	**RIB**ose	-	+	+	-	+	+	+	+	+	+	+	+
6	**D-XYL**ose	-	-	-	-	**d**	**d**	-	-	-	-	-	-
7	**L-XIL**ose	-	-	-	-	-	-	-	-	-	-	-	-
8	**ADO**nitol	-	-	-	-	-	-	-	-	-	-	-	-
9	β-**M**ethyl-**D**-**X**yloside	-	-	-	-	-	-	-	-	-	-	-	-
10	**GAL**actose	-	-	+	-	+	+	+	+	+	+	+	+
11	**GLU**cose	-	**d**	+	**d**	+	+	+	+	+	+	+	+
12	**FRU**ctose	-	**d**	+	**d**	+	+	+	+	+	+	+	+
13	**M**a**N**nos**E**	-	-	+	**d**	+	+	+	+	+	+	+	+
14	**S**or**B**os**E**	-	-	-	-	-	-	-	-	-	-	-	-
15	**RHA**mnose	-	-	-	-	**d**	**d**	-	-	-	-	-	-
16	**DUL**citol	-	-	-	-	-	-	-	-	-	-	-	-
17	**INO**sitol	-	-	-	-	-	-	-	-	-	-	-	-
18	**MAN**nitol	-	-	+	-	+	+	-	-	-	-	-	-
19	**SOR**bitol	-	-	-	-	+	+	-	-	-	-	-	-
20	1-**M**ethyl-**D**-**M**annoside	-	-	-	-	**d**	+	-	-	-	-	-	-
21	1-**M**ethyl-**D**-**G**lucoside	-	-	-	-	-	-	-	-	-	-	-	-
22	**N**-**A**cetyl-**G**lucosamine	-	**d**	+	**d**	+	+	+	+	+	+	+	+
23	**AMY**gdaline	-	-	+	-	+	+	-	-	-	-	+	+
24	**ARB**utin	-	-	-	-	+	+	+	+	+	+	+	+
25	**ESC**ulin	**d**	**d**	+	+	+	+	+	+	+	+	+	+
26	**SAL**icin	-	-	+	**d**	+	+	+	+	+	+	+	+
27	**CEL**lobiose	-	-	+	**d**	+	+	+	+	+	+	+	+
28	**MAL**tose	-	-	+	**d**	+	+	+	+	+	+	+	+
29	**LAC**tose	-	-	-	-	+	+	+	+	+	+	+	+
30	**MEL**ibiose	-	-	-	-	+	+	-	-	-	-	**d**	**d**
31	**SAC** sucrose	-	-	+	**d**	+	+	-	-	-	-	-	-
32	**TRE**halose	-	**d**	+	-	+	+	+	+	+	+	+	+
33	**INU**lin	-	-	-	-	-	-	-	-	-	-	-	-
34	**M**e**L**e**Z**itose	-	**d**	+	-	+	+	-	-	-	-	-	-
35	**RAF**finose	-	-	-	-	-	**d**	-	-	-	-	-	-
36	**AMD** starch	-	-	-	-	-	-	-	-	-	-	-	-
37	**GLY**co**G**en	-	-	-	-	-	-	-	-	-	-	-	-
38	**X**y**L**i**T**ol	-	-	-	-	-	-	-	-	-	-	-	-
39	**GEN**tiobiose	-	-	-	**d**	+	+	+	+	+	+	+	+
40	D-**TUR**anose	-	-	-	-	+	+	-	-	-	-	-	-
41	D-**LYX**ose	-	-	-	-	-	-	-	-	-	-	-	-
42	D-**TAG**atose	-	+	+	**d**	+	+	+	+	+	+	+	+
43	**D-FUC**ose	-	-	-	-	-	-	-	-	-	-	-	-
44	**L-FUC**ose	-	-	-	-	-	-	-	-	-	-	-	-
45	**D-AR**abito**L**	-	-	-	-	-	-	-	-	-	-	-	-
46	**L-AR**abito**L**	-	-	-	-	-	-	-	-	-	-	-	-
47	**G**luco**N**a**T**e	-	-	+	-	-	**d**	-	-	-	-	-	-
48	**2-K**eto-**G**luconate	-	-	-	-	-	-	-	-	-	-	-	-
49	**5-K**eto-**G**luconate	-	-	**d**	-	-	-	-	-	-	-	-	-

+ positive reaction - negative reaction d uncertain reaction

Table 2: Inhibitory capacity of LAB against foodborne pathogens using the agar diffusion test.

Specie	Strain	Average diameter of the inhibition zone (mm)		
		STEC*	*S. Typhimurium*	*S. aureus*
L. paracasei	LC6	15.0±1.0^{aB}	19.0±1.0^{bA}	10.0±1.0^{cB}
L. plantarum	LP48	24.0±1.0^{aA}	18.3±0.6^{bA}	23.7±0.6^{aA}
L. lactis subsp. *hordniae*	LL69	13.7±0.6^{aB}	12.3±0.6^{aB}	8.3±0.6^{bB}
L. mesenteroides	LM99	10.3±0.6^{aB}	12.0±1.0^{aB}	8.3±0.6^{aB}

*STEC: Shiga-toxin *Escherichia coli*
Lowercase letters indicate significant differences between the zones of inhibition generated by each LAB against the pathogens. Capital letters indicate significant differences between the zones of inhibition generated by all LAB against each pathogen.

Table 3: Viable counts (log_{10}CFU/mL) of LAB strains under optimal growth conditions (C) and under gastric solution conditions (GS) over time.

Specie	Strain	T0		T1		T2		T3	
		C	GS	C	GS	C	GS	C	GS
L. paracasei	LC6	9.30	9.15^{B}	9.44	8.10^{A}	8.43	7.90^{A}	8.29	8.05^{C}
L. plantarum	LP48	9.59	9.30^{A}	9.45	8.34^{B}	8.79	8.35^{A}	8.30	8.09^{C}
L. lactis subsp. *hordniae*	LL69	9.17	8.99^{B}	8.87	8.84^{D}	8.87	8.87^{B}	8.92	8.17^{A}
L. mesenteroides	LM99	9.77	9.61^{B}	9.11	8.87^{C}	9.06	8.60^{A}	8.91	8.48^{B}

T0: initial time; T1: one hour under GS conditions; T2: two hours under GS conditions and T3: three hours under GS conditions.
* Capital letters per column (C-GS) indicate significant differences in treatment effect between different LAB.

Table 4: Resistance of LAB to different salts and concentrations estimated by turbidity and confirmed by plate counting (CFU/mL).

Specie	Strain	NaCl									KCl								
		1%			2%			3%			1%			2%			3%		
L. paracasei	**LC6**	**+**	**+**	**+**	**d**	**d**	**d**	**-**	**-**	**-**	**+**	**+**	**+**	**d**	**d**	**d**	**-**	**-**	**-**
L. plantarum	LP48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. lactis* subsp. *hordniae	LL69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. mesenteroides	LM99	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: turbidity presence; -: turbidity absence; d: weak turbidity (confirmed by plate counting)

Table 5: Resistance of LAB to different pH conditions estimated by turbidity.

Specie	Strain	pH											
		4.5			5.0			5.5			6.4		
L. paracasei	LC6	d	d	d	+	+	+	+	+	+	+	+	+
L. plantarum	LP48	+	+	+	+	+	+	+	+	+	+	+	+
L. lactis subsp. *hordniae*	LL69	d	d	d	d	d	d	d	d	d	+	+	+
L. mesenteroides	LM99	d	d	d	d	d	d	d	d	d	+	+	+

+: turbidity presence; -: turbidity absence; d: weak turbidity (confirmed by plate counting)

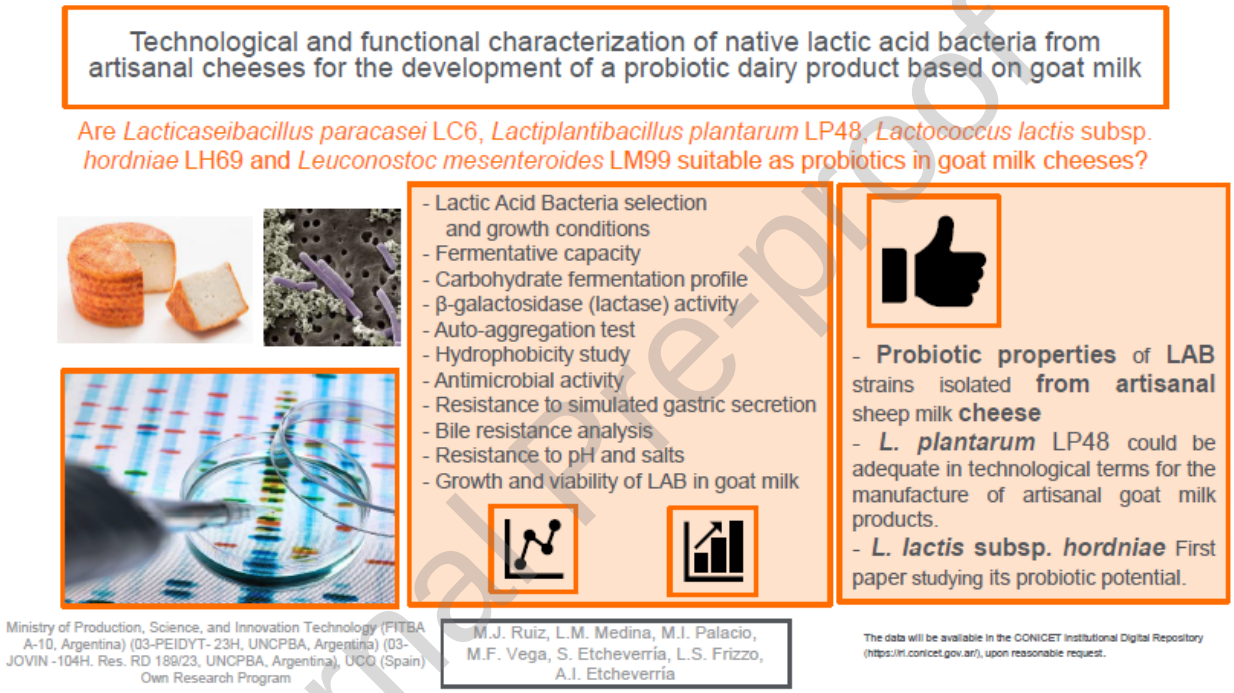
Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of interests

☒ 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.

Graphical abstract



Highlights

- Probiotic properties of LAB strains isolated from artisanal sheep milk cheese.
- *L. plantarum* LP48 could be adequate in technological terms for the manufacture of artisanal goat milk products.
- First study on *L. lactis subsp. hordniae* probiotic potential.