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Research article

Characterization of autochthonous lactobacilli from goat dairy products with probiotic potential for metabolic diseases

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HIGHLIGHTS

- New probiotic consortium (CRL1449, CRL1472, and *CRL1446) was selected.*
- The probiotic consortium showed *in vitro* immuno and adipomodulatory properties.
- *Lactobacillus delbrueckii subsp. indicus* CRL1447 was selected as a starter culture for fermented milk elaboration.
- Manufacturing of a functional fermented goat milk with a new probiotic consortium.

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ABSTRACT

The present study aimed to design functional fermented goat milk with probiotic potential for metabolic diseases. Thereby, autochthonous lactobacilli from goat dairy products that target improving the inflammatory, lipid, and glycemic profile were characterized. We designed fermented goat milk using *Lactobacillus delbrueckii* subsp. *indicus* CRL1447 as starter strain, supplemented with different probiotic consortia formed by *Limosilactobacillus fermentum* CRL1446, *Lactiplantibacillus paraplantarum* CRL1449, and CRL1472 strains. These lactobacilli were selected for their positive effects on inhibition of α-glucosidase, bile salts hydrolase activity, cholesterol assimilation, and decreased triglyceride percentage in *Caenorhabditis elegans*. Furthermore, the lactobacilli oral administration to obese mice caused a significant decrease in body weight gain and ameliorated hyperglycemia and hyperlipemia. These results reveal the potential of this goat dairy product as a functional food to prevent obesity and related pathologies. Goat milk-derived products stand out for their marketing potential. Hence, fermented goat milk incorporating novel probiotics represents a group of food products with broad prospects by their promising nutritive and therapeutic properties for metabolic diseases. The goat dairy product designed in this study could be used in the prevention of dyslipidemia and hyperglycemia in obese people.

1. Introduction

Significant scientific efforts are currently focused on the selection and characterization of probiotic cultures. In addition, several scientific investigations hold the importance of probiotics as part of a healthy diet for humans and animals (Shehata et al., 2016). According to the FAO/WHO, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001). The vast quantity of current knowledge with scientific proof of certain microorganisms strains with beneficial properties is why the broad and increasing global demand for probiotics.

Most bacteria used as probiotics are strains of lactic acid bacteria (LAB), mainly composed of species previously assigned to the genus *Lactobacillus*. However, a new taxonomic classification was proposed for this genus by Zheng et al. (2020), including the emended genus *Lactobacillus*, *Paralactobacillus,* and 23 novel genera. This new classification groups lactobacilli into clades that share ecological and metabolic properties. In this current nomenclature, *Lacticaseibacillus rhamnosus,*

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Lactiplantibacillus plantarum, Lacticaseibacillus casei, Limosilactobacillus fermentum, *Lactobacillus delbrueckii* subsp. *bulgaricus* are the most typical strains used as probiotics (Mulaw et al., 2019). It is known that different probiotics have extensive applications in treating, controlling, or preventing diseases in humans and animals (Bermudez-Brito et al., 2012). In this context, several authors have reported the beneficial anti-obesity effects of probiotics (Cani and Van Hul, 2015; Sivamaruthi et al., 2019). Indeed, positive effects of lactobacilli strains on the metabolism were demonstrated (Wang et al., 2020). Hence, they have been considered suitable for the management of obesity and related diseases. Furthermore, we previously reported hypocholesterolemic and hypoglycaemic effects, reduced leptin levels, and pro-inflammatory cytokines associated with the oral administration of *Limosilactobacillus fermentum* CRL1446 in the obesity mice model (Russo et al., 2020).

Considering the importance of probiotic selection criteria, we established a screening method for microorganisms with specific effects on metabolic diseases in this study. For selecting these beneficial strains, it is also fundamental to evaluate their technological properties in food matrices because they can be taken advantage of in the design of functional food formulations (Huys et al., 2013). In particular, dairy products are considered the primary sources for isolating probiotics (da Silva et al., 2019) and are the main carriers or delivery vehicles (Ranadheera et al., 2018). Among these dairy products, we can mention yogurts, fermented milk, cheeses, sour creams, among others (Attri et al., 2020). Because of its extensive consumption and healthy nutritional composition, milk has been widely used as a basis for developing functional foods. Their bioactive properties could be associated with the microorganisms themselves or with the metabolites resultant from the fermentation (Iraporda et al., 2014). Although the vast majority of probiotic milk drinks are prepared from bovine milk, other species of mammals (goat, sheep, camel, etc.) are being used due to their intrinsic nutritional composition (Turkmen et al., 2019). Currently, 13.5% of world non-bovine milk corresponds to goat milk production, and this milk differs from cow milk in having better digestibility, distinct alkalinity, higher buffering capacity, and specific therapeutic values in human medicine and nutrition (Ranadheera et al., 2019).

A recent trend in manufacturing functional food is the multi-strain combination since it may have advantages derived from the synergism between the different specific effects contributed by each of them (Ranadheera et al., 2016). Besides the above, many scientific reports highlighted the advantages of utilizing autochthonous starters to ferment different matrixes (dairy, vegetables, and fruits) to obtain related fermented products with advantageous technological and functional properties (Torres et al., 2020; Yasmin et al., 2020). Autochthonous microorganisms that carry probiotic properties could produce postbiotics that allow them to transform the fermented product into functional foods.

This work aimed to characterize the probiotic potential of eight lactobacilli strains isolated from goat dairy products. We select strains with the potential to modulate adipokine and cytokine production, triglyceride concentration, cholesterol assimilation, bile salts hydrolase, and α-glucosidase activity to elaborate functional fermented goat milk with the potential to be used for the prevention of dyslipidemia and glycemia in obese people.

2. Material and methods

2.1. Bacterial strains, genotypic characterization, and culture conditions

Eight lactobacilli strains previously isolated from goat's milk and cheese from Tucumán, Argentina (Oliszewski et al., 2006), were used in this study. These strains were molecularly identified by sequencing of the 16S rRNA gene (Table 1) and the respective sequences were deposited in the Culture Collection of the Centro de Referencia para Lactobacilos (CERELA-CONICET, Tucumán, Argentina). They were previously characterized for their technological properties and selected for having suitable metabolic activities (esterase activity, citrate utilization, production of esters) in milk (Medina et al., 2011). These potential probiotic strains are listed as follow: *Lacticaseibacillus rhamnosus* CRL1425 (CRL1425), *Lactiplantibacillus paraplantarum* CRL1427 (CRL1427), *Lactiplantibacillus paraplantarum* CRL1428 (CRL1428), *Lactiplantibacillus paraplantarum* CRL1449 (CRL1449), *Lactiplantibacillus paraplantarum* CRL1472 (CRL1472), *Lactiplantibacillus paraplantarum* CRL1430 (CRL1430), *Limosilactobacillus fermentum* CRL1446 (CRL1446), and *Lactobacillus delbrueckii* subsp. *indicus* CRL1447 (CRL1447) (Table 1). Bacteria were cultured in de Man Rogosa Sharpe (MRS) broth for 18 h at 37 °C in aerobiosis, except for the CRL1447 strain that was cultivated at 42 °C. Strains were maintained in MRS containing 20% (v/v) glycerol and stored at -80 °C until used.

Table 1. Lactobacilli strains included in this study.

Origin	Species	Strain	Accession number
Goat's cheese	*Lacticaseibacillus rhamnosus*	CRL1425	OM755575
Goat's milk	*Lactiplantibacillus paraplantarum*	CRL1427	OM755576
Goat's milk	*Lactiplantibacillus paraplantarum*	CRL1428	OM755577
Goat's milk	*Lactiplantibacillus paraplantarum*	CRL1449	OM755580
Goat's cheese	*Lactiplantibacillus paraplantarum*	CRL1472	OM755581
Goat's cheese	*Lactiplantibacillus paraplantarum*	CRL1430	OM755578
Goat's cheese	*Limosilactobacillus fermentum*	CRL1446	MF350659
Goat's milk	*Lactobacillus delbrueckii* subsp. *indicus*	CRL1447	OM755579

CRL: Centro de Referencia para Lactobacilos Culture Collection.

2.2. In vitro general probiotic properties

2.2.1. Resistance to simulated gastrointestinal tract (GIT) conditions

The strain's resistance to simulated gastric and intestinal juice was carried out according to Zárate et al. (2000). Briefly, pellets of active bacterial cultures were resuspended in artificial gastric juice and then in synthetic intestinal fluid with incubations at 37 °C for 2 h every step. Survival percentage was calculated using the following equation: Survival% = log CFU_f/log CFU_0 x 100, where log CFU_f represents the total viable cells after treatment and log CFU_0 represents the starting number of microorganisms.

2.2.2. Resistance to bile salts

The method proposed by Bao et al. (2010) was used to estimate strain tolerance to bile salts. MRS broth tubes supplemented with 0.3% (w/v) oxgall (Sigma-Aldrich, MO, USA) were inoculated with the tested strains. MRS without oxgall was used as control. Survival percentage was calculated using the following equation: Survival% = log $CFU_{MRSoxgall}$/log CFU_{MRS} x 100, where log $CFU_{MRSoxgall}$ represents the total viable cells after treatment with bile salts, and log CFU_{MRS} means the total number of viable cells in control.

2.2.3. Bacterial surface properties

The bacterial surface properties were studied following the methodology described by Maldonado et al. (2012). The hydrophobicity percentage (% H) was calculated using the following equation: % H = $[(A_0 - A_f)/A_0]$ x 100. A_0 and A_f are the OD_{600} before and after extraction with n-hexadecane, respectively. For the bacterial autoaggregation, the results were expressed as a percentage of autoaggregation (%A) = $[(A_0 - A_f)/A_0]$ x 100. A_0 and A_f are the OD_{600} before and after the 2 h. The score of hydrophobicity and auto-aggregation applied was high (60–100%), medium (30–60%), and low (0–30%).

2.2.4. Antagonistic activity of the lactobacilli strains

The antagonistic activities of cell-free supernatants (CFSs) of lactobacilli were determined using the method of Shehata et al. (2016). Tested pathogens were: *Salmonella* (*S) enterica* serotype Typhimurium (provided

by the Instituto de Microbiología de la Universidad Nacional de Tucumán, Argentina), *Listeria innocua* 12 (obtained from the Unité de Recherches Laitières et Génétique Appliquée, INRA, France) and *Escherichia (E.) coli* ATCC 700728™.

2.3. In vitro functional properties

2.3.1. Bile salts hydrolase (BSH) activity

Qualitative BSH activity was determined according to Sedláčková et al. (2016). Bacterial cell suspensions (5 μL) of each strain were spotted on the BSH selection medium (MRS agar supplemented with sodium salt at 0.5% (w/v) taurodeoxycholic acid (Sigma-Aldrich, MO, USA) and $CaCl_2$ 3 mM and incubated at 37 °C for 72 h. A precipitation zone around a colony indicates positive BSH activity of the strain.

2.3.2. Cholesterol assimilation

Cholesterol assimilation was determined using a modified method from Tomaro-Duchesneau et al. (2014). MRS broth supplemented with 100 μg ml^{-1} cholesterol-polyethylene glycol (PEG) 600 (Sigma-Aldrich, MO, USA) was inoculated with a bacterial cell suspension of each strain. After 24 h incubation, the remaining cholesterol was determined using Enzymatic Colestat kit (Wiener lab., Rosario, Argentina). The following equation was applied: % Assimilated cholesterol = $[(C_0–C_f)/C_0]$ x 100, where C_0 and C_f are initial and final cholesterol, respectively.

2.3.3. Inhibition of α-glucosidase (α-glu) activity

The inhibitory capacity of α-glu activity was determined using the method described by Li et al. (2016), using *p*-nitrophenyl α-*D*-glucopyranoside (*p*-NPG) as a substrate. The reaction mixture containing sterile phosphate-buffered saline (PBS) pH 6.5 (200 μl), *p*-NPG 20mM (8 μl), bacterial suspension (10 μl) and α-glu (0.17 U ml^{-1} (Sigma-Aldrich, MO, USA.) was incubated at 37 °C for 20 min. The amount of p-nitrophenol released was quantified by measuring OD_{405}.

2.3.4. Immunomodulatory capacity. Lactobacilli stimulation of macrophages and cytokine detection

Mouse macrophage cell line RAW 264.7 was used to evaluate the immunomodulatory capacity of different strains according to the methodology used by Fabersani et al. (2017). Briefly, Raw cells were stimulated with 1×10^7 CFU ml^{-1} of each strain (relation Raw cells/strain cells, 1:10) for 24 h. Lipopolysaccharide (LPS) purified from *E. coli* serotype 0111:B4 (Sigma-Aldrich, MO, USA) at a final concentration of 1 μg ml^{-1} and unstimulated Raw 264.7 cells were used as positive and basal controls of cytokine production, respectively. Cell culture supernatants were collected and stored at -80 °C until cytokine determination. Tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Monocyte chemoattractant protein-1 (MCP-1) were quantified using the CBA Mouse Soluble Protein Flex Set Kit (BD Bioscience, CA, USA).

2.3.5. Adipomodulatory capacity. Lactobacilli stimulation of murine adipocytes and leptin detection

Adipocytes of eight nine-week-old male C57BL/6 mice from the closed random-bred colony maintained at CERELA, were isolated by digestion of epididymal adipose tissue as previously described by Fabersani et al. (2017). Adipocyte stimulation assay and cytokine determination were performed as previously described for macrophages. Leptin concentration was determined in stimulated adipocyte supernatant by enzyme-linked immunoadsorption assay (ELISA) (DuoSet, R&D Systems, MN, USA).

2.4. In vivo functional properties

2.4.1. Effect of lactobacilli on triglyceride (TG) contents in caenorhabditis (C.) elegans

C. elegans wild-type N2 were propagated on a nematode growth medium (NGM, USBiological, USA) agar plates seeded with *E. coli* OP50 as standard food for nematode culture (Stiernagle, 2006). The methodology described by Yen et al. (2010) with modifications was used to extract and determine TG in nematodes. Briefly, synchronized worms (Porta de la Riva et al., 2012) were fed either with *E. coli* OP50 (control group) or a combination of *E. coli* OP50 and each lactobacilli strain in a ratio of 25:75. Incubation was performed at 20 °C until they reached stage L4/adult. The worms were collected and washed with PBS. The worm pellet was resuspended in a 5% solution of Triton X-100 (Sigma-Aldrich, MO, USA) and then sonicated. Lipids were solubilized by heating the lysate at 90 °C for 5 min, followed by centrifugation. The TG quantification kit (Wiener Lab, Rosario, Argentina) was used to analyze the TG content in the supernatant.

Futhermore, we also determinated the effect of lactobacilli mixes on TG contents in nematodes as described above. Mixes were developed with selected strains based on the other studied functional properties. These mixes were combined as follows: mixture 1 (Mix 1) was formed by CRL1446, CRL1449, and CRL1472. Mixture 2 (Mix 2) was formed by CRL1446 and CRL1449. Mixture 3 (Mix 3) was formed by CRL1446 and CRL1472. Mixture 4 (Mix 4) was formed CRL1449 and CRL1472.

2.4.2. Effect of oral administration of CRL1446, CRL1449, and CRL1472 in a diet-induced obese (DIO) mice

Adult male C57BL/6 mice were obtained from the closed random-bred colony maintained at CERELA. After the adaptation period (7 days), the mice were randomly separated into the following groups (n = 9 mice per group): (a) control group (Control) that was fed a standard diet (SD) and water *ad libitum*; (b) obese group (Ob) that was fed a high-fat diet (HFD) and drinking water *ad libitum*; (c) group that received HFD and a daily dose of CRL1446 (Ob + CRL1446); (d) group that received HFD and a daily dose of CRL1449 (Ob + CRL1449: HFD) and (e) group that received HFD and a daily dose of CRL1472 (Ob + CRL1472). The mice received a daily probiotic dose of each lactobacilli suspension in drinking water (1×10^8 CFU ml^{-1}). These feeding regimes were maintained throughout the experiment (10 weeks). The SD provided 3.21 kcal/g diet (Association of Argentine Cooperatives, Buenos Aires, Argentina) and the HFD provided 5.21 kcal/g diet.

Body weight was measured weekly. At the end of the study, animals fasted for 12 h and were subsequently anesthetized and sacrificed by cervical dislocation. Blood samples were collected in tubes containing EDTA, centrifuged to obtain plasma, and was kept at -20 °C to analyze metabolic parameters. Plasma glucose levels and total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density cholesterol (LDL), and triglycerides (TG) were carried out by enzymatic methods using commercial kits (Wiener Lab, Rosario, Argentina).

Previously and according to Levit et al. (2018), an acute oral toxicity study was performed to evaluate the probiotic's safety. After 7 days of treatment, livers and spleens of three mice from each group were homogenized, plated in Mac Conkey, BHI, and MRS agar plates, and incubated at 37 °C for 48 h. Results were expressed as positive or negative translocation.

2.5. Technological properties

2.5.1. Acidifying capacity

Acidifying capacity was determined following the methodology described by Ruiz Rodríguez et al. (2019) with modifications. Overnight culture strains were inoculated in 5 ml of 10% reconstituted skim goat milk powder (RSM) and incubated for 24 h at 37 °C or 42 °C. The pH of RSM was determined at 0, 4, 8, 12, and 24 h. Based on the pH curves obtained, the following rates were calculated: ΔpH8 = pH (0 h) – pH(8 h); ΔpH24 = pH (0 h) − pH (24 h); maximum acidification rate (V_{max}, speed of the pH decrease): the slope of the curve where the pH decrease is linear and maximum: $\Delta pH/\Delta t = (pH2-pH1)/(t2- t1)$.

2.5.2. Diacetyl production

Diacetyl production in MRS was determined according to Ruiz Rodríguez et al. (2019). Briefly, 2 ml of the culture was supplemented

with 1 ml of 4% (w/v) α-naphthol dissolved in isoamyl alcohol-ethanol (10:90) and 1 ml of KOH (30%, w/v) and incubated at 30 °C for 30 min. The results were qualitatively defined as negative (-), weak (+), medium (++), or strong (+++) production of diacetyl according to the intensity of the color of the red/pink ring formed.

2.5.3. NaCl resistance

Tolerance to NaCl salt of lactobacilli strains was determined by the technique developed by Prado et al. (2015). Each strain's active cultures were inoculated in MRS broth containing 4 or 10% NaCl and incubated for 24 h at 37 °C or 42 °C. The absence or presence of bacterial growth was evaluated by OD_{590} measurements at the beginning and at the end of the incubation.

2.6. Formulation of a probiotic goat milk

2.6.1. Elaboration of fermented goat milk (FGM) supplemented with the selected probiotic mixes

For goat milk (GM) fermentation, commercial GM powder (La Primera, Córdoba, Argentina) reconstituted in 10% (w/v) sterile distilled water was used. Sterile flasks were filled with 400 ml of reconstituted GM, and it was then pasteurized in a thermostatic bath at 90 °C for 15 min and cooled to 45 °C. The CRL1447 strain was used as the single starter for the fermentation of the GM. Eighteen-hour-old (overnight) cells cultivated in MRS broth at 37 °C were harvested by centrifugation, inoculated (4%, v/v) in pasteurized GM, and incubated at 42 °C for 8–10 h. These optimal growth conditions were previously established with pH monitoring up to 5.4–5.2. Four FGM were developed as follows: at the end of fermentation, Mix 1, Mix 2, Mix 3 and Mix 4 were added in a concentration of 1×10^8 CFU mL^{-1}. FGM without bacterial supplementation was used as the control. Aliquots of GM and FGM were stored at 4 °C for nutritional composition analysis.

2.6.2. Carbohydrates, organic acids and fatty acid methyl esters (FAMEs) profile in GM and FGM

The concentration of carbohydrates and organic acids was determined by high-performance liquid chromatography (UHPLC). Samples of GM and FGM were deproteinized using the methodology described by Vrancken et al. (2008). The separation of organic acids and saccharides was carried out by injecting 20 μl of the sample on an Aminex HPX-87H column (Bio-Rad Labs., USA). The chromatographic run was performed at a flow rate of 0.6 mL/min using 5 mM H_2SO_4 as mobile phase at a temperature of 45 °C and a pressure of ~50 bar. Based on comparing retention times with standards, the detection of organic acids and carbohydrates was carried out with a UV detector (210 nm) and a refractive index detector, respectively. Organic acids and sugar peaks were identified using commercial analytical standards as references (Sigma-Aldrich Chemical Co.).

Fatty acid methyl esters (FAMEs) were determined by gas chromatography coupled to a mass spectrometer (GC-MS). Samples of GM and FGM were derivatized in FAMEs following the protocol of Méndez Antolín et al. (2008). FAMEs were determined using a Thermo Scientific TRACE 1300 Mainframe MS 230V gas chromatograph with a Thermo Scientific ISQ mass detector. 0.5 μL of samples were injected at a flow rate of 1 mL/min. Injector, ion source, and connector temperatures were maintained at 250 °C, 200 °C, and 200 °C, respectively. The oven temperature was set at an initial temperature of 40 °C for 2 min, increased to 200 °C at a rate of 10 °C/min, and then held for 5 min. The concentration of individual fatty acids was quantified according to the peak area and expressed as a percentage of total fatty acids.

2.6.3. Microbiological analysis

The FGM and FGM supplemented with the different consortia were analyzed microbiologically at days 1 and 21, time corresponding to the shelf life (Ranadheera et al., 2016), to ensure the strains viability and hygienic conditions of milk. Serial dilutions were plated in agarized media. The following media and conditions were used: lactobacilli in MRS agar medium at 37 °C for 48 h, gram-negative bacteria in Mac Conkey agar at 37 °C for 48 h and fungi and yeasts in Sabouraud agar at 25–30 °C for 5–7 days. pH was determined in the different kinds of milk on days 1 and 21 with a digital pH meter (Altronix TPX I, NY, USA).

2.7. Statistical analysis

Statistical analyses and graphics were performed using XLSTAT 2018.6 (Addinsoft Company, Paris) and GraphPad Prism version 6.0 (GraphPad Software, Inc., CA, USA). Data were normally distributed, and significant differences were determined by applying one-way ANOVA followed by Tukey's test. p-values < 0.05 were considered statistically significant. Every assay in this study was performed in two independent experiments, and each parameter was determined in triplicate.

2.8. Ethics approval

The experimental mice protocol for adipocytes extraction, biochemical parameters determination, and bacterial translocation test complied with current Argentinean laws and was approved by CERELA's Ethical Committee of Animal Care (CRL-BIOT-EF-2012/2B).

3. Results and discussion

The selection of native bacteria with probiotic and technological properties is fundamental for developing functional dairy products. Although many strains are available on the market, it is desirable to characterize and select new native and safe starter and functional cultures, which may have unique properties for making novel fermented foods. The goat milk's bacteria, present as a beneficial microbiota, become a valuable source of strains with probiotic potential. In this work, we have screened and selected lactobacilli strains with probiotic phenotypic characteristics, which could be candidates for functional food.

3.1. In vitro general probiotic properties

3.1.1. Simulated GIT and bile salts resistance and adherence ability

According to Vinderola et al. (2017), despite a lack of standardized *in vitro* protocols, GIT and bile tolerance are functional properties that continue to be used as a suitable selection criterion for potential probiotic strain. Results of survival strains to the simulated GIT and bile salts tolerance were presented in Table 2. According to other authors, our results showed that these properties are variable and strain-dependent (Vinderola and Reinheimer, 2003). Survival at pH 3 is generally considered the absolute minimum necessary property for a probiotic strain to remain viable *in vivo* (Davoren et al., 2019). Although some studies consider 80% survival as a reasonable survival rate (Bao et al., 2010), *in vitro* static experiments might be much more inhibitory than *in vivo* gastric digestion (Vinderola et al., 2017); therefore, we used a survival rate above 70% as standard. Among studied lactobacilli strains, only CRL1425 and CRL1447 strains could not withstand the conditions of GIT (lower than 70%), being CRL1449 and CRL1472 the most tolerant to the simulated gastric juice (higher than 80%). Similarly, previous studies have shown better tolerance of gastric conditions for *Lactiplantibacillus plantarum* species than other potentially probiotic strains (Jitpakdee et al., 2020; Verón et al., 2017).

Another barrier that probiotics have to overcome is bile salts, which disorganize bacterial cell membranes (Ruiz et al., 2013). All strains, except CRL1447, showed considerable tolerance to bile salts within the normal physiological range found in the human intestine (0.1–0.3%) (Dunne et al., 2001). A similar response to bile salts was frequently observed in *Lactiplantibacillus plantarum, Lacticaseibacillus. rhamnosus*, or *Levilactobacillus brevis* (Jitpakdee et al., 2020; Verón et al., 2017). The microorganism's growth inhibition by bile salts is quite common, though it is known that the bile salt tolerance is a strain-specific feature. Some

Table 2. Viability and survival rate of autochthonous lactobacilli strains after in vitro simulated gastrointestinal tract and bile salts conditions.

	Resistance to Gastrointestinal Tract				Bile tolerance		
Strains	Saline solution†	Gastric solution pH3†	Intestinal solution pH8†	Survival rate (%)	MRS†	MRS+ Oxgall†	Survival rate (%)
CRL1425	8.88 ± 0.01^{a}	7.49 ± 0.03^{b}	5.30 ± 0.03^{c}	59.69	8.81 ± 0.01^{a}	8.67 ± 0.01^{a}	98.41
CRL1427	10.00 ± 0.01^{a}	8.72 ± 0.02^{b}	7.69 ± 0.02^{c}	76.99	9.72 ± 0.01^{a}	9.34 ± 0.01^{a}	96.09
CRL1428	9.00 ± 0.02^{a}	8.17 ± 0.01^{b}	7.00 ± 0.01^{c}	77.78	9.25 ± 0.01^{a}	9.03 ± 0.03^{a}	97.62
CRL1449	9.48 ± 0.01^{a}	9.46 ± 0.01^{a}	8.07 ± 0.01^{b}	85.13	9.79 ± 0.01^{a}	9.49 ± 0.02^{a}	96.94
CRL1472	9.48 ± 0.01^{a}	9.43 ± 0.01^{a}	8.00 ± 0.01^{b}	84.41	9.39 ± 0.01^{a}	9.27 ± 0.01^{a}	98.72
CRL1430	9.34 ± 0.01^{a}	7.40 ± 0.02^{b}	6.69 ± 0.01^{b}	71.63	9.34 ± 0.01^{a}	9.23 ± 0.01^{a}	98.82
CRL1446	9.33 ± 0.01^{a}	8.08 ± 0.02^{b}	6.88 ± 0.01^{c}	73.74	9.34 ± 0.01^{a}	9.24 ± 0.01^{a}	98.92
CRL1447	8.08 ± 0.02^{a}	8.00 ± 0.02^{a}	5.30 ± 0.03^{b}	65.61	9.45 ± 0.01^{a}	3.76 ± 0.02^{b}	38.41

† log CFU ml^{-1}. Data are expressed as mean ± standard error of the mean (SEM) of each strain determined in two independent experiments (n = 3). Values with different superscript letters in the same row are significantly different ($p < 0.05$) as assessed by Tukey's test.

research showed that bile salt resistance of some strains was related to their activity of BSH, which can hydrolyze combined bile salt and thus reduces its toxic and side effects (Bao et al., 2010).

The hydrophobicity and autoaggregation (cell surface properties) can predict their probiotic's ability to adhere to the epithelial cells of mucosal tissues (Polak-Berecka et al., 2014). The bacterial surface characterization results are shown in Table 3. The highest hydrophobicity values were presented by the strains CRL1446 and CRL1472 (30.52 and 31.35%, respectively). These values are lower than that observed in a commercial probiotic *Lacticaseibacillus rhamnosus* GG (approximately 70%) but higher than hydrophobicity values of others species of lactobacilli, *Lactiplantibacillus plantarum* S-811 and *Lactiplantibacillus plantarum* DGK-17 (Khan and Kang, 2016; Verón et al., 2017). All strains presented a low autoaggregation score (<30%). The highest autoaggregation value was observed in the CRL1472 strain (14.30%).

3.1.2. Antagonistic activity of the lactobacilli strains

Lactobacilli species are used as probiotics because they can modulate the intestinal microbiota and prevent the colonization and growth of pathogenic bacteria in the GIT. These bacteria are characterized by producing a wide range of antibacterial compounds, including sugar catabolites (such as organic acids), oxygen catabolites, and proteinaceous compounds (de Melo Pereira et al., 2018). Strain CRL1447 did not inhibit the growth of any of the studied pathogenic bacteria. Strains CRL1430 and CRL1446 did not induce the growth inhibition of *Salm.* Typhimurium. The other strains inhibited the growth of all studied pathogenic bacteria (Table 3). These results agree with the extensive information concerning lactobacilli's antimicrobial activity. In particular, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus,* and *Limosilactobacillus fermentum* have proved an inhibitory effect against Gram-negative and Gram-positive pathogens (de Melo Pereira et al., 2018).

3.2. In vitro functional properties

3.2.1. BSH activity, cholesterol assimilation, and inhibition of α-glu activity

Table 4 shows the results of BSH activity, cholesterol assimilation, and inhibition of α-glu activity**.** BSH is the enzyme produced by intestinal microorganisms responsible for 5–10% of the bile salt's deconjugation (Jeun et al., 2010). This enzyme is a critical component of the removal cholesterol mechanism, which enables the reduction of blood cholesterol. LAB carrying BSH can disrupt the cholesterol micelle's formation and prevent its absorption by decomposition of bile salts (Lye et al., 2010a). Among the different probiotic properties of bacteria, the hypocholesterolemic effect is of great significance for maintaining human health (FAO/WHO, 2001). Although some authors consider that BSH cannot be produced by bacteria isolated from environments without bile salts (Sedláčková et al., 2016), several strains isolated from non-bile salts environments may also possess BSH activity. Effectively, all strains studied in this work were isolated from goat milk and have BSH activity, except for CRL1447, which was also unable to grow in this selective medium.

Furthermore, probiotics can lower blood cholesterol through bacterial assimilation, reducing intestinal absorption availability (Lye et al., 2010a). In this sense, apart from the ability to deconjugate bile salts, all studied strains successfully assimilated cholesterol. The assimilation ability ranging from 27.49% (CRL1425) to 59.48% (CRL1449). These strains could assimilate cholesterol at similar levels to those reported for LAB isolated from goat cheese (de Oliveira et al., 2021).

Several *in vitro* and *in vivo* studies showed that some LAB strains inhibit the α-glu enzyme (Chen et al., 2014a; Li et al., 2016a). This enzyme hydrolyzes glycosidic bonds, releasing glucose in the gut, and therefore increasing postprandial hyperglycemia. Hence, its inhibition decreases the absorption of carbohydrates in the intestine and reduces blood glucose

Table 3. Bacterial surface properties and antagonistic activity of autochthonous lactobacilli strains.

	Bacterial surface properties†		Antagonistic activity‡		
Strains	Hydrophobicity (%)	Autoaggregation (%)	*S. typhimurium*	*E. coli* O 157:H7	*List. innocua 12*
CRL1425	25.99 ± 2.59^{b}	3.94 ± 1.94^{bc}	-	-	-
CRL1427	15.13 ± 1.80^{a}	2.96 ± 0.02^{ab}	-	-	-
CRL1428	16.96 ± 2.44^{a}	6.62 ± 0.74^{cd}	-	-	-
CRL1449	14.38 ± 4.38^{a}	0.07 ± 0.02^{a}	-	-	-
CRL1472	31.35 ± 0.47^{b}	14.30 ± 1.26^{e}	-	-	-
CRL1430	14.30 ± 2.62^{a}	3.08 ± 1.08^{ab}	+	-	-
CRL1446	30.52 ± 0.52^{b}	3.06 ± 0.94^{ab}	+	-	-
CRL1447	26.25 ± 1.76^{b}	7.52 ± 0.18^{d}	+	+	+

† Data are expressed as mean ± SEM of each strain determined in two independent experiments (n = 3). Values with different superscript letters in the same column are significantly different ($p < 0.05$) as assessed by Tukey's test.

‡ (+) growth or (-) growth inhibition of the pathogenic strain.

Table 4. *In vitro* functional properties of autochthonous lactobacilli strains.

Strains	BSH activity[†]	Cholesterol assimilation (%)[‡]	α-glu inhibitory activity[‡]
CRL1425	++	27.49 ± 3.94[a]	ND
CRL1427	+++	51.18 ± 0.97[bc]	ND
CRL1428	+++	47.68 ± 2.24[bc]	ND
CRL1449	++++	59.48 ± 1.97[d]	49.71 ± 5.01[a]
CRL1472	+++	53.77 ± 1.40[cd]	45.79 ± 5.00[a]
CRL1430	+++	45.21 ± 2.49[b]	ND
CRL1446	++	48.28 ± 1.56[bc]	97.28 ± 0.70[b]
CRL1447	-	ND	ND

[†] (-) negative, (+) weak, (++) medium or (++ +) strong BSH activity.
[‡] Data are expressed as mean ± SEM of each strain determined in two independent experiments (n = 3). Values with different superscript letters in the same column are significantly different ($p < 0.05$) as assessed by Tukey's test. (ND: Not determined).

levels after an intake. Recently, Russo et al. (2016) demonstrated that CRL1446 shows hypoglycemic capacity in the metabolic syndrome mice model. This effect could be due to the high inhibition of α-glu induced by this strain (97%), as observed in the present study. The CRL1449 and CRL1472 strains inhibited enzyme activity by 50%.

3.2.2. Immuno- and adipo-modulatory capacity

Beyond doubt, the relation between metabolic disorders such as obesity and metabolic syndrome with chronic low-grade inflammation is known. In these pathologies, the cytokines play a pivotal role. Extensive evidence has highlighted the benefits of probiotics in metabolic disorders by modulating gut microbiota and ameliorating host immune status (Torres et al., 2019). We evaluated the ability of the lactobacilli to modulate the pro- and anti-inflammatory molecules' secretion involved in metabolic inflammation (TNF-α, MCP-1, IL-6, IL-10, and leptin). Figures 1a and 1c show that all strains decreased the secretion of the pro-inflammatory TNF-α (except CRL1428) and IL-6 molecules in macrophages compared to that triggered by LPS stimuli. However, this decline in cytokine values did not reach the ones of the basal controls. The secretion of the pro-inflammatory chemokine MCP1 did not undergo significant changes (Figure 1b). Regarding the effect on anti-inflammatory cytokine IL-10, all strains, except for CRL1446 and CRL1447, showed levels similar to basal cytokine production. These strains induced IL-10 values higher than the basal control of production (Figure 1d). In adipocyte cells, most strains induced an intermediate production level of TNF-α, MCP-1, and IL-6 (Figures 2a, 2b, 2c), significantly different from LPS stimulus. Regarding leptin production by adipocytes, CRL1427, CRL1428, and CRL1430 were able to induce similar levels to LPS stimulus. Otherwise, CRL1446, CRL1447, CRL1449, and CRL1472 displayed similar values to baseline control (Figure 2 d). These values are in agreement with previous outcomes obtained in mouse adipocytes or macrophage cell lines stimulated with *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus, and Lactiplantibacillus plantarum*, which induced reduction of IL-6, TNF-α, and MCP-1 secretion (Fabersani et al., 2017). Furthermore, these results showed that the inflammatory modulation is strain-dependent due to differences observed in the inflammatory profile among tested strains.

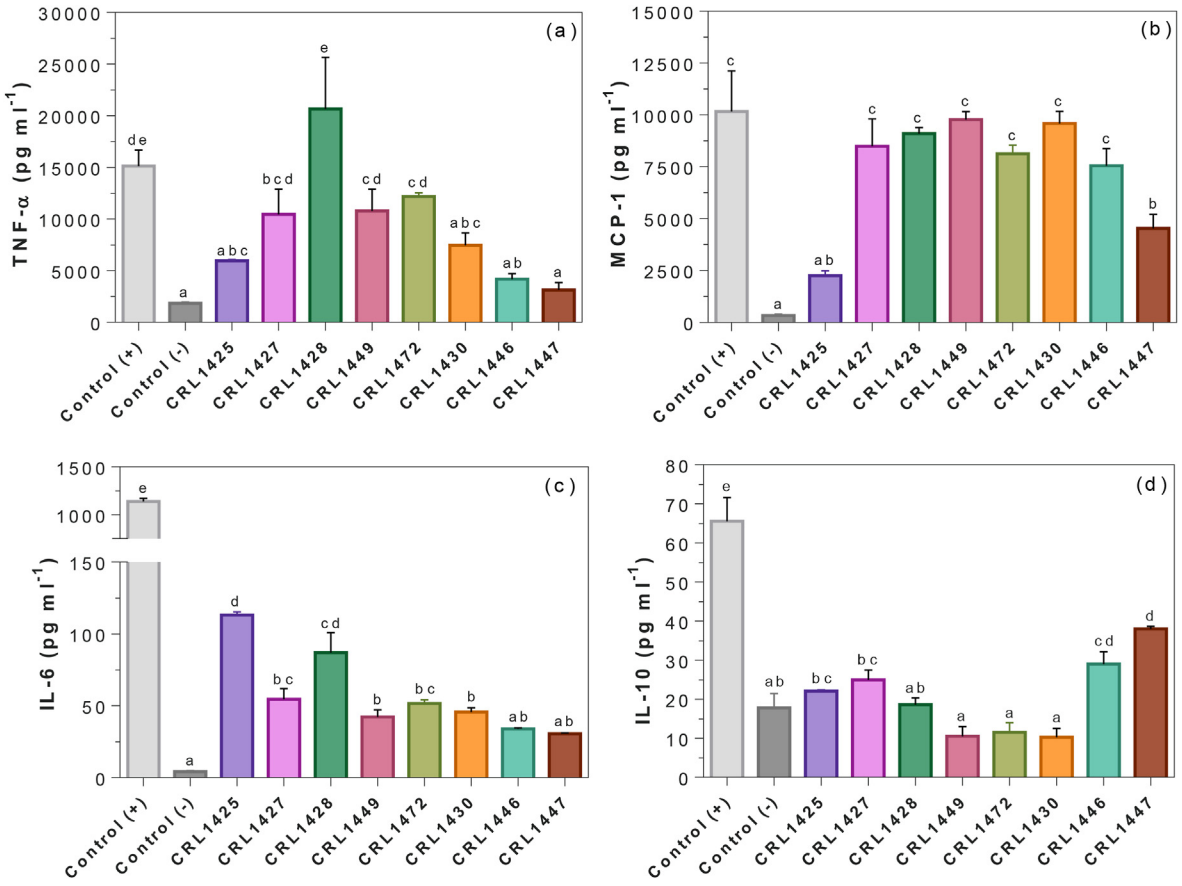

Figure 1. Anti- and pro-inflammatory cytokines/chemokine produced by macrophages (RAW 264.7) stimulated with lactobacilli strains. (a) TNF-α, (b) MCP-1, (c) IL-6, and (d) IL-10 production levels by macrophages. The cells were stimulated with CRL1425, CRL1427, CRL1428, CRL1449, CRL1472, CRL1430, CRL1446 and CRL1447 strains. Control (-): macrophages without stimulation. Control (+): macrophages stimulated with LPS. Data are expressed as mean ± standard error of the mean (SEM) of each strain determined in two independent experiments (n = 3). Different letters indicate significant differences ($p < 0.05$) between levels of cytokine/chemokine production for different stimuli [strains, control (+) and control (-)], as assessed by Tukey's test.

According to the cytokines secretion induced in macrophages, the principal component analysis (PCA) (Figure 3a) revealed an association between strains with similar behavior, which allowed a separation into three groups. Cluster I included CRL1425, CRL1446, and CRL1447; these strains had a low inflammatory profile near the negative control, representing the baseline cytokine secretion. Cluster II included CRL1430 and CRL1427, CRL1449, and CRL1472, with a medium inflammatory profile. Cluster III only comprised CRL1428, which is positioned distinctively from the other strains due to their higher inflammatory profile.

According to the studied variables, the PCA performed with cytokines and leptin production by adipocytes revealed two different lactobacilli groups with varying capacities for modulating adipokine production (Figure 3b). Cluster I include CRL1427, CRL1428, and CRL1430 strains, which represent the intermediate cytokine production. In cluster II, we observed strong correlations between CRL1425, CRL1446, CRL1447, CRL1449, and CRL1472. This last group also includes the control with basal production of adipokines. The positive control is far from all strains, indicating that the studied strains are not highly inflammatory like LPS. It is known that high levels of cytokines and leptin favor the state of obesity-associated chronic inflammation. In this context, the use of microorganisms with low and middle inflammatory properties and the ability to modulate leptin levels could be a strategy for treating metabolic diseases (Fabersani et al., 2017).

3.3. *In vivo functional properties*

3.3.1. *TG reduction in C. elegans fed with individual and mixes of lactobacilli strains*

The involvement of the studied LAB in fat metabolism was investigated using *C. elegans*. This nematode is regarded as a simple model to assess the *in vivo* effects of probiotics, especially concerning the study of fat metabolism due to its ability to store lipids in intestinal and skin-like hypodermal cells (Schifano et al., 2020). The main constituents in fat droplets stored in this nematode are TG, and so, lipid accumulation in *C. elegans* has been associated with an increase in TG (Zhang et al., 2011). Figure 4a shows the TG values in nematodes grown up with different lactobacilli strains. CRL1427 and CRL1428 strains did not change TG concentration compared to the control (nematodes fed with *Escherichia coli* OP50). However, a significant TG level reduction was observed when nematodes were grown up with the other strains. Our results align with other studies that report an anti-obesity effect of some lactobacilli species (Park et al., 2013; Savcheniuk et al., 2014). In recent research carried out with *Lactiplantibacillus plantarum*-fermented barley β-glucan, the fat deposition reduction was associated with increased energy consumption and an increase in nematodes' locomotive behavior (Xiao et al., 2020). Furthermore, these authors considered that probiotics affect lipid accumulation in *C. elegans* in different pathways, such as activating fatty acids β-oxidation. Considering previous results of the anti-cholesterolemic and hypoglycemic effects of CRL1446 in mice with metabolic syndrome (Russo et al., 2020) and taking the studied properties into account in this

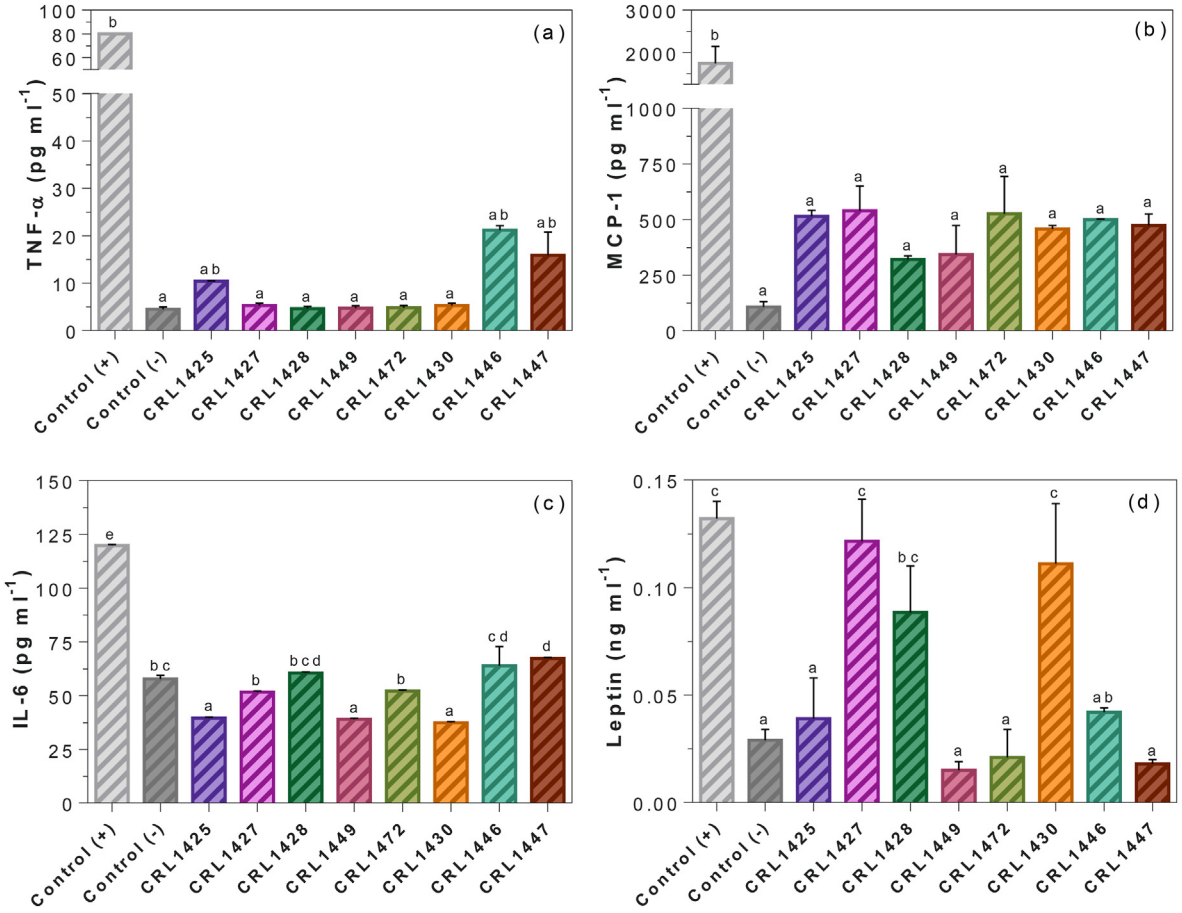

Figure 2. Leptin, anti-and pro-inflammatory cytokines/chemokine produced by adipocytes stimulated with lactobacilli strains. **(a)** TNF-α, **(b)** MCP-1, **(c)** IL-6, and **(d)** Leptin production levels by adipocytes. The cells were stimulated with CRL1425, CRL1427, CRL1428, CRL1449, CRL1472, CRL1430, CRL1446 and, CRL1447. Control (-): adipocytes without stimulation. Control (+): adipocytes stimulated with LPS. Data are expressed as mean ± SEM of each group determined in two independent experiments (n = 3). Different letters indicate significant differences ($p < 0.05$) between levels of cytokine/chemokine production for different stimuli [strains, control (+) and control (-)], as assessed by Tukey's test.

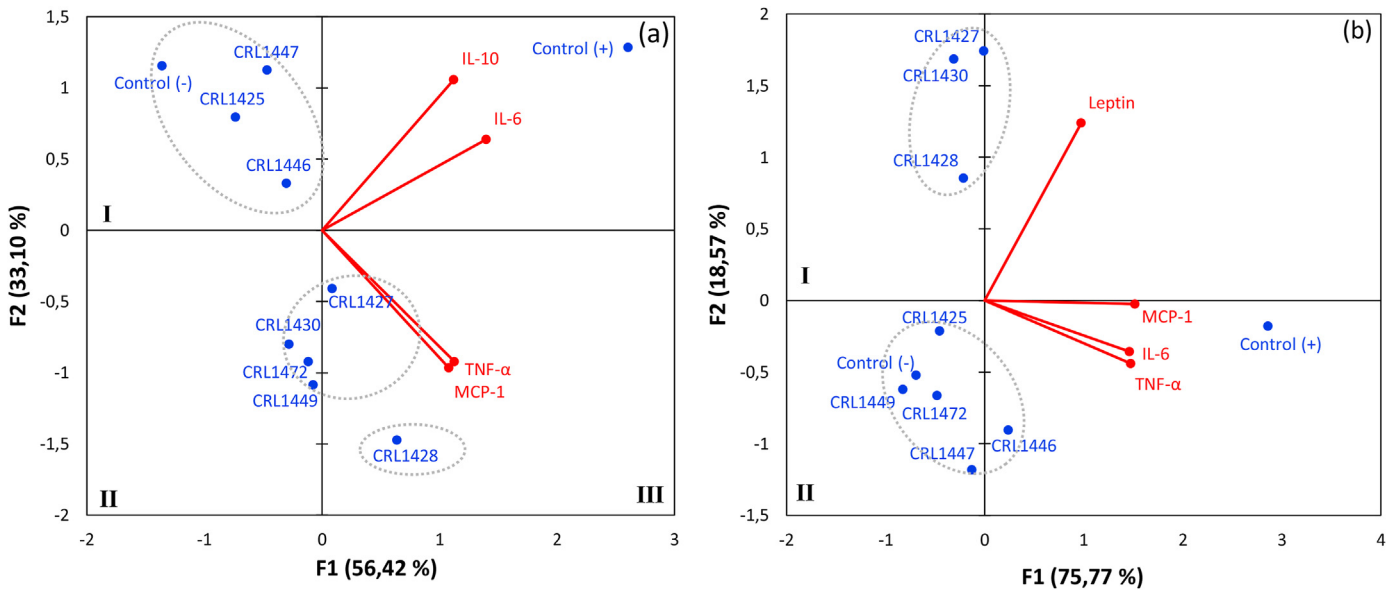

Figure 3. Principal component analysis (PCA) based on data regarding inflammatory features of the lactobacilli strains. The PCA biplot was obtained from **(a)** the levels of TNF-α, MCP-1, IL-6, and IL-10 produced by macrophages and **(b)** the levels of TNF-α, MCP-1, IL-6, and leptin produced by adipocytes stimulated with CRL1425, CRL1427, CRL1428, CRL1449, CRL1472, CRL1430, *CRL*1446, and CRL1447. The points for each strain and the controls mean (•) Active variables and (•) Active observations. The position of some points was slightly modified to avoid the overlapping of the labels.

work, we decided to combine this strain with CRL1449 and CRL1472. Mix 1 (CRL1446, CRL1449, and CRL1472), Mix 2 (CRL1446 and CRL1449), and Mix 3 (CRL1446 and CRL1472) caused a lower TG content than the nematode control. However, no significant differences were observed between these mixes (Figure 4b). Mix 4 (CRL1449 and CRL1472) did not induce any change compared with nematode control. These *in vivo* results indicate that CRL1446 could be the strain most effective in reducing TG in obesity.

3.3.2. Body weight gain and biochemical parameters in diet-induced obesity mice fed with CRL1446, CRL1449 and CRL1472

A preliminary study was conducted to evaluate the effect of CRL1446, CRL1449, and CRL1472 in a model of DIO (Table 5). The body weight gain (BWG) of the Ob group was 40% higher than the Control group, and it decreased by 19, 14, and 15% with the administration of CRL1446, CRL1449, and CRL1472, respectively, reaching values similar to the Control group. The glucose values of the Ob group were 41% higher compared to the Control group. These values decreased by 36, 34, and 22% with the administration of CRL1446, CRL1449, and CRL1472, respectively. Increased blood glucose levels often result in diabetes (Namekawa et al., 2017), so it is crucial to control blood glucose in the early treatment of diabetes. Decreased glucose absorption and circulation can be achieved by inhibiting α-glu (Chen et al., 2014b; Li et al., 2016b). This work demonstrated that CRL1446 induced a high inhibition of α-glu *in vitro* (97%), whereas CRL1449 and CRL1472 strains inhibited the enzyme activity by 50%. Therefore, both results *in vitro* and *in vivo* suggested that these strains could significantly increase glucose tolerance. Regarding lipid profile, compared with the Control group, TG levels increased 2 fold in the Ob group, and these values were significantly reduced by 22, 18, and 20% when administered CRL1446, CRL1449, and CRL1472, respectively. The TC levels in the Ob group increased by 33% compared to the animals in the Control group, while in the Ob + CRL1446 group mice, TC levels were significantly reduced by 18% compared with the Ob group. LDL values were 1.8 times higher in the Ob group than the Control group, and no significant differences were observed between the different treatment groups regarding the Ob group.

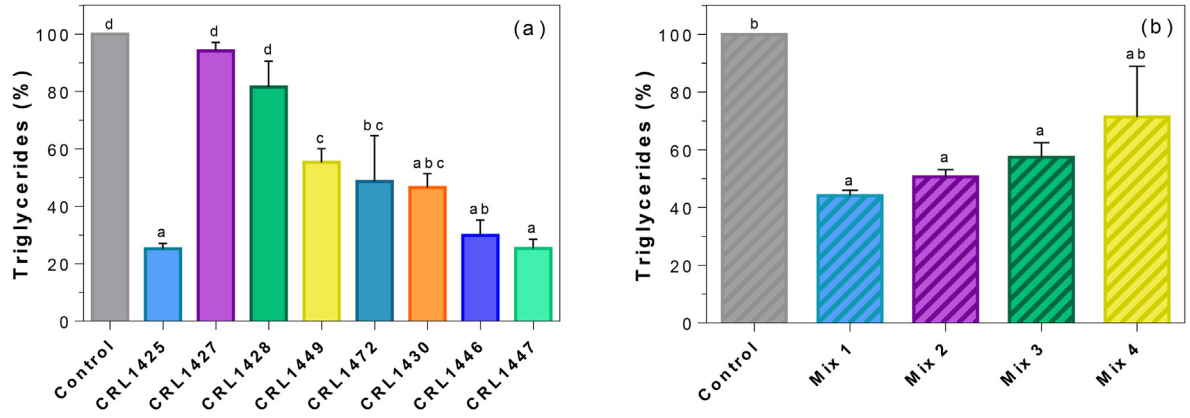

Figure 4. Effects of lactobacilli strains on triglyceride (TG) content of *C. elegans*. The nematode was fed with **(a)** CRL1425, CRL1427, CRL1428, CRL1449, CRL1472 CRL1430, CRL1446 and CRL1447 and **(b)** Mix 1: CRL1446, CRL1449 and CRL1472; Mix 2: CRL1446 and CRL1449; Mix 3: CRL1446 and CRL1472 and Mix 4: CRL1449 and CRL1472. The content of TG quantified to the control (*C. elegans* fed with *E. coli* OP50) represents 100%. Data are expressed as mean (percentage) ± SEM of two independent experiments (n = 3). Different letters indicate significant differences between TG contents levels of (a) strains and control and (b) mixes and control ($p < 0.05$), as assessed by Tukey's test.

Table 5. Effect of CRL1446, CRL1449 and CRL1472 administration on body weight gain, glucose and lipid profile in diet-induced obesity mice.

Groups	BWG[†]	Glucose[‡]	TG[‡]	TC[‡]	LDL[‡]	HDL[‡]
Control	7.63 ± 0.28^{a}	0.95 ± 0.05^{a}	0.28 ± 0,02^{a}	0.46 ± 0.01^{a}	0.23 ± 0.02^{a}	0.11 ± 0.01^{ab}
Ob	10.68 ± 0.58^{c}	1.34 ± 0.06^{b}	0.54 ± 0.01^{c}	0.61 ± 0.05^{b}	0.41 ± 0.03^{b}	0.10 ± 0.01^{a}
Ob + CRL1446	8.60 ± 0.42^{ab}	0.86 ± 0.10^{a}	0.42 ± 0.03^{b}	0.50 ± 0.03^{a}	0.32 ± 0.06^{ab}	0.17 ± 0.02^{c}
Ob + CRL1449	9.20 ± 0.40^{b}	0.88 ± 0.04^{a}	0.44 ± 0.04^{b}	0.55 ± 0.01^{ab}	0.42 ± 0.04^{b}	0.15 ± 0.02b^{c}
Ob + CRL1472	9.03 ± 0.46^{ab}	1.05 ± 0.10^{a}	0.43 ± 0.02^{b}	0.53 ± 0.03^{ab}	0.41 ± 0.004^{b}	0.13 ± 0.01^{abc}

[†] Body weight gain (g). [‡]TG: triglycerides; TC: total cholesterol; LDL: high-density lipoprotein cholesterol; LDL: low-density cholesterol (g l^{-1}). Data are expressed as mean ± SEM. Values with different superscript letters in the same column are significantly different ($p < 0.05$) as assessed by Tukey's test.

Regarding HDL levels, values in the Ob + CRL1446 and Ob + CRL1449 groups were higher than those observed in the Control group. High cholesterol levels are commonly associated with dyslipidemia in DIO models (Namekawa et al., 2017). Several studies reported that probiotic products could lower total plasma TC and LDL-cholesterol levels (Russo et al., 2020). This cholesterol-lowering capacity could be due to different mechanisms such as bile salt deconjugation and membrane assimilation (Lye et al., 2010b). In this study, CRL1446, 1449, and 1472 showed *in vitro* BSH activity and cholesterol-lowering ability.

To incorporate these strains into food, they must be innocuous. The translocation test was performed for CRL1446, CRL1449, and CRL1472, selected by their functional properties. These lactobacilli strains were not detected in any of the organs studied (data no shown). Therefore, it is considered that the strains are innocuous because they did not favor the colonization of intestinal bacteria at the extra-intestinal level in the acute security test in mice.

3.4. Technological properties

The acidification rate, diacetyl production, and resistance to NaCl were evaluated to study the performance of strains as starters or adjunct cultures in functional dairy products (Table 6). In the manufacturing of fermented dairy products, it is preferred to use autochthonous than allochthonous starter cultures since native bacteria have a lower latency phase of growth and are better at acidifying the matrix (Torres et al., 2019). Therefore, in selecting starter cultures, bacteria capable of rapidly lowering the pH of the food matrix at pH values below 4.5 are meaningful in inhibiting the growth of undesirable microorganisms at the beginning of the fermentation process. Fast acid-producing LAB strains are preferred and frequently selected as starters for milk or fermented vegetable products (Medina et al., 2001). Of the strains evaluated in this work, CRL1447 presented the highest acidification rate at 8 h, detecting a ΔpH of 1.9 (Vmax 0.23). The rest of the strains were slow acidifiers (Vmax 0.06–0.11). Some lactobacilli species can ferment citrate present in milk, leading to the biosynthesis of aroma compounds, such as diacetyl, which positively impact fermented dairy products' flavor (Smid and Kleerebezem, 2014). Under these considerations, the biosynthesis of diacetyl in milk was tested in the studied strains, and only CRL1446 and CRL1447 strains could not produce diacetyl. The starter or adjunct cultures used in cheesemaking must tolerate high osmotic pressures generated by the addition of NaCl during the manufacture of the cheeses. The lactobacilli strains evaluated were only able to grow in the presence of 4% NaCl and did not show growth at 10% NaCl. Resistance to salt is an evident technological requisite for cheese cultures (Briggiler-Marcó et al., 2007). However, salt sensitivity to moderate salt concentrations is also appreciated (Yanachkina et al., 2020). Similar to the salt-tolerance results of studied LAB were observed in *Lactococcus lactis* strains (starters in low salt cheese manufacture) (Yanachkina et al., 2020).

3.5. Fermented goat milk analysis

3.5.1. Nutritional composition of GM and FGM

The strain CRL1447 used to ferment GM increased one logarithmic unit during fermentation, reaching an approximate growth of 7.4 log CFU ml^{-1} after 10 h of incubation, with a decrease in pH from 6.62 to 5.18 (Figure 5). Table 7 shows the pH, carbohydrates, organic acids, and fatty acids of GM and FGM. Regarding the analysis of sugar consumption in milk, low glucose levels were found (<0.1%), while lactose was the mayor sugar detected in the medium. The basal level of lactose in milk is 2.9%, and after fermentation showed a concentration of 2.4%. These results indicated that fermentation was achieved based on lactose consumption since it decreased significantly by 16% concerning unfermented goat milk. Lactose consumption produced a decrease in pH values, from 6.62 to 5.18, which is associated with the production of organic acids during fermentation. High lactic acid production (100 times higher than unfermented milk), and small acetic acid production

Table 6. Technological properties of autochthonous lactobacilli strains.

	Acidifying parameters in milk[†]			Diacetyl production[‡]	Growth in MRS+ NaCl[§]	
Strains	ΔpH8	ΔpH24	Vmax (ΔpH h^{-1})		4 % (w/v)	10% (w/v)
CRL1425	0.75 ± 0.14^{b}	1.70 ± 0.32^{b}	0.10 ± 0.02^{b}	+++	+	-
CRL1427	0.50 ± 0.12^{b}	1.20 ± 0.27^{b}	0.06 ± 0.01^{b}	++	+	-
CRL1428	0.63 ± 0.10^{b}	1.60 ± 0.26^{b}	0.09 ± 0.03^{b}	++	+	-
CRL1449	0.80 ± 0.07^{b}	1.20 ± 0.12^{b}	0.08 ± 0.04^{b}	++	+	-
CRL1472	0.90 ± 0.14^{b}	1.60 ± 0.21^{b}	0.11 ± 0.03^{b}	++	+	-
CRL1430	0.52 ± 0.14^{b}	1.50 ± 0.17^{b}	0.09 ± 0.02^{b}	+++	+	-
CRL1446	0.90 ± 0.21^{b}	1.40 ± 0.30^{b}	0.11 ± 0.03^{b}	-	+	-
CRL1447	1.90 ± 0.20^{a}	2.55 ± 0.15^{a}	0.23 ± 0.05^{a}	-	+	-

[†] Data are expressed as mean ± SEM of each strain determined in two independent experiments (n = 3). Values with different superscript letters in the same column are significantly different ($p < 0.05$) as assessed by Tukey's test.

[‡] (-) negative, (+) weak, (++) medium or (+++) strong diacetyl production.

[§] (-) negative or (+) positive growth in NaCl.

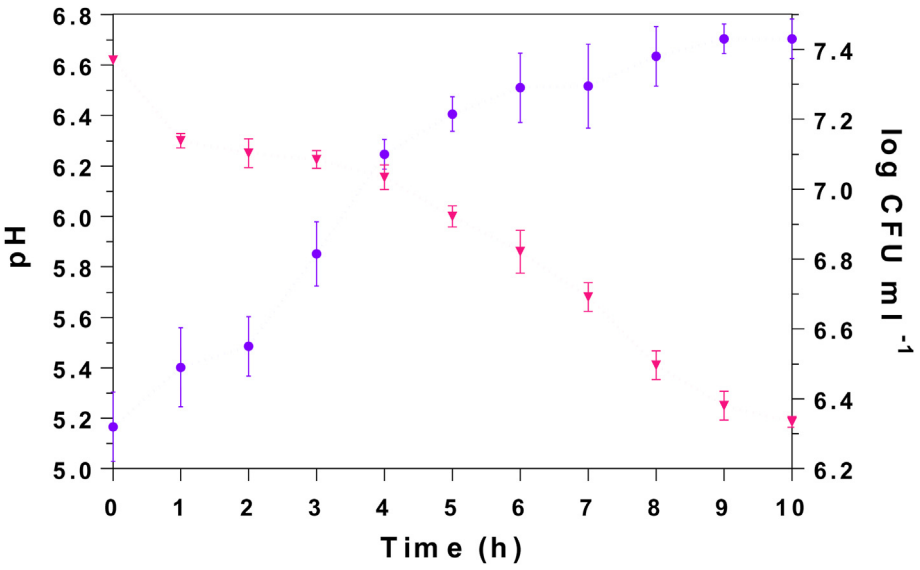

Figure 5. Viable CRL1447 cell count (●) and pH measurement (▾) during goat milk fermentation with CRL1447 strain. Data are expressed as mean ± SEM of two independent experiments (n = 3).

were observed. Lactic acid plays an essential role as a natural preservative in fermented dairy products and contributes to sensory properties; in contrast, large amounts of acetic acid result in a vinegar flavor, decreasing consumer acceptability (Rodrigues et al., 2011). The propionic acid concentration was lower than 0.1% in both samples.

The fatty acid profile for the two samples reveals a wide variety of saturated fatty acids from the C8: 0 to C18: 0 series. The primary fatty acid present in the samples was palmitic acid (C16: 0) (33.3 and 33.1% in GM and FGM, respectively), followed by capric acid (C10: 0) (22.4 and 22.7% in GM and FGM, respectively). Significant higher levels of myristic (C14: 0) and oleic acid (C18:1 n-9) were observed in FGM (14.1 and 7.3%, respectively) related to GM (11.9 and 6.2%), whereas caprylic (C8: 0) and stearic (C18: 0) acids were higher in GM. Lauric acid (C12: 0) percentage was similar in the FGM and GM. The most acceptable fatty acid composition characterizes goat milk in terms of healthy nutrition, and its lipid components can have many benefits in preventing atherosclerosis (Voblikova et al., 2020). Among unsaturated fatty acids, oleic acid plays an essential role in preventing cardiovascular diseases (Perdomo et al., 2015).

Table 7. Nutritional composition of GM and FGM by CRL1447 strain.

	GM	FGM
pH	6.62 ± 0.01[b]	5.17 ± 0.02[a]
Carbohydrates (% w/v)		
Lactose	2.90 ± 0.07[b]	2.44 ± 0.06[a]
Glucose	<0.1	<0.1
Galactose	ND	ND
Organic acids (mM)		
Lactate	0.20 ± 0.01[a]	26.65 ± 0.65[b]
Acetate	0.10 ± 0.0[a]	1.00 ± 0.02[b]
Propionate	<0.1	<0.1
Fatty acids[†] (g/100g FAMEs)		
C8:0	5.4 ± 0.2[b]	3.5 ± 0.1[a]
C10:0	22.4 ± 0.5[a]	22.7 ± 0.6[a]
C12:0	8.2 ± 0.4[a]	9.3 ± 0.6[a]
C14:0	11.9 ± 0.2[a]	14.1 ± 0.4[b]
C16:0	33.3 ± 0.8[a]	33.1 ± 0.7[a]
C18:1 n-9	6.2 ± 0.1[a]	7.3 ± 0.2[b]
C18:0	13.3 ± 0.3[b]	10.8 ± 0.3[a]

[†] C8:0 caprylic acid; C10:0 capric acid; C12:0 lauric acid; C14:0 myristic acid; C16:0 palmitic acid; C18:1 n-9 oleic acid; C18:0 stearic acid. Data are expressed as mean ± standard error of the mean (SEM) (n = 3). Values with different superscript letters in the same row are significantly different ($p < 0.05$) as assessed by Tukey's test. GM: goat milk; FGM: fermented goat milk; ND: not detected.

Table 8. Viable cell count and pH in FGM and FGM supplemented with different Mixes stored 21 days at 4 °C.

	Day 1		Day 21	
Sample[†]	Viable cells[‡]	pH	Viable cells[‡]	pH
FGM	7.43 ± 0.07[a]	5.18 ± 0.01[a]	7.28 ± 0.09[a]	5.07 ± 0.05[a]
FGM + Mix 1	8.60 ± 0.13[a]	5.15 ± 0.03[b]	8.41 ± 0.06[a]	4.98 ± 0.01[a]
FGM + Mix 2	8.46 ± 0.07[a]	5.17 ± 0.02[a]	8.25 ± 0.04[a]	5.08 ± 0.03[a]
FGM + Mix 3	8.37 ± 0.06[a]	5.20 ± 0.02[a]	8.07 ± 0.09[a]	5.06 ± 0.02[a]
FGM + Mix 4	8.32 ± 0.04[a]	5.16 ± 0.02[a]	8.12 ± 0.09[a]	5.18 ± 0.03[a]

[†] FGM: fermented goat milk; .Mix 1:CRL1446, CRL1449 and CRL1472; Mix 2: CRL1446 and CRL1449; Mix 3: CRL1446 and CRL1472; Mix 4: CRL1449 and CRL1472. [‡]log CFU ml^{-1}. Data are expressed as mean ± standard error of the mean (SEM) (n = 3). Values with different superscript letters for each parameter in the same row are significantly different ($p < 0.05$) as assessed by Tukey's test.

3.5.2. Microbial growth and viability in FGM and FGM supplemented with the selected mixes

Total viable lactic bacteria and pH were determined on day 1 and day 21 in FGM and FGM supplemented with the selected mixes (Table 8). It can be observed that the microbiological count remained constant during the shelf life at 4 °C; this indicates that probiotic strains remain viable. Although on day 1 there were slight variations in the counts in the different kinds of milk (between 7.4 and 8.6 log CFU ml^{-1}), the strains were able to maintain high viable counts (between 7.3 and 8.2 log CFU ml^{-1}) in all the samples at the end of storage. This product contains an adequate amount of viable probiotic cells, which should exceed 10^6 CFU ml^{-1} (>6 log CFU ml^{-1}) at the time of consumption to exert its beneficial effects on the consumer (Dan et al., 2019). In addition, no significant differences were observed in pH during storage of the samples, except in FGM + Mix 1, with a pH decrease of 0.2 units.

The presence of fungi, yeasts, or specific bacteria indicates poor hygiene in food production and potential microbiological contamination. In the FGM products prepared in this study, no coliform microorganisms were detected, and fungi and yeasts were detected in values $<10^2$ UFC ml^{-1} at

the end of the storage period (data not shown), probably due to the antimicrobial potential of lactic acid bacteria (de Melo Pereira et al., 2018), thus guaranteeing the safety of the product.

4. Conclusion

In summary, based on the obtained results in strain characterization, we elaborated a fermented goat milk with CRL1447 strain (highlighted by the high rate of milk acidification) supplemented with different functional combinations of adjunct cultures (CRL1446, CRL1449, and CRL1472). These selected strains demonstrated positive effects on inhibition of α-glucosidase, HSB activity, cholesterol assimilation, decreased % TG in *C. elegans*, and metabolic markers in DIO mice. These parameters suggest that these strains could efficiently prevent the development of hyperglycemia and lipid alterations in obese individuals. Furthermore, these strains could have a higher potential for dietary interventions with probiotics due to their immune and adipo-modulatory characteristics. Fermented goat milk products elaborated in this study are currently being tested in diet-induced obesity mice (pre-clinical studies).

Declarations

Author contribution statement

Antonela Marquez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Estefanía Andrada: Performed the experiments; Wrote the paper.

Matias Russo, María Lujan Bolondi, Emanuel Fabersani: Performed the experiments.

Roxana Medina, Paola Gauffin-Cano: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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