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Characterization and Technological Properties of Lactic Acid Bacteria Isolated from Traditional Argentinean Goat's Milk Products

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A total of 286 lactic acid bacteria isolated from goat's dairy products in northwest of Argentina were characterized. Lactobacilli (38%) and cocci (62%) were identified according to morphological, physiological, and relevant technological properties. *L. plantarum* (14%) and *Enterococcus* (34%) were the predominant species. *S. thermophilus, Pediococcus* and *L. plantarum* were the highest acid producers. Eight strains of *L. fermentum* produced bacteriocins or metabolites similar to bacteriocins. The API-ZYM test was applied to 39 isolates. Eight strains were selected from their both technological properties and enzymatic activities for use as starter or adjunct culture in the manufacture of artisanal goat cheeses.

Key Words: goat's milk products; lactic acid bacteria; technological properties

INTRODUCTION

The worldwide goat livestock population is about 86 million animals, with South America accounting for 2.5% of the population. Argentina is the second largest producer in South America (FAO, 2010), where the goat livestock is mainly distributed in the northwest region (Medina et al., 2011). These goats adjust well to prevailing climatic conditions (Paz, 2002) and very high

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temperatures (yearly highest average temperature is between 40 and 45°C) (Núñez et al., 2009) and ensure the production of meat, milk and hide.

The production and processing of goat's milk into cheese is of vital economic and social importance in the northwest of Argentina. Artisanal goat cheeses are freshly made from unpasteurized milk with the addition of natural rennet, without the addition of any selected starter culture, thus fermentation is spontaneously obtained by natural lactic acid bacteria (LAB). Most goats' milk cheeses are produced under artisanal conditions; however, a large amount is manufactured on an industrial scale and for hygienic reasons are made from pasteurized milk (López Alzogaray et al., 2007). This thermal process inactivates both enzymes and native microbiota present in raw milk (Buffa et al., 2004). Therefore, one way of preserving the native lactic acid microflora is with the addition of indigenous starter and adjunct cultures in cheese manufacturing. LAB may play different roles in cheese making: some species participate in the fermentation process, whereas others are implicated in the maturation of cheese. In the first case, LAB rapidly ferment lactose producing high concentrations of lactic acid and are designated as starter LAB (SLAB), while LAB responsible for the ripening process are indicated as non-starter LAB (NSLAB) or adjunct cultures (Settanni and Moschetti, 2010).

The group of SLAB mainly includes *Lactococcus lactis* and *Leuconostoc* spp. among mesophilic species and *Streptococcus* (*S.*) *thermophilus, Lactobacillus* (*L.*) *delbrueckii,* and *L. helveticus* among thermophilic species (Fox and McSweeney, 2004). The group of NSLAB is particularly heterogeneous with lactobacilli being mostly represented: *L. farciminis* among obligately homofermentative species, *L. casei, L. paracasei, L. plantarum, L. pentosus, L. curvatus,* and *L. rhamnosus* among facultatively heterofermentative species and *L. fermentum, L. buchneri, L. parabuchneri,* and *L. brevis* among obligately heterofermentative species. The non-Lactobacillus species of NSLAB commonly isolated during cheese ripening are *Pediococcus* (*P.*) *acidilactici, P.* pentosaceus, *Enterococcus* (*E.*) *durans, E. faecalis, E. faecium,* and *Leuconostocs,* which are the same species that act as starter cultures (Settanni and Moschetti, 2010).

Several studies have demonstrated that traditional cheeses made with autochthonous LAB exhibit a greater overall intensity of flavor, and broader flavor profiles and the typical sensorial properties of these cheeses are a result of the diversity of species and strains of local and specific indigenous milk microflora (Casalta et al., 2005; Awad et al., 2007, Randazzo et al., 2008; Rasouli Pirouzian et al., 2012).

The potential effect of LAB on the flavor intensity of the cheese is linked to increased secondary proteolysis, metabolism of remaining carbohydrates, and the capacity to produce free amino acids and associated metabolism to form flavor compounds (Burns et al., 2012). No less important is the involvement of these strains in making the environment of the food less favorable for the development of undesirable microorganisms, potentially harmful, thereby

contributing to the safety and quality of the cheeses (Leroy and De Vuyst, 2004). The preservative effect exerted by LAB is mainly due to the production of organic acids (such as lactic acid), which results in lowered pHs (Šušković et al., 2010). LABs also produce antimicrobial compounds, including hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, D-isomers of amino acids, reuterin, and bacteriocins (Cintas et al., 2001). Bacteriocins are ribosomally synthesized antimicrobial peptides that are active against other bacteria, either of the same species (narrow spectrum), or across genera (broad spectrum) (Yang et al., 2012).

Characterization and identification of the microflora from goat milk and artisanal goat's milk cheeses have been analyzed in different countries (Xanthopoulos et al., 2000; Terzic-Vidojevic et al., 2002; Sanchez et al., 2005; Terzic-Vidojevic et al., 2009; Colombo et al., 2010). In Argentina, little information is available (Savoy de Giori et al., 1987; Oliszewski et al., 2006). Therefore, the aim of this study was to isolate LAB from goat´s dairy products and identify the LAB species responsible for fermentation and specific organoleptic qualities of traditional cheeses. Characterization of isolated LAB based on phenotypic, physiological, and technological criteria would help in the selection of autochthonous strains which could be potentially used as starter or adjunct cultures for the manufacture of safe dairy products as well as to improve the quality and attractiveness of these artisanal cheeses.

MATERIALS AND METHODS

Samples

A total of 108 samples were collected at 12 farmhouses from 4 specific geographic locations in Northwest Argentina over a 9-month period. The goat herds were composed of indigenous goats. From each farmhouse, fresh raw milk, whey and one-week-old cheese were taken from each batch. Artisans removed the whey from the cheese vat during cheese making. This whey was a very complex microbial association constituted of a predominant lactic microflora and certain nonlactic microflora as contaminants with its pH in the range of 4–4.7. The raw milk was inoculated with this whey in variable proportions. Most of the artisanal goat milk cheese are made with natural rennet and are consumed fresh (after one to two days), and they are not salted (López Alzogaray et al., 2007). The samples were chilled at 4°C and transported immediately to the laboratory to be analyzed.

Microbiological Analyses

For the cheese, after removing the rind, representative 10 g samples were homogenized in 90 mL of a warm (40°C) sterile 2% (w/v) sodium citrate solution in a Stomacher 4000 (A. J. Seward Ltd., London, UK). Decimal dilutions

of whey, milk, and the cheese homogenates in quarter-strength Ringer's solution were processed by the pour-plate method. *Lactococcus* and *S. thermophilus* strains were isolated on M17 agar plates (pH 7.1) (Biokar Diagnostic, Beauvais, France) (Terzaghi and Sandine, 1975) after incubation at 30°C for 120 h (*Lactococcus*) and at 45°C for 48–72 h (*S. thermophilus*). *Lactobacillus, Enterococcus* and *Pediococcus* strains were isolated on MRS agar plates (pH 6.5) (Biomerieux,Marcy L'Etoile, France) (De Man et al., 1960) after incubation at 35°C for 48–72 h (mesophilic lactobacilli and enterococci), at 45°C for 48–72 h (thermophilic lactobacilli), and at 30–35°C for 48 h (*Pediococcus*). *Leuconostoc* strains were isolated from MSE agar plates (pH 6.9) (Biokar Diagnostics) after incubation at 30–35°C for 48–120 h. Plates were incubated under semi-anaerobic conditions. Representative colonies (10) were randomly selected from each sampling point and from each medium corresponding to the last dilution at which growth occurred. Purity of the isolates was checked by streaking again and subculturing in MRS broth (Difco) as well as MRS agar, followed by microscopic examinations. The short-term conservation of the pure isolates was done in the respective liquid media. After growth at optimal temperature, the culture was maintained at 4°C and cultures were renewed every month. Stock cultures were kept frozen (–20°C) in a protective medium (Badis et al., 2004). Presumptive *Leuconostoc* colonies were inoculated into MSE agar (Biokar Diagnostics) to confirm dextran production. Total viable counts of LAB in raw milk and cheese were made in MRS agar in autumn, spring, and summer. Isolates that were Gram-positive, catalase-negative, nonspore forming, nonmotile, indol- and nitrate-negative were selected and considered as LAB (Axelsson, 2004).

Phenotypic Characterization

Gram-positive, homofermentative, cocci grouped in pairs or short chains, which grew at 10°C, 35°C, 40°C, and usually at 45°C, grew with a 6.5% salt concentration, at a pH of 9.6, and in 40% bile, were capable of hydrolysis of esculin, were considered to be enterococci (Konrad et al., 2003). Gram-positive, heterofermentative, catalase negative cocci which did not hydrolyze arginine and did not grow at 45°C were taken to be *Leuconostoc* (Navidghasemizad et al., 2009). Gram-positive, homofermentative, cocci grouped in short or long chains, which grew at 35°C, 40°C, and 45°C and were able to grow rapidly in litmus milk at 42°C but did not grow at 4% salt concentration, did not grow at a pH of 9.6 and did not grow in 40% bile were considered to be presumptive *S. thermophilus* (Facklam, 2002). Gram-positive, homofermentative, cocci grouped in tetrads which did not hydrolyze arginine were considered to be presumptive pediococci (Pfannebecker and Fröhlich, 2008; Baroei et al., 2011). Gram-positive, catalase-negative rods, whether homofermentative or heterofermentative, which were capable of growing at 15°C and/or 45°C, were

considered to be lactobacilli (Xanthopoulos et al., 2000). The fermentation of carbohydrates was determined based on previous studies of Parente et al. (2001). The carbohydrates tested (1% w/v) were lactose, glucose, sucrose, galactose, sorbitol, mannose, melezitose, raffinose, arabinose, xylose, melibiose, maltose, rhamnose, salicin, ribose, mannitol, trehalose, fructose, and cellobiose.

Technological Properties

Acidifying activity (Abeijón et al., 2006) and the production of diacetyl and acetoin in goat milk (Medina et al., 2001) were measured. To determine the level of adaptation of the isolates in goat milk, actively growing cultures were inoculated (2% w/v) in tubes containing 10 mL of 10% (w/v) pasteurized reconstituted goat milk powder with sodium citrate at 0.4% (w/v) and incubated at 35°C for 16 h; acidifying ability (% lactic acid in milk) and diacetyl-acetoin were determined. To determine proteolytic activity and to distinguish between fast- and slow-growing cultures, the type of growth in fast-slow differentiation agar was determined (Medina et al., 2001). Citrate utilization (Oliszewski et al., 2006) was considered positive when zones of clearing around colonies (> 3 mm) on calcium citrate medium were observed.

Antimicrobial and Bacteriocin Activities

Agar well diffusion assay (Castellano et al., 2004) was used to study the antibacterial activity of the 109 lactobacilli isolated from goat milk, whey, and artisanal cheese. For this purpose, eight reference strains were used to check sensitivity to the antimicrobial substances produced by the lactobacilli strains, these being *L. plantarum* CRL 691, *Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus, Listeria monocytogenes, Bacillus subtilis, Escherichia coli, Enterobacter* sp., *and Salmonella enteritidis. L. casei* CRL705 was used as bacteriocin-producer indicator strain (Vignolo et al., 1993). Lactobacilli strains isolated from goat dairy sources and the reference varieties used as indicator strains, that is *Lactobacillus plantarum* CRL 691 and *L. casei* CRL705 were grown in MRS broth at 35°C for 24 h, *Listeria monocytogenes* in BHI broth (Merck, Darmstadt, Germany) at 30°C for 24 h, and the others were grown in Nutrient broth (Merck, Darmstadt, Germany) at 35°C for 24 h. Antibacterial activity may often be due to the production of organic acids, with a consequent reduction in pH, or to the production of hydrogen peroxide. It may also be due to the production of bacteriocins or bacteriocin-like substances (BLIS). Hence, antimicrobial activity was evaluated by a well diffusion assay after excluding inhibition due to organic acids and hydrogen peroxide. Seventy (70) μL of the indicator strain (with approximately 5 x 10^5 cfu/mL), cultured in MRS broth (*L. plantarum* and *L. casei*), BHI broth (*L. monocytogenes*) and Nutrient broth (*Pseudomonas aeruginosa,*

Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Eschericchia coli, and Salmonella enteritidis) for 24 h at corresponding temperature, were inoculated into 7 mL of soft MRS agar, BHI agar, and Nutrient agar (each broth plus 0.7% bacteriological agar), respectively, and incubated at 45°C. The resultant mixtures were overlaid onto MRS agar, BHI agar and Nutrient agar plates, respectively. After solidification of the agar, wells 4 mm in diameter were cut into it, and 30 μL of supernatant fluid from a culture of the strain under test for antibacterial activity, obtained as indicated above, was added to each well. The plates were kept at 3–4°C for 4 h to ensure diffusion of the fluid into the agar and examined for inhibition after incubation at either 30°C or 35°C for 24 h. If inhibition was found to be present when this was done, it was deemed to be due to the production of bacteriocins or bacteriocin-like compounds. Inhibition was considered positive when the inhibition halo around the well was more than 2 mm.

LAB strains for testing were cultured overnight in MRS broth. Cells were then removed by centrifuging at 14,000*g* for 5 min. The supernatant fluid was filtered through a filter with a pore size of 0.22 μm (Millipore Corporation, Billerica, Mass., USA), and adjusted to pH 6.5 with sterilized 1 and 4 M NaOH. The possible inhibitory action of hydrogen peroxide was eliminated by adding a sterile solution of catalase (1 mg/mL) and leaving at 25°C for 30 min. In all the assays made in this study, this extract was placed into the wells.

To confirm the production of proteinaceous substance, proteolytic enzymes (Sigma, Chemie GmbH, Deisenhofen, Germany) were used; saturated solutions of protease IV, trypsin III, α chymotrypsin, pronase E, protease XV, and trypsin were prepared. The BLIS producers with the widest spectrum of antimicrobial activity were selected for this test. Plates obtained as indicated above, using *Lactobacillus plantarum* CRL 691as indicator strain, were prepared. In a plate, two wells were made close to each other (4 mm) in the lawn of agar; in a well, 20 μL of enzymatic solution were placed; in the other well, 30 μL of the supernatant obtained from the selected isolate were placed. The plates were kept at 3–4°C for 2 h to ensure diffusion of the fluid into the agar and examined for inhibition after incubation at 35°C for 24 h. Absence of the inhibition zone in vicinity of enzymatic solution well was taken as an indication of proteinaceous nature of produced antimicrobial substance, that is, a potencial bacteriocin-like compound.

Api Zym Test

The strains were grown in MRS agar at 35°C for 24 h in semianaerobic conditions. The culture was then removed from the surface of the agar plates and resuspended in 2 mL of distilled water to obtain a dense suspension. Two drops of cell suspension were added to each cupule in the API ZYM strip (API System S.A., La Balme les Grottes, France), which was then placed in a moist

chamber and incubated at 37°C for 4 h. After incubation one drop of each of the API reagents A and B was added to each of the cupules. Enzyme activity was measured by comparing the color developed with the chart provided by the manufacturer and expressed on a scale of 0 (no activity) to 40 (maximum activity, ≥40 nM of chromophore released).

Statistical Analysis

ANOVA analysis (InfoStat 2011, Grupo Infostat, FCA, Universidad Nacional de Córdoba, República Argentina) was carried out to determine statistical differences ($P \leq 0.05$) between the seasonal mean number of total viable LAB. Tukey test was used ($P \leq 0.05$). All experiments were repeated twice.

RESULTS AND DISCUSSION

Enumeration and Isolation of LAB

A total of 286 isolates from goat dairy samples were classified as cocci (62%) and rods (38%). The general characteristics of the isolates are shown in Table 1. Other workers reported different groups of cocci and lactobacilli in goat dairy products (González et al., 2007; Nikolic et al., 2008; Medina et al., 2011). Lactobacilli (109 isolates) were classified into eight species. Homofermentative (*L. delbrueckii* subsp. *bulgaricus, L. helveticus, L. acidophilus*) (11%), facultatively heterofermentative (*L. plantarum, L. rhamnosus, L. casei*) (59%), and obligately heterofermentative (*L. brevis, L. fermentum*) (30%) species were identified. The species were primarily differentiated by their patterns of carbohydrate fermentation. However, when the fermentation patterns of these isolates were compared with the identification key in Bergey's Manual, significant differences were noted. Of all lactobacilli isolated, *L. plantarum* was the predominant species (36.70%) over *L. fermentum* (21.10%), *L. rhamnosus* (11%), *L. casei* (11%), *L. brevis* (9.17%), *L. delbrueckii* subsp. *bulgaricus* (5.50%), *L. acidophilus* (1.83%), and. *L. helveticus* (3.67%).

Cocci (177 isolates) were classified into five species: *Enterococcus* (*E.*) *faecium* (47.46%), *Leuconostoc* (27.12%), *S. thermophilus* (13.56%), *E. faecalis* (7.34%), and *Pediococcus* (4.52%). Lactococci were not isolated. Enterococci occurred in 90% of the samples examined and predominated over the other LAB in most samples. Enterococci (97 isolates) were classified into two species, *E. faecium* (84 isolates) and *E. faecalis* (13 isolates); they were primarily differentiated by their patterns of carbohydrate fermentation. *E. faecium* fermented arabinose and melibiose; melezitose and sorbitol were not fermented and the capacity to grow at 50°C. Eight isolates were identified as presumptive pediococci of which four strains were isolated from milk. The best capacity to grow was observed at 30°C.

Table 1: Phenotypic characterization of lactic acid bacteria isolated from goat dairy products.

Strains	Hydrolysis of Esculine	Production of ADH	Bile salt 40%	Growth at						Gas from		Growth in NaCl		
				10°C	15°C	35°C	40°C	45°C	50°C	glucose	gluconate	4%	6.5%	10%
Enterococcus faecium (84[A])	+	ND	+	+	ND	+	ND	+	+	–	ND	+	+	ND
Leuconostoc sp. (48)	–	–	ND	–	ND	+	+	V	ND	+	ND	+	–	ND
S. thermophilus (24)	–	–	ND	–	ND	+	+	+	ND	–	ND	–	–	ND
Enterococcus faecalis (13)	+	ND	+	+	ND	+	+	+	–	–	ND	+	+	ND
Pediococcus sp. (8)	ND	–	–	–	ND	+	+	+	ND	–	ND	–	–	ND
L. plantarum (40)	+	–	ND	ND	+	+	ND	+	ND	–	+	+	ND	ND
L. fermentum (23)	–	–	ND	ND	–	+	ND	+	ND	+	+	+	ND	+
L. casei (12)	+	–	ND	ND	+	+	ND	+	ND	–	+	+	ND	ND
L. rhamnosus (12)	+	–	ND	ND	+	+	ND	+	ND	–	+	+	ND	ND
L. brevis (10)	+	+	ND	ND	+	+	ND	+	ND	+	+	–	ND	ND
L. bulgaricus (6)	–	–	ND	ND	–	+	ND	+	ND	–	–	–	ND	ND
L. helveticus (4)	–	–	ND	ND	–	+	ND	+	ND	–	–	–	ND	ND
L. acidophilus (2)	+	–	ND	ND	–	+	ND	+	ND	–	–	–	ND	ND

[A]Number of strains isolated from goat's dairy samples.
(+) More than 90% of positive reactions, (–) less than 10% of negative reactions, V more than 10% and less than 90% positive reactions.
ADH, arginine dihydrolase.
ND, not detected.

Table 2: Counts of lactic acid bacteria isolated from goat dairy samples.

	Mean and SD of bacterial counts (log CFU/ml or g)[A]		
Period	**Milk**	**Whey**	**Cheese**
Autumn	1.86 ± 0.40^{B}	2.88 ± 0.81^{B}	6.14 ± 0.67^{B}
Spring	2.26 ± 0.40^{B}	5.61 ± 0.81^{B}	8.32 ± 0.67^{B}
Summer	4.93 ± 0.40^{C}	4.59 ± 0.81^{B}	7.74 ± 0.67^{B}

[A]Counts are expressed as log CFU per milliliter and log CFU per gram.
[B–C]Values in the same column with different superscript differ significantly ($P < 0.05$).

The species identified in this study have been found in fermented dairy products by other workers (Badis et al., 2004; Terzic-Vidojevic et al., 2009; Colombo et al., 2010).

Since lactococci strains were not isolated, it is also possible that the selected media and incubation conditions did not provide complete selectivity.

In raw goat milk, the highest population of LAB was found in summer (4.93 ± 0.40 log cfu/mL) (Table 2). In the artisanal goat cheese, the main population of LAB was found in spring (8.32 ± 0.67 log cfu/g). The primary cause of these microbial counts in spring and summer could be the high temperatures prevailing during sampling. These results suggest that significant seasonal variation in the number of LAB was found. Artisanal cheese was the main source of LAB (200 isolates), followed by milk (45 isolates) and whey (41 isolates) (Fig. 1). Pediococci were isolated mostly in raw milk.

The high number of enterococci found in milk and cheese can be due to poor hygienic conditions during milking or manufacture and the ability of these bacteria to adapt to adverse conditions, like high temperatures. *L. plantarum*

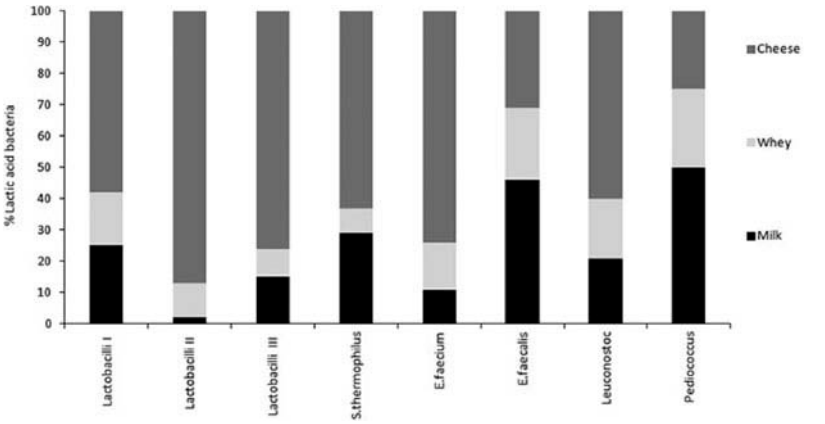

Figure 1: Distribution of species of lactic acid bacteria isolated from raw goat milk, whey and artisanal cheese.

and *E. faecium* were the predominant species in cheese. Similar results were reported by other workers (Hatzikamari et al., 1999; Terzic-Vidojevic et al., 2002; Oliszewski et al., 2006) in artisanal cheese. However, no relationship between diversity of species and their geographical origin was found (data not show).

Technological Properties

Acidification activity is a very important parameter in the selection of starter strains (Mäyrä-Mäkinen and Bigret, 2004). Studied strains were classified into three groups according to the level of acidification reached: low ($\leq$ 0.5% lactic acid), medium (between 0.5% and 0.7% lactic acid), and high ($\geq$ 0.7% lactic acid) as shown in Table 3. The same percentage of *S. thermophilus* and *Pediococcus* strains with high acidifying activity (38%) was observed. Of all studied strains, facultatively heterofermentative lactobacilli were present in the highest percentage as strains with high acidifying activity (62%). *S. thermophilus, E. faecium, Pediococcus,* and obligately heterofermentative lactobacilli showed the highest percentage of rapid fermentation. The majority of the studied strains utilized citrate and produced diacetyl and acetoin in goat milk. The strains with high acidifying activity play an important role in the initial milk coagulation process, so they can be used as starter culture. The strains which are able to ferment citrate that is naturally present in milk and thus produce aromatic substances such as diacetyl, confer the typical butter aroma (Centeno et al., 1996).

Nonstarter lactic acid bacteria (NSLAB) dominate cheese microbiota during ripening since they tolerate the hostile environment well and strongly influence the biochemistry of curd maturation, contributing to the development of the final characteristics of cheese. Several NSLAB are selected on the basis of their health benefits and are employed in cheese making (Settanni and Moschetti, 2010). Also NSLAB influence flavor and texture development especially of homemade fermented dairy products manufactured at specific ecological localities (Terzic-Vidojevic et al., 2009). *Pediococcus* and *L. plantarum* strains are usually selected as NSLAB.

Antimicrobial and Bacteriocin Activity

It was confirmed that 8 out of 109 analyzed LAB isolates showed antimicrobial activity (Table 4) and were phenotypically classified as *L. fermentum*. The 8 BLIS producers showed antimicrobial activity against the reference strain *L. plantarum* CRL691 and only one strain did not inhibit *Listeria monocytogenes* (UNSE236). Tested isolates exhibited clear or turbid zones of inhibition on indicator strains including the bacteriocin-producer strain (*L. casei* CRL705). Among these BLIS producers the widest spectrum

Table 3: Technological properties of lactic acid bacteria isolated from goat dairy products.

Strains	Acidification capacity [A]			FSDA[B] Fast %	Citrate utilization[C]	DA[D]
	Low	Medium	High			
S. thermophilus (24[F])	(33)[E]	(29)[E]	(38)[E]	(54)[E]	(54)[E]	(88)[E]
E. faecium (84)	50	18	32	48	79	82
E. faecalis (13)	70	15	15	23	69	62
Leuconostoc (48)	60	17	23	38	85	94
Pediococcus (8)	38	24	38	50	100	100
Homofermentative lactobacilli (12)	33	8	59	25	92	92
Facultatively heterofermentative lactobacilli (64)	19	19	62	39	95	95
Obligately heterofermentative lactobacilli (33)	33	18	49	67	82	85

[A]Acidification capacity, low (≤ 0.5 % lactic acid), medium (between 0.5 and 0.7% lactic acid), high (≥ 0.7% lactic acid).
[B]FSDA, fast-slow differentiation agar.
[C]Citrate utilization, zones of clearing around colonies (> 3 mm) on calcium citrate medium.
[D]DA, diacetyl and acetoin in goat milk.
[E]Percentage of strains with positive response.
[F]Number of strains isolated from goat's dairy samples.

of antimicrobial activity appeared to be displayed by isolates UNSE212 and UNSE53A, that gave zones of inhibition on 4 out of 10 indicator strains used in the test. These are very important data since *Salmonella enteritidis, Escherichia coli,* and *Listeria monocytogenes* are associated with food borne diseases (Notermans and Hoogenboom-Verdegaal, 1992).

Experiments with proteolytic enzymes revealed a proteinaceous nature of antimicrobial compounds produced by the isolates UNSE212 and UNSE53A; the activity of the antibacterial compound from *L. fermentum* UNSE 212 and UNSE53A was destroyed by protease IV, trypsin III, α chimotrypsin, pronase E, trypsin, and protease XV (absence of the inhibition zone in vicinity of enzymatic solution well). Consequently, the inhibitory effect that these strains demonstrated might be due to the presence of bacteriocins or metabolites similar to them. For further elucidation and detailed analysis of the nature of antimicrobial compounds produced by the tested strains is required. This study has relevance for the application of LAB and their antimicrobial metabolites in the prevention of food spoilage and the extension of the shelf life of food that is ready to eat, fresh-tasting, nutrient and vitamin rich, minimally processed and bio-preserved, as these are the major challenges for the current food industry (Šušković et al., 2010).

Table 4: Antimicrobial activity of lactic acid bacteria isolated from goat's dairy products.

	Isolates							
Indicator microorganisms	**UNSE200**	**UNSE212**	**UNSE204**	**UNSE199**	**UNSE 236**	**UNSE 224**	**UNSE218A**	**UNSE53A**
Enterobacter sp[A]	–	Clear zone (2mm)	–	–	–	–	–	–
Micrococcus luteus[A]	Clear zone (2mm)	Clear zone (2mm)	–	Clear zone (2mm)	–	–	–	–
Listeria monocytogenes[A]	Clear zone (2mm)	Clear zone (3mm)	Clear zone (3mm)	Clear zone (3mm)	–	Clear zone (3mm)	Clear zone (2mm)	Clear zone (2mm)
Pseudomonas aeruginosa[A]	–	–	–	Clear zone (2mm)	Clear zone (2mm)	Clear zone (2mm)	–	Clear zone (2mm)
Salmonella enteritidis[A]	–	–	–	–	–	–	–	Turbid zone (2mm)
E. coli[A]	Clear zone (2mm)	Clear zone (2mm)	–	+	–	–	Turbid zone (2mm)	Clear zone (2mm)
L. casei CRL705[B]	Clear zone (2mm)	Clear zone (3mm)	–	–	Clear zone (2mm)	Clear zone (2mm)	–	–

[A], Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, UNT, Tucumán, Argentina.
[B], CERELA-CONICET-; no inhibition zone.
Three more indicator strains, *L. plantarum* CRL691, *B. subtilis* and *S. aureus* were used in the test, but none of BLIS producers showed antimicrobial activity towards *B. subtilis* and *S. aureus* and all the BLIS producers showed antimicrobial activity towards *L. plantarum* CRL691.

API ZYM Test

The Api Zym test was applied to 39 strains that were selected because of their relevant technological properties (they exhibited high or medium acidification activity, were fast acid producers, produced diacetyl and acetoin in goat milk and metabolized citrate). These were classified as 4 homofermentative lactobacilli strains (UNSE115, UNSE150, UNSE267, and UNSE291), 16 facultatively heterofermentative lactobacilli strains (UNSE84, UNSE132b, UNSE141, UNSE143, UNSE148b, UNSE191, UNSE195, UNSE201, UNSE213, UNSE225, UNSE238, UNSE241, UNSE269, UNSE287, UNSE290, and UNSE293), 14 obligately heterofermentative lactobacilli strains (UNSE47, UNSE58, UNSE184, UNSE199, UNSE200, UNSE204, UNSE212, UNSE214, UNSE218a, UNSE219, UNSE226, UNSE236, UNSE237, and UNSE239), and 5 *Pediococcus* strains (UNSE22, UNSE85, UNSE117, UNSE216, and UNSE253). The studied strains produced a wide spectrum of enzymes (Figs. 2 and 3). All the lactobacilli evaluated (Fig. 2) showed high values of alkaline (28 nmol) and acid (30 nmol) phosphatases and phosphohydrolase (24 nmol), expressed as nmol of chromophore released. Acid phosphatase and phosphohydrolase are essential for the hydrolylisis of phosphopeptides in cheese ripening (Akuzawa and Fox, 2004; Serio et al., 2010). All the lactobacilli exhibited esterase and esterase/ lipase activity but obligately

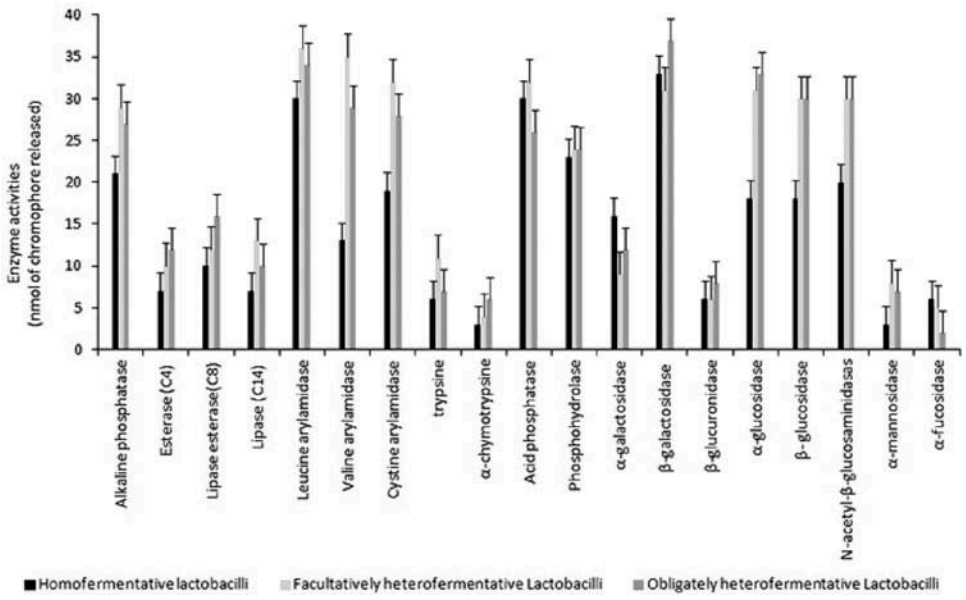

Figure 2: Enzyme profiles of lactobacilli isolates from goat dairy sources. Results are the average values of API ZYM results of homofermentative (4 strains), facultatively heterofermentative (16 strains) and obligately heterofermentative (14 strains) lactobacilli (thin bars denote standard deviations).

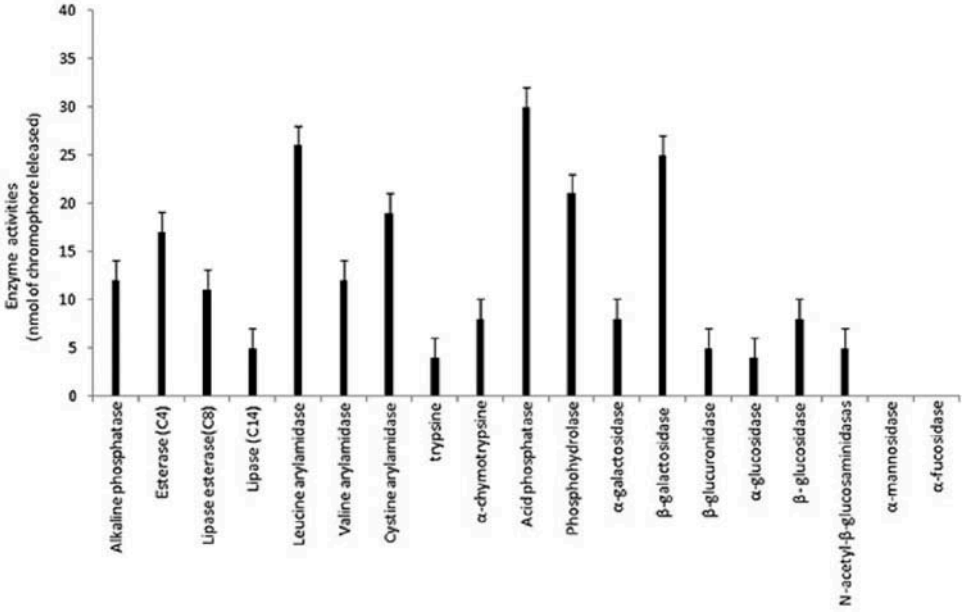

Figure 3: Enzyme profiles of pediococci isolates from goat dairy sources. Results are the average values of API ZYM results of 5 strains (thin bars denote standard deviations).

heterofermentative strains being the major producers. The highest lipase activity (13 nmol) was detected in facultatively heterofermentative strains. High levels of lipase activity is a nondesirable trait in production of cheese flavorings; the accumulation of long chain fatty acids causes the soapiness as a flavor defect. Esterase/ lipase increase short fatty acid concentration, contributing to piquant flavors especially in cheeses obtained with goats' milk (McSweeney and Sousa, 2000). Our results are contrary to that reported by Requena et al. (1991) and Herreros et al. (2003), who detected very weak or no lipase, esterase/lipase or esterase activities in *L. plantarum* strains isolated from goat dairy sources.

The strains evaluated exhibited low levels of activity of proteases (5 nmol of trypsin and 2.5 nmol of chymotrypsin) and higher activity of peptidases (36 nmol of leucine, 35 nmol of valine and 32 nmol of cystine-aminopeptidase). These properties are important in reducing bitterness and improving body and textural defects in cheese (Serio et al., 2010). Besides, the soapiness defect caused by the accumulation of long chain fatty acids in many ripened cheese varieties can be removed by using strains with intermediate esterase and lipase activities.

All of the studied lactobacilli presented high ß-galactosidase activity (37 nmol), which explained the high acidifying levels detected (Table 3). This

is the main enzyme whereby homofermentative lactobacilli transformed lactose in lactic acid, responsible for the coagulation of milk. Similar results were obtained by Oliszewski et al. (2006) in *L. plantarum* and *L. rhamnosus*. The strains producing glucosidases are able to hydrolyze milk sugars, providing carbohydrates for their metabolism (Serio et al., 2010). These enzymes were detected in high levels especially in facultatively heterofermentative and obligately heterofermentative strains. Low activities toward mannose, fucose and glucuronides were detected.

Activities values of pediococci (5 isolates) strains were lower than in lactobacilli (34 isolates) (Fig. 3). All the pediococci studied showed high values of acid phosphatases (30 nmol) and phosphohydrolase (22 nmol). All the strains exhibited higher esterase (18 nmol) and esterase/ lipase (12 nmol) than lipase activity (5 nmol). Peptidase activities (26 nmol of leucine, 12 nmol of valine and 18 nmol of cystine) were stronger than proteinases (5 nmol of trypsin, 8 nmol of chymotripsin).ß-galactosidase (25 nmol) activities were lower than in lactobacilli, according to the low acidifying levels (Table 3). The interest in ß-galactosidase activity lies in the fact that k-casein is glycosylated and the release of these sugars may provide a potential energy source to the microorganisms. The presence of sugars may also impede proteolytic enzyme activity and their removal would also facilitate more effective proteolysis (Herreros et al., 2003). No mannosidase nor fucosidase were detected. In all studied strains, no strong variations in the enzyme activities between strains of the same species were observed, as shown by the low standard deviations (Figs. 2 and 3).

CONCLUSION

The present work represents a contribution to the characterization of LAB population in goat milk and goat milk cheese from northwest of Argentina. LAB distribution showed a high diversity of species that are dominant and frequently described in various dairy products. The predominant species were *L. plantarum, E. faecium, E. faecalis, L. fermentum, L. delbrueckii* subsp. *bulgaricus, Pediococcus sp,* and *S. thermophilus*. The strains had physiological properties of biotechnological interest such as the ability of lactic acid production in a short period of time, good proteolytic activity, production of antimicrobial compounds and production of diacetyl in goat milk. Since some of the strains of isolated LAB not only had the required technological characteristics but also the ability to produce bacteriocins active against pathogenic microorganisms or which lead to spoilage, these high metabolically versatile cultures of these LAB would have major applications for mass production and for safety of cheese. *L. fermentum* UNSE200, UNSE212, UNSE204, UNSE199, UNSE236, UNSE224, UNSE218A, and UNSE53A strains, which

produce bacteriocins or metabolites similar to bacteriocins should be carefully evaluated for their potential applications in dairy fermented products.

Based on these results, eight strains were selected from their both technological properties and enzymatic activities for use as starter or adjunct culture in the manufacture of artisanal goat cheeses. They have been identified by metabolic tools as: UNSE308 (*L. rhamnosus*), UNSE309 (*L. delbrueckii* subsp. *bulgaricus)*, UNSE316 and UNSE317 (*L. plantarum*), UNSE22, UNSE253, UNSE315 (*P. pentosaceus),* and UNSE314 (*S. thermophilus).*

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