Bacteriocinogenic Lactic Acid Bacteria of Caprine Products from Chaco – Argentina

F.P. Rivas[1,2], M.P. Castro[1,2], M.E. Cayré[1] and C.A. Campos[2,3,*]

[1]*Laboratorio de Microbiología de Alimentos, Universidad Nacional del Chaco Austral, Comandante Fernández 755, (3700) Sáenz Peña, Chaco, Argentina*

[2]*Conicet, Consejo Nacional de Investigaciones Científicas y Técnicas*

[3]*Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires, Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Argentina*

Abstract: The microbiota of artisanal caprine products is essential for the manufacture of fermented products, such as cheeses and dry sausages, conferring them particular and distinctive flavors and generating high value-added products. Many of the bacteria comprising this microbiota are able to produce bacteriocins and antimicrobial substances. The finding of bacteriocinogenic strains within this microbiota could be the first step to introduce biopreservation into these products. Hence, ten lactic acid bacteria (LAB) (6 *Lactobacillus paracasei*, 2 *Lactococcus lactis*, 1 *Leuconostoc mesenteroides* and 1 *Lactobacillus rhamnosus*) isolated from artisanal caprine products from Chaco (Argentina) were screened for antagonistic activity against other LAB and some spoilage and pathogenic microorganisms, such as *Listeria innocua* (in lieu of *Listeria monocytogenes*), *Staphylococcus aureus* and *Escherichia coli*. The final goal was to investigate LAB antibacterial activity within this unexplored ecological niche and to select interesting strains for the role of bio-preservatives. Bacteriocin-like substances (BLIS) produced by the isolated strains inhibited three species of *S. aureus*, *L. innocua* and *Brochothrix thermosphacta*. Titles of these antibacterial substances were within the range 100-200 AU.mL^{-1}. BLIS produced by the isolated strains were heat stable and effective after refrigerated storage and freeze/thaw cycles. Moreover, BLIS activity was higher at acidic pH values, showing a decrease when these values were closer to neutrality or they become alkaline. BLIS did not adsorb to the producer cells which is expected for future production and application on food systems. The results presented in this study could contribute to characterize the antimicrobial activity of the indigenous flora of artisanal caprine products manufactured in the province of Chaco, Argentina. The isolated bacteriocinogenic strains showed a regular production of BLIS in culture broth, which offers promising applications for the biopreservation of these products.

Keywords: Caprine artisanal products, Bacteriocinogenic strain, Argentina, Goat meat, Goat milk cheese.

1. INTRODUCTION

Caprine industry in South America has a long tradition beginning with the arrival of Spanish conquerors in the 16th century. However, large commercial development was not achieved due to continuous economical fluctuations. During the last decade, the establishment of programs to enhance the activity gave a major boost to goats' products elaboration and, as a consequence, the studies on the microbiota of caprine products started, mainly in Argentina and Brazil [1-5]. This microbiota is essential for the manufacture of fermented products, such as cheeses and dry sausages, conferring them particular and distinctive flavors and generating high value-added products.

Goat's milk possesses unique properties which distinguishes it from cow milk and makes it a valuable alternative. Goat milk differs from cow milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition [6, 7]. In Argentina, goat's milk is mostly processed into cheeses, but powdered and UHT milks are also manufactured. Since most milk production is processed into fermented products, Argentina has turned into the country in which research on indigenous lactic microbiota has reached the largest development [1]. Lactic acid bacteria (LAB) isolated from Argentinean goat's milk and cheeses were: lactobacilli (60%), enterococci (35%) and pediococci (5%). Isolated species were identified as *L. plantarum* (35%), *L. rhamnosus* (15%), *L. delbrueckii* subsp. *bulgaricus* (5%), *L. fermentum* (5%), *Enterococcus faecium* (35%) and *Pediococcus pentosaceus* (5%) [8]. Nevertheless, the microbiota associated to goat dairy products from the province of Chaco (North-East of the country) has not been investigated yet.

Regarding goat meat, process and technological innovation are scarce and goat meat dishes appear to be difficult to prepare and cook for urban people. However, opportunities exist for goat meat thanks to a good ecological image, its dietetic and healthy quality

*Address correspondence to this author at the Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Buenos Aires, Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Argentina; Tel,/Fax: +54 11 4576 3366; E-mail: carmen@di.fcen.uba.ar

(low saturated fat, low cholesterol level, low caloric, etc.), cultural tendency of consumers towards natural foods, and recent food crises [9]. In Argentina, the level of production and consumption of goat meat remains below other meats, emulating the overall context seen in other countries of the region. As manufacturing-grade meat can constitute up to half of the meat from a carcass, it is important to examine the potential uses of goat meat for producing value-added products. Madruga & Bressan [4] stated that there are indications that it can be used in any recipe or processed product instead of beef. In fact, goat meat dry sausage production has become an accessible alternative to take advantage of the meat of those animals which are not within the scope of retailers or consumers. The specific characteristics of this product mainly arise from the raw food materials used and the adventitious microflora associated with its production. Oki *et al*. [2] described the diversity of LAB in ethnic chevon (goat) meat products of the Western Himalayas. The LAB isolates were identified as *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, and *Weissella cibaria*. In Chaco, dry sausages made with goat meat comprise part of the artisanal regional products with high appealing to local consumers. As their production is seasonal and restricted, the microbiota of these products has not been described yet.

Similar to many fermented artisanal food products, goat derived products are prone to the occurrence of undesirable microorganisms such as *Staphylococcus aureus* and *Listeria monocytogenes*. The need to control these pathogens without altering the organoleptic characteristics of the products leads us to the use of a new tool from biopreservation processes: bacteriocins. Bacteriocins are peptides with antibacterial properties produced by lactic acid bacteria. These peptides can reduce or inhibit the growth of other Gram-positive bacteria; thereby they can be used to control the growth of food borne pathogens in dairy and meat products [10]. Bacteriocinogenic LAB originally isolated from traditionnal products are probably the best candidates for improving their microbiological safety, because they are well adapted to the conditions in these products and should therefore be more competitive than LAB from other sources [11]. Furthermore, the production of bacteriocins during fermentation plays an important role in enhancing the functional value of the products.

In this paper, we report on the screening of antibacterial activity and on the characterization of antibacterial compounds of twenty-nine LAB isolated from artisanal cheeses and dry sausages manufactured with goat milk and meat, respectively. The goal was to investigate LAB antibacterial activity within these unexplored ecological niches in order to select suitable strains for the role of bio-preservatives. This is the first report on bacteriocinogenic microbiota from goat's milk cheese and goat's meat dry sausages manufactured in this region of Argentina.

2. MATERIALS AND METHODS

2.1. Bacterial Strains and Culture Conditions

The following spoilage and pathogenic bacterial strains were selected for the screening of antagonistic activity: *Listeria innocua* ATCC 33090 was used instead of *Listeria monocytogenes*, because of their similar response to stress factors [12]; *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213; *Enterococcus faecium* TW 20 (Universidad Nacional de la Patagonia San Juan Bosco, Chubut, Argentina); *Pseudomonas aeruginosa* FBUNT; *Staphylococcus aureus* FBUNT; *Escherichia coli* FBUNT (these last three strains were isolated from clinical samples were identified by the Microbiology Department of Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (FBUNT), Argentina); *Lactobacillus curvatus* 268; *Lb. curvatus* ACU-1 and *Brochotrix thermosphacta* 405. These latter strains were isolated from cooked meat products in our laboratory. All strains were maintained as frozen stocks at -30 °C. Prior to being used as bacterial lawns in the screening assay, all the indicator strains were recovered in Brain Heart Infussion (BHI; Biokar Diagnostics, Beauvois, France) at the convenient temperature for each one (at 25 °C: *B. thermosphacta*; at 30 °C: *L. innocua* and *P. aeruginosa* FBUNT; at 37 °C: *S. aureus* and *E. coli*), with the exception of the lactobacillus strains which were recovered in MRS broth (de Man, Rogosa & Sharpe; Biokar Diagnostics, France) at 30 °C.

2.2. Caprine Product Samples

2.2.1. Dry Sausage

Dry fermented products were collected from the cities Charata and Pampa del Infierno (both located in the north-eastern region of the province of Chaco), Argentina. Ten sample units were purchased from each small-scale facility producing traditional dry sausages without the addition of starter cultures. Sausages were all alike in their formulations: goat meat, beef and bacon in an average proportion of 60:30:10 thoroughly

mixed together with salt (~2%), wheat starch or milk powder (~1%), sucrose (~2%), spices (~2%) and nitrite/nitrate salt (0.03%). The meat dough obtained after mixing was used to fill natural casings (sheep gut). Sausages were fermented and ripened under non-standardized environmental conditions. For analytical purposes, sausage casing was aseptically removed and 10 g of the sample derived as cross section was homogenized with 90 ml of sterile solution containing 0.1% ($w.v^{-1}$) peptone (Britania, Argentina) and 0.85% ($w.v^{-1}$) NaCl (Anedra, Argentina) using a domestic blender (Braun, Germany). Ten-fold serial dilutions of the meat homogenate were prepared in peptone water to spread plate the sample on de Man Rogosa Sharpe agar (MRS, Biokar Diagnostics, France) with 1 $g.L^{-1}$ of sorbic acid (Sigma-Aldrich, USA) to inhibit yeast and molds growth. Plates were incubated for 72 h at 30°C.

2.2.2. Cheese

Goat cheese produced in a small dairy industry (Cooperativa Trento Chaqueña, Pampa del Infierno, Chaco, Argentina) was chosen for this study. The cheese is entirely made from caprine milk by a traditional method. The milk is standardized to a fat content of 5.6–6.0%; then it is pasteurized (73.4 °C, for 15 s) and immediately cooled at 32–34 ◦C for renneting. The milk is curdled for 8 h; curdle is left to drain and the cheese is shaped into moulds. The cheese is immersed in brine (25% w/v) for 6 h and then it is drained off, turning it alternatively on both faces. Ripening times oscillate between 45 and 60 days at 10 °C. Samples were purchased as whole pieces (average weight 0.250 - 0.500 kg). One hundred grams of cheese was ground aseptically and then 10 g homogenized with 90 mL of a previously warmed (40 ± 2 °C) sterile 2% (w/v) sodium citrate solution in a domestic blender for 2 min. Serial dilutions were made in the same solution and plated onto MRS agar. Plates were incubated under aerobic conditions for 72 h at 30 °C.

2.3. Counts, Isolation and Maintenance of Lactic Acid Bacteria

After incubation, ten colonies with different macroscopic morphology were randomly picked from each plate. Isolates were re-inoculated in MRS broth, incubated at 30 °C and checked for purity by streaking on MRS agar. Plates with pure cultures were used to test Gram stain, catalase formation and cell morphology by phase contrast microscopy, Gram positive and catalase negative strains were selected. Isolated bacteria were maintained as frozen stocks in MRS broth suplemented with 10% ($v.v^{-1}$) glycerol at −18 °C for one month. Before experimental use, all LAB strains were recovered in MRS broth and were incubated at 30°C.

2.4. Screening for Antagonistic Activity

Detection of antagonistic activity in isolated LAB strains was performed by the agar well diffusion assay (AWDA) [13]. Plates were made with MRS for lactobacilli and with BHI agar for the rest of the indicator bacteria. Briefly, Petri dishes were overlaid with 15 ml of molten agar (1% agar), inoculated with 30 µl of an overnight culture of the indicator microorganism, in which wells of 5 mm were formed by carving the agar with a cork borer. Afterwards, 30 µl of an overnight culture of the putative inhibitor strain were placed in each well. The plates were then incubated for 24 h at a temperature conductive to growth of the indicator microorganism and were examined for zones of inhibition. Inhibition was recorded as negative if no zone was observed around the agar well (6 mm clear or larger zones around the well were scored as positive inhibition). A positive control for antimicrobial activity, *i.e*. bacteriocin-producing *Lb. plantarum* ATCC 8014, was included.

2.5. Characterization of the Antibacterial Compounds

Isolates exhibiting antagonistic activities against the indicator microorganisms were investigated for their antibacterial compounds. These isolates were grown for 12 h at 30 °C in MRS broth. Then, bacterial culture was harvested by centrifugation (4000×g, 10 min at room temperature) to obtain a cell-free supernatant (CFS) followed by a filtration through a 0.22 µm-pore-size cellulose acetate filter (Sartorius, Goettingen, Germany). Acid inhibition was ruled out by the adjustment of pH samples to pH 6.5 with 1 N of NaOH (Merck, Argentina). Inhibitory activity from hydrogen peroxide was ruled out by the addition of catalase (300 $IU.mL^{-1}$) (C9322, Sigma-Aldrich Chemie, Steiheim, Germany). The antagonistic activities of these samples were determined for each isolate by the AWDA as it was previously mentioned using *S. aureus* FBUNT as indicator microorganism. Proteinaceous nature of the antimicrobial compounds was confirmed by the addition of Trypsin (T 1426, Sigma-Aldrich Chemie, Steiheim, Germany) and Proteinase K (P6556, Sigma-Aldrich Chemie, Steiheim, Germany) at a final concentration of 1 $mg.ml^{-1}$ to the CFS. The samples were incubated for 3 h at 37 °C and immediately after, the residual activity

was determined by the AWDA for the indicator strain mentioned above.

2.6. Determination of Bacteriocin Title

The antimicrobial activity of each bacteriocin-like substance was quantified as means of the bacteriocin title. This number was determined from serial dilutions in sterile peptone water (0.1% $w.v^{-1}$) by the AWDA previously described. Arbitrary units (AU) per ml were calculated as AU=(1000/v)/d; being v: volume seeded in the well and d: dilution [14].

2.7. Adsorption of the Antimicrobial Substance to the Producer Cell

Following the method described by Yang *et al*. [15], the adsorption of antimicrobial substances to producer cells was studied. After 18 h of growth at 30 °C, each culture was adjusted to pH 6.0, the cells were harvested (10000×g, 15 min, 4 °C) and washed with sterile 0.1 M phosphate buffer (pH 6.5). The cells were resuspended in 10 mL 100 mM NaCl, adjusted to pH 2.0, stirred for 1 h at 4 °C and then harvested (12000×g, 15 min, 4 °C). The cell-free supernatant was neutralized to pH 7.0 with sterile 1 N NaOH and tested for activity as described elsewhere [16].

2.8. Effect of pH and Temperature on Bacteriocin Activity

The effect of pH on the bacteriocin-like substances was determined by adjusting the cell-free supernatant (CFS) to pH range between 2.0–10.0 with sterile 1 N HCl or 1 N NaOH. The pH values were measured with a glass electrode attached to a pHmeter (Oakton®, Eutech Instruments, Singapore). Then, the samples were sterilized by filtration, incubated for 2 h at 30 °C, and antimicrobial activity was determined by the AWDA. Negative controls, in order to elucidate the role of acid pH values in the inhibition of *S. aureus* FBUNT, were prepared by testing portions of non-inoculated MRS broth whose pH values were adjusted to 2.0-10.0. CFS from the bacteriocin-producer *Lactobacillus plantarum* ATCC 8014 was used as positive control [17].The effect of temperature on the bacteriocins was tested by heating the cell-free supernatants to 60, 80 and 100 °C, during 30 and 60 minutes. Autoclaving conditions were also tested, *i.e*. 121°C for 15 minutes. As control, sterile MRS were exposed to these temperatures and pH and tested against an indicator microorganism. To test the stability of the different CFSs during three freeze–thaw cycles, they were frozen at −20 °C during 24 h and thawed for 20 min at 5 °C. In all instances, a positive control, consisting of freshly prepared CFS was tested in parallel.

2.9. Phenotypic Characterization

Biochemical characterization was carried out using API 50 CH galleries (API System, BioMérieux, Montalieu Vercie, France) following the manufacturer's recommendations. The APILAB Plus computer-aided identification program version 4.0 (BioMérieux) was used to analyze the carbohydrate fermentation profiles obtained with the identification strips.

2.10. Statistical Analyses

The antimicrobial activity of LAB strains against different indicators was performed in three independent experiments in duplicate. The sensitivity of the cell-free supernatants to proteolytic enzymes, heat treatments and different pH values was performed in two independent experiments in duplicate. The sensitivity of supernatants to refrigerated storage and freeze–thaw cycles was performed in three independent samples in duplicate. Data were analyzed by analysis of variance (one way ANOVA) and by Tukey's test using a statistical software Statgraphics 5.0. Probability level was fixed to $p<0.05$.

3. RESULTS

3.1. Isolation and Preliminary Characterization of Lab

According to the selection criteria, namely those that characterize LAB, ten isolates (named after strains 1 to 10) were obtained from dry sausage samples and nineteen (named after strains 11 to 29) from cheese samples. Among the twenty-nine isolates, twenty-five showed to be bacilli while the rest showed *cocci* morphology.

3.2. Spectrum of Antimicrobial Activity

All the isolates were tested against the previously mentioned indicator strains. It is remarkable that four of the indicator strains, *i.e*. *S. aureus* FBUNT, *S. aureus* 65, *B. thermosphacta* 405 and *Ent. faecium* TW 20, were inhibited by the twenty-nine isolates. The information collected in this step is depicted in Table **1** where data can be appreciated in a more friendly fashion. Remarkably, the three pathogenic strains of the species *S. aureus* had been inhibited by almost the whole group of isolates (isolates 15 and 27 did not inhibit *S. aureus* ATCC 29213). Moreover, twenty-two

Table 1: Inhibitory Spectrum of LAB Isolates from Goat Products

Indicator Strain	Isolated Strains																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
S. aureus FBUNT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus ATCC 6538	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus ATCC 29213	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
L. innocua ATCC 33090	-	-	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. coli FBUNT	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. thermosphacta 405	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. faecium TW 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. curvatus ACU-1	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
L. sakei 268	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. curvatus 304	-	-	+	-	-	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Positive signs (+) indicate antimicrobial activity. Negative signs (-) indicate nule antimicrobial activity.

isolates inhibited *L. innocua* ATCC 33090 growth and twenty-one of them inhibited *E. coli* FBUNT. As these inhibitions could be the cause of acid, hydrogen peroxide, catalase and/or bacteriocin production, a characterization of the antimicrobial substance produced by the bacteria was done.

3.3. Characterization of Antibacterial Compounds

Among the isolates that exhibited antagonistic properties against target microorganisms, only twenty kept their antimicrobial activity in the CFS. From this group, half of the isolates lost their antimicrobial activity when samples were treated with catalase. Moreover, the inhibitory substances were not sensitive to proteolytic enzymes showing that inhibition was due to the production of hydrogen peroxide. Isolates 5, 8, 14, 19, 21, 22, 23, 24, 26 and 29 kept their antibacterial activity against *L. innocua* ATCC 33090, *S. aureus* FBUNT and *S. aureus* ATCC 6538 in the CFS and they were also sensitive to proteolytic enzymes. Therefore, the CFSs from these strains were considered to contain bacteriocin-like substances (BLIS) and they were chosen for further studies. The titles of BLIS were within the range 100-200 $AU.mL^{-1}$.

3.4. Adsorption of Bacteriocins to Producer Cells

No activity of bacteriocins was detected after treatment of 18 h-old cells of the ten selected strains with 100 mM NaCl and adjusted to pH 2.0. Therefore, bacteriocins did not adsorb to the surface of the producer cells during fermentation.

3.5. Effect of pH And Temperature on Bacteriocin Activity

Bacteriocin-like substances from the selected ten strains remained stable after incubation (30 °C) within the range of pH values tested. Antimicrobial activity of BLIS is reported in Table **2**, where it can be observed that this activity was higher at acidic pH values, showing a decrease when these values were closer to neutrality or they become alkaline.

Treatment of the extracellular extracts of these strains at the selected temperatures did not elicit any loss of antimicrobial activity against the indicator strains (data not shown). The results of bacteriocin stability throughout the refrigerated storage time showed that the maximum inhibitory activity remained constant up to 21 days when the supernatant was stored at 5 °C. In addition, 100% of the initial activity was observed after the three freeze-thaw cycles to which the CFSs were subjected.

3.6. Phenotypic Identification

To establish the preliminary identity of the isolates, they were submitted to further biochemical characterization using API 50 CH galleries. The strains were identified as: *Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*, *Lactobacillus paracasei* ssp. *paracasei*, *Lactococcus lactis* ssp. *lactis* and *Lactobacillus rhamnosus* (Table **3**), being *L. paracasei* ssp. *paracasei* the more recurrent species since six of the strains corresponded to this characterization.

Table 2: Influence of pH on Free-Cell Supernatant Antimicrobial Activity of the Isolated Strains

pH Treatment	Strain Producers									
	5	8	14	19	21	22	23	24	26	29
2	8.0±1.0[A a]	9.5±0.5[a]	10.1±0.5[c]	12.2±0.5[d]	8.4±0.5[b]	8.5±0.5[b]	8.1±0.6[b]	9.3±0.5[d]	8.4±0.5[b]	10.3±0.5[b]
3	8.5±0.5[a]	9.0±0.5[a]	7.5±0.5[b]	9.5±0.5[c]	8.3±0.2[b]	7.6±0.3[b]	8.2±0.5[b]	9.1±0.7[d]	8.0±0.5[b]	8.5±0.5[a]
4	8.4±0.5[a]	8.5±1.0[a]	6.5±0.5[a]	8.5±0.5[b]	8.5±0.1[b]	7.4±0.5[b]	8.1±0.4[b]	8.5±0.5[c]	8.0±0.5[b]	8.2±0.5[a]
5	8.0±1.0[a]	7.7±0.5[a]	6.0±0.5[a]	8.0±0.4[b]	6.5±0.5[a]	7.5±0.5[b]	6.5±0.4[a]	7.4±0.5[b]	8.1±0.3[b]	8.3±0.3[a]
6	8.1±0.7[a]	-[B]	-	7.2±0.6[a]	6.0±0.5[a]	6.5±0.3[a]	6.5±0.1[a]	6.5±0.6[a]	7.1±0.5[a]	8.0±0.5[a]
7	8.0±0.5[a]	-	-	7.0±0.5[a]	-	-	-	6.5±0.5[a]	7.0±0.5[a]	8.1±0.1[a]
8	8.1±0.5[a]	-	-	7.0±0.2[a]	-	-	-	-	-	8.2±0.2[a]
9	8.0±0.5[a]	-	-	-	-	-	-	-	-	8.0±0.5[a]
10	8.0±0.5[a]	-	-	-	-	-	-	-	-	8.1±0.1[a]
Control[C]	8.5±0.5[a]	7.4±0.5[a]	7.3±0.4[b]	8.0±0.5[b]	8.3±0.3[b]	7.1±0.2[b]	8.0±0.5[b]	7.2±0.5[b]	7.5±0.5[a]	8.4±0.5[a]

Antimicrobial activities were performed in two independent experiments by duplicate.
Residual activity was determined by agar diffusion assay against *S. aureus* FBUNT.
[A]. Inhibition halos (mm) by the agar well diffusion assay.
[B]. No inhibition zone.
[C]. Control samples consisting of freshly prepared cell-free supernatants without treatment.
[a-d]. The same superscript lowercase letters within a column denote no significant differences (P > 0.05) between values according to Tukey's method

Table 3: Characteristics of the Ten Strains Producing Bacteriocin-like Substances Isolated from Goat Artisanal Products

Isolate	Goat Food Source	Cell Morphology	Presumptive Genotipic Identification
5	Dry sausage	bacilli	*Leuconostoc mesenteroides* ssp. mesenteroides/dextranicum
8	Dry sausage	cocci	*Lactococcus lactis ssp. lactis*
14	Cheese	bacilli	*Lactobacillus rhamnosus*
19	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*
21	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*
22	Cheese	cocci	*Lactococcus lactis ssp. lactis*
23	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*
24	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*
26	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*
29	Cheese	bacilli	*Lactobacillus paracasei ssp. paracasei*

4. DISCUSSION

Numerous studies can be found on the isolation of LAB from goat milk cheeses manufactured worldwide [1, 18-21]. Despite that, to our knowledge, little evidence has been found on the microbiota of dry sausages made with goat meat [2]. Regardless the source of the bacteria, whether if it is from milk or meat derived products, the results presented herein can be considered the first report on goat products microbiota belonging to the province of Chaco, Argentina. Twenty-nine LAB strains were isolated from both sources. All these bacteria had the ability to inhibit at least five of the ten indicator microorganisms chosen for this work. More than twenty of the isolates had antimicrobial activity against all the pathogenic strains tested. Moreover, all the isolated strains inhibited the growth of *B. thermosphacta* 405, a ubiquitous spoilage bacteria associated to cooked meat products. Although this inhibition could not be attributed immediately to the production of bacteriocins, it showed us the potential application of these strains in the control of foodborne pathogens since they produce antimicrobial metabolites such as lactic acid, hydrogen peroxide and catalase.

Ten of the isolates tested produced bacteriocin-like inhibitory substances (BLIS). As a consequence, the study of several technological attributes was carried out in order to determine whether these substances would be useful biopreservatives when they were applied to food products. Firstly, adsorption to producer cells was examined for all the isolates. The results showed that no adsorption took place in any of the bacteriocinogenic strains in concordance with other authors [22-24]. This fact is of great importance if the substance is meant to be produced *ex situ* since the bacteriocin would be easily removed from the batch culture by peptides precipitation with 60% ammonium sulphate [25]. Secondly, the influence of pH environment and thermal treatments on BLIS was analyzed. Among other environmental conditions, a bacteriocin should resist pH variations so as to be used as a potential antimicrobial agent. The pH variations (2.0-10.0 range) influenced the antimicrobial activity of the ten BLIS. Maximum inhibitory effects were registered at low pH values, findings that are in agreement with previous reports referring to bacteriocinogenic LAB from fermented products which described the highest antimicrobial activity at acidic pH values [26, 27]. Acidification enhances the antibacterial activity of bacteriocins due to the increase in net charge of bacteriocins at low pH which might facilitate translocation of bacteriocin molecules through the cell wall. The solubility of bacteriocins may also increase at lower pH, facilitating diffusion of bacteriocin molecules [28]. However, it should be noted that strains 5 and 29 kept their antimicrobial activity without any changes throughout the pH range tested; similar results were observed by Noonpakdee *et al*. [29] and Todorov and Dicks [16]. Finally, it was observed that BLIS antimicrobial activity was not significantly affected by the different thermal treatments applied to them, even the autoclaving process. This stability is remarkable since many authors reported a complete loss of activity after certain thermal treatments. For example, *L. lactis* lowered its antimicrobial activity after an exposure time of 10 and 20 minutes at 60 and 80°C [30, 31]. Thermal resistance could be advantageous if BLIS are expected to be effective in products subjected to cooking or smoking processes.

The production of artisanal dry sausages implies no use of starter cultures. The indigenous microbiota found in these products comes mainly from environmental bacterial contamination after slaughtering and manufacturing processes [32]. Preliminary genetic characterization was carried out using fermentation profiles (API CH50 galleries). From goat's meat dry sausages two different LAB species were characterized. In accordance with the findings from Oki *et al*. [2], *Leuconostoc mesenteroides* was one of the strains, while the other corresponded to the species *Lactococcus lactis.* Although this species is generally encountered in dairy products, few authors reported its presence in fresh meat or meat products [33, 34, and 35]. Ben Belgacem *et al*. [36] reported on the isolation of *L. lactis* MMZ25 from artisanal Tunisian fermented meat. The rest of the bacterial species corresponded to the ones found in the sampled goat's milk cheeses and they represent the typical bacteria frequently found in these ecological niches. Papanikolaou *et al*. [37] reported on the isolation of *Lactobacillus paracasei* ssp. *paracasei* from Melichloro cheese, a Greek hard cheese made from fresh sheep and goat milk. Besides, Sánchez *et al*. [19] identified ten species with predominance of *Lactobacillus paracasei* subsp. *paracasei* in Spanish goat cheeses. Oliszewski *et al*. [38] reported that 15% of the isolated LAB strains from Argentinian goat's milk and cheeses were *L. rhamnosus*. Finally, several strains of *L. lactis* act as mesophilic starter cultures in Greek traditional cheeses made with goat's milk or a mixture of goat and sheep milks [39].

5. CONCLUSION

The results presented in this study could contribute to preliminarily characterize the microbiota of artisanal caprine products manufactured in the north-eastern region of the province of Chaco, Argentina. Furthermore, this is the first report on bacteriocinogenic microbiota from goat's milk cheese and goat's meat dry sausages manufactured in this region. New ways of preservation would have a positive impact on the evolution and development of caprine products in Argentina, which still have a long road ahead. The ten bacteriocinogenic strains isolated herein could be used as protective or starter cultures, as part of hurdle technology. However, more experiments must be carried out to study maximum bacteriocin production under specific environmental conditions (nutrient availability, NaCl concentration, presence of additives, among others), and to elucidate chemical structure of the molecules produced to facilitate massive production and purification procedures. The next necessary stage would be the molecular identification of the strains in order to confirm their genetic identity.

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