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ORIGINAL ARTICLE

Bacteriocinogenic Bacteria Isolated from Raw Goat Milk and Goat Cheese Produced in the Center of México

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Abstract Currently, there are few reports on the isolation of microorganisms from goat milk and goat cheese that have antibacterial activity. In particular, there are no reports on the isolation of microorganisms with antibacterial activity from these products in central Mexico. Our objective was to isolate bacteria, from goat products, that synthesized antimicrobial peptides with activity against a variety of clinically significant bacteria. We isolated and identified *Lactobacillus rhamnosus*, *L. plantarum*, *L. pentosus*, *L. helveticus* and *Enterococcus faecium* from goat cheese, and *Aquabacterium fontiphilum*, *Methylibium petroleiphilum*, *Piscinobacter aquaticus* and *Staphylococcus xylosus* from goat milk. These bacteria isolated from goat cheese were able to inhibit *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *L. inoccua*, *Pseudomona aeruginosa*, *Shigella flexneri*, *Serratia marcescens*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. In addition, bacteria from goat milk showed inhibitory activity against *B. cereus*, *L. lactis*, *E. coli*, *S. flexneri*, *E. cloacae* and *K. pneumonia*; *S. aureus*, *L. innocua*, *S. agalactiae* and *S. marcescens.* The bacteriocins produced by these isolates were shown to be acid stable (pH 2–6) and thermotolerant (up to 100 °C), but were susceptible to proteinases. When screened by PCR for the presence of nisin, pediocin and enterocin A genes, none was found in isolates recovered from goat milk, and only the enterocin A gene was found in isolates from goat cheese.

Keywords Goat milk · Goat cheese · Bacteriocins · Enterocin A · Pathogenic bacteria

Introduction

Different types of commercial milk, including goat, cow and sheep milk, are produced worldwide for human consumption. Goat milk is consumed less than cow milk and represents ~2 % of the global milk source. In 2012, goat milk production worldwide was ~16.2 billion liters, with México

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being the largest producer (~155.5 million liters) in Latin America [1]. Although goat milk is mainly consumed as a raw product, it has been used for production of cheese and yogurt, usually at the farm level, in small dairies and in informal retail sales [2]. Recently, goat milk has gained interest mainly because of its iron bioavailability, higher concentration of fatty acids and lower allergenicity [3].

It has been reported that the microbiota in goat milk is composed primarily of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus* species, bacteria with known probiotic and bacteriocinogenic properties. In addition, goat milk samples have been shown to harbor halophilic bacteria (e.g. *Jeotgalicoccus psychrophilus* and *Salinicoccus* sp.), though this finding may be atypical [3–5]. The isolation of potential pathogenic bacteria in raw goat milk, such as *Staphylococcus aureus* and *Escherichia coli* [6], have also been reported. The antimicrobial activity of bacteria isolated from goat milk has been linked to different compounds, including lactic acid, hydrogen peroxide, and bacteriocins (antimicrobial peptides) [7]. Bacteriocins, in particular, are ribosomally synthesized proteins with known biopreservative and antimicrobial activities. Recently, nisin, lacticin, and enterocin AS-48 were found to be produced by lactic bacteria isolated from raw milk of ewes, goats and cows, collected from different farms in Central Spain [8].

In Mexico, only a few reports on the microbiota of goats, and the physical and chemical properties of goat milk have been published [2, 9]. These include an analysis of cheese produced in a desert rangeland and activity of bacteriocins against potential etiological agents of mastitis [2, 10]. Although it has been shown that the dominant bacterial population in goat milk is lactic bacteria, the microbiota can vary depending on goat breeds, nutrition, weather conditions and animal health [11]. To our knowledge there are no reports on the isolation of microorganisms with antibacterial activity from goat milk and cheese produced in central Mexico. Here we report on the isolation of bacteria from these products, and show that they produce peptides active against a wide variety of clinically significant bacteria.

Materials and Methods

Goat Milk and Cheese Sampling

Samples of raw goat milk were collected using aseptic conditions directly from the udder of Saneen goats in dairy farms located in Apaseo el Alto, Guanajuato, México, and samples were stored at 4 °C until further testing. Goat cheese samples (natural and ash type) were obtained from local supermarkets in the state of Guanajuato, but they were manufactured in a national company located in the state of Querétaro, México. As our purpose was to isolate bacteriocinogenic bacteria, we only obtained two samples of each type of cheese. Ten grams of each cheese was aseptically collected and homogenized in 90 mL of sterile 0.85 % (w/v) NaCl and used for further microbiological analysis.

Bacterial Isolation

Milk and cheese samples were diluted (10^{-5}–10^{-6}) using sterile 0.85 % (w/v) NaCl, plated on MRS (Man, Rogosa and Sharpe) agar (Difco, Becton–Dickinson, Franklin Lakes, NJ, USA), MRS-1 % (w/v) glucose, MRS-1 % (w/v) lactose, and peptonized milk agar. Cultures were incubated at 37 °C for 24–72 h under both aerobic and anaerobic conditions. For primary selection, 300 colonies were selected and grown on MRS agar at 37 °C for 24 h. Then based on colony morphology, Gram stain, and catalase test, 44 colonies (15 from milk, 20 from ash style cheese and 9 from natural cheese) were selected for further study.

Preliminary Identification of Bacteria by the Overlay Method

A single colony of the 44 isolates was taken and inoculated in duplicate parallel streaks (1 cm) on MRS agar plates at 37 °C. One of the MRS plates was overlaid with trypticase soy agar (0.75 % w/v) containing 105 µL of each indicator strain with ~1×10^8 CFU/mL of the indicator bacterium and incubated at 37 °C for 24 h. Antibacterial activities were detected by the formation of zones of inhibition around the colonies. The other plate was used to select bacteriocinogenic bacteria that showed inhibitory effects in the overlay assay [12]. Indicator bacteria were: *Bacillus cereus* 183, *Listeria monocytogenes*, *L. innocua*, *Micrococcus luteus*, *S. aureus* and *E. coli* ATCC 25922 (Gram-negative bacterium commonly used as control strain for antimicrobial susceptibility test) (www.atcc.org). From the 44 colonies, we selected 12 isolates (7 from cheese and 5 from milk), which showed the relatively higher inhibitory activity against the inhibitor bacteria. These isolates were labeled as MAe1, MAe2, MAe5, LPAe1, LPAn2, LC20, LC22, MN24, MN26, MN27, MN28, and MN29 (Table S1), which were subsequently identified by sequencing of the 16S rDNA.

Bacterial Identification Amplification and Sequencing of the 16S rDNA

The twelve bacteria selected were cultivated in 3 mL of MRS at 37 °C overnight and DNA was extracted as described before [13]. Oligonucleotides UBF (F: 5′-

AGAGTTTGATCCTGGCTGAG-3′) and 1492 (R: 5′-GGTTACCTTGTTACGACTT-3′) [14] were used to amplify 16S rDNA sequences with the following conditions: 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 58 °C and 90 s at 72 °C, with a final extension of 5 min at 72 °C in a C1000 Touch™ thermocycler (Bio-Rad). Amplicons were purified from agarose gels using the QIAprep Spin Miniprep Kit (250) (Qiagen) and sequenced at the National Laboratory of Genomics for Biodiversity (Langebio, at CINVESTAV-Irapuato, México). Once sequences were obtained, the ambiguous bases from the 5′ and 3′ terminal were deleted, and the resultant sequences were submitted to the GenBank nucleotide database (www.ncbi.nlm.nih.gov) for comparison.

Screening for Nisin, Enterocin A, Pediocin Genes by Polymerase Chain Reaction (PCR)

DNA from the twelve bacteria was used for PCR amplification of nisin, enterocin A and pediocin genes using Taq polymerase (Invitrogen, Carlsbad CA, USA) and gene specific primers; for nisin, nisRF (5′-CTATGAAGTTGC GACGCATCA-3′), nisRR (5′-CATGCCACTGATACCC AAGT-3′); for enterocin A, entAF (5′-GGGTACCACT CATAGTGGAA-3′); enterocin, entAR (5′-CCAGCAGTT CTTCCAATTTCA-3′); and pediocin, pedF 5′-GGTAAG GCTACCACTTGCAT-3′), pedR (5′-CTACTAACGCT TGGCTGGCA-3′) [15]. Amplification was performed in a C1000 Touch Thermal Cycler (Bio-Rad) using the following conditions: 5 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 58 °C and 90 s at 72 °C, with a final extension of 5 min at 72 °C. Amplicon sizes were compared in an agarose gel with those obtained from *Lactococcus lactis* subsp. *lactis* ATCC 19435 (Microbial Culture Collection, Micro 500 CINVESTAV Mexico City), *Enterococcus faecium* UQ1 (provided by Dr. Blanca Garcia Almendarez, Autonomous University of Queretaro, México) and *Pediococcus acidilactici* (provided by Dr. Blanca Escudero-Abarca, North Carolina State University, USA), which synthesize nisin (608 bp), enterocin A (412 bp) and pediocin (332 bp), respectively. Amplicons were submitted for sequencing to the Langebio laboratory (CINVESTAV-Irapuato, México) and sequences were compared with those reported in the NCBI databases (www.ncbi.nlm.nih.gov).

Bacteriocins Production

To study the kinetics of bacteriocins production in the twelve selected bacteria, and to detect the time where the highest inhibitory activities were produced in growth curves, strains were cultured in MRS for 24 h at 37 °C to achieve $\sim 10^8$ -cells/mL. Then 250 µL of the culture was added to 250 mL fresh MRS and incubated at 37 °C, and duplicate samples were collected at 2 h intervals over a 24-h period. One of the samples was monitored spectrophotometrically at O.D. = 600 nm. The second sample was centrifuged and the pH of the supernatant was adjusted to 6.8 with 5 N NaOH, filtered through a 0.20-mm filter and the antibacterial activity was evaluated using the well-diffusion method [12, 16]. Twenty-five millilitre of TSB with soft agar 0.7 % (w/v) was mixed with 50 µL (1×10^9 cells/mL) of the indicator bacteria and plated. As *E. coli* ATCC 25922 was one of the most susceptible bacterium in this work, it was used as indicator bacterium to study the kinetics of the bacteriocins production. Once the medium solidified, wells, 8 mm diameter, were dug into the agar under sterile conditions. Plates were stored 2 h at 27 °C to dehumidify, and 90 µL of the supernatants were added to the wells and incubated for 12 h at 4 °C to allow diffusion of the liquid, followed by incubation for 24 h at 37 °C before diameters of zones of inhibition were measured. The minimum detectable zone measured was 1 mm beyond the well diameter. Assays were repeated in triplicate and the average was recorded. One arbitrary unit of bacteriocin activity (U) was defined as equal to 1 mm^2 of the zone of inhibition of growth of the indicator bacterium [12].

Partial Purification of Proteins with Antimicrobial Activity

The twelve bacteria were cultivated in 200 mL of MRS broth for the time where the highest inhibitory activities were detected in growth curves. Cell-free culture supernatants were concentrated with ammonium sulfate to 80 % saturation at 4 °C with constant stirring overnight. Precipitated proteins were pelleted by centrifugation at 16,000×*g* for 30 min at 4 °C, resuspended in 100 mM phosphate buffer (pH 6.8), and dialyzed overnight against the same buffer using a mini-dialysis kit with a 1 kDa cut-off (Amersham Biosciences). Protein concentration was determined using the Quick Start Bradford 1× Dye reagent (BioRad, Hercules CA, USA) in a SINERGY HTX multimode reader™ (BioTek, USA). Antimicrobial activity was determined by the well-diffusion method against different bacteria (e.g. *M. luteus*, *B. cereus*, *Streptococcus agalactiae*, *L. lactis*, *E. coli* ATCC 25922, *Shigella flexneri*, *Serratia marcescens*, *Enterobacter cloacae* and *Klebsiella pneumoniae*, among others) (Tables S2 and S3), as previously reported [12].

Sensitivity to Enzymes, Heat and pH

To confirm the proteinaceous nature inhibitory substances, partially purified protein samples were treated with different enzymes, including proteinase K (New England BioLabs, Ipswich, MA), peptidase (Sigma-Aldrich Co., St.

Louis, MO), trypsin (Sigma-Aldrich Co., St. Louis, MO), protease (Sigma-Aldrich Co., St. Louis, MO), lysozyme (Sigma-Aldrich Co., St. Louis), and amylase (Sigma-Aldrich Co., St. Louis, MO), each at the final concentration of 1 mg/mL. Untreated samples with buffer, buffer alone or enzyme solutions were used as controls. Reactions were incubated at 37 °C for 2 h according to manufacturer's protocol. Treated samples and controls were assayed by the well diffusion method against the indicator bacterium [12].

To determine the effect of temperature on inhibitory activity, aliquots of partially purified peptides at pH 4 were stored at different temperatures (60, 70, 80, 90 and 100 °C) for 30 min and then assayed by the well diffusion method. The pH stability of the partially purified substances was estimated after 24 h of storage at 4 °C in 100 mM of citrate (citric acid + sodium citrate, pH 2, 4) and phosphate (KH_2PO4 + K_2HPO4, pH 6, 8) buffers. Activity was evaluated by the well diffusion assay [12].

Results

Bacterial Identification and Determination of the Time with the Highest Inhibitory Activity

We isolated seven bacterial strains from goat cheese and five bacterial strains from goat milk (Table S1). Based on 16S rDNA sequences, isolates from goat cheese were identified as *Lactobacillus rhamnosus* (LC20), *L. plantarum* (LC22), *L. pentosus* (MN24), *L. plantarum* (MN26, MN29), *L. helveticus* (MN27) and *E. faecium* (MN28). In addition, isolates from goat milk were identified as *Aquabacterium fontiphilum* (strain MAe1), *A. fontiphilum* (MAe2), *Methylibium petroleiphilum* (MAe5), *Staphylococcus xylosus* (LPAe1) and *Piscinibacter aquaticus* (LPAn2) (Table S1).

When bacteria were cultivated and assayed for inhibitory activity at different times, each isolate showed highest inhibitory effect against *E. coli* ATCC 25922 at ~22 h. Bacterial isolates from goat cheese showed similar behavior in the growth curve. For example, with *L. helveticus* (MN27), *E. faecium* (MN28), and *L. plantarum* (MN29), the bacteriocin activity was observed in sample collected at middle of the logarithmic phase and achieved the highest level at the stationary period. Alternatively, bacteria isolated from goat milk also showed the highest inhibitory effect against *E. coli* in the stationary phase (data not shown), except *M. petroleiphilum* (MAe5), which showed the highest inhibitory activity at the death phase (Fig. 1).

Screening of Bacteriocin Genes

When we amplified genes from the seven isolates obtained from goat cheese, enterocin A amplicons were detected (~0.4 kbp) (Fig. S1). Amplicons of nisin (0.608 kbp) or pediocin (0.412 kbp) genes were not observed in these bacterial strains. Additionally, amplicons of nisin, enterocin A and pediocin were not obtained from the five isolates from goat milk.

Inhibitory Activity of Partial Purified Antimicrobial Peptides

The seven bacteria isolated from goat cheese inhibited *M. luteus*, *S. aureus*, *B. cereus* and *E. coli*, but were not active against *E. faecalis*, *S. pyogenes* and *Salmonella* spp. (Table S2). Strains also showed inhibitory activity against *S. aureus*, *L. monocytogenes*, *L. inoccua*, *Pseudomona aeruginosa*, *S. flexneri*, *S. marcescens*, *E. cloacae* and *K. pneumoniae.* In addition, all bacteria isolated from goat milk showed inhibitory activity against *M. luteus*, *B. cereus*, *L. lactis*, *E. coli*, *S. flexneri*, *E. cloacae* and *K. pneumoniae*. Only *A. fontiphilum* Mae2 (or Mae1), *M. petroleiphilum* MAe5 and *P. aquaticus* LPAn2 showed activity against *S. aureus*, *L. innocua*, *S. agalactiae* and *S. marcescens* (Table S3).

To compare the activity of crude bacteriocins, we determined the protein concentration in each sample and then tested them against *E. coli* ATCC 25922 using approximately the same protein concentration. Activity was evaluated using arbitrary units (U). As explained previously, *E. coli* ATCC 25922 was selected for this assay, as it was one of the most susceptible bacterium observed in this work. When we compared the activity of crude bacteriocins of *A. fontiphilum* Mae1, *S. xylosus* LPAe1 and *P. aquaticus* LPAn2, the highest activity (~126 U) was observed with *A. fontiphilum* Mae1. Antimicrobial peptides produced by bacteria isolated from goat cheese had activities of ~200 U, but the highest value (~296 U) was observed with sample from *L. pentosus* MN24 (Table 1).

Effect of Enzymes, Temperature, pH and Solvents in the Inhibitory Activity

To determine the nature of partially purified antimicrobial peptides, preparations were digested with various enzymes and treated at different temperatures, pH and chemicals, and then the inhibitory activity were tested against *E. coli* ATCC 25922. The inhibitory effect were completely lost or drastically reduced after treatment with protease K, and trypsin indicating the proteinaceous nature of these bactericidal compounds. Antimicrobial peptides were thermoresistant, even at 100 °C. Most of the crude bacteriocin samples had activity at low pH but lost their inhibitory effect at pH 8. Antimicrobial peptides were resistant to methanol and ethanol (Table 2).

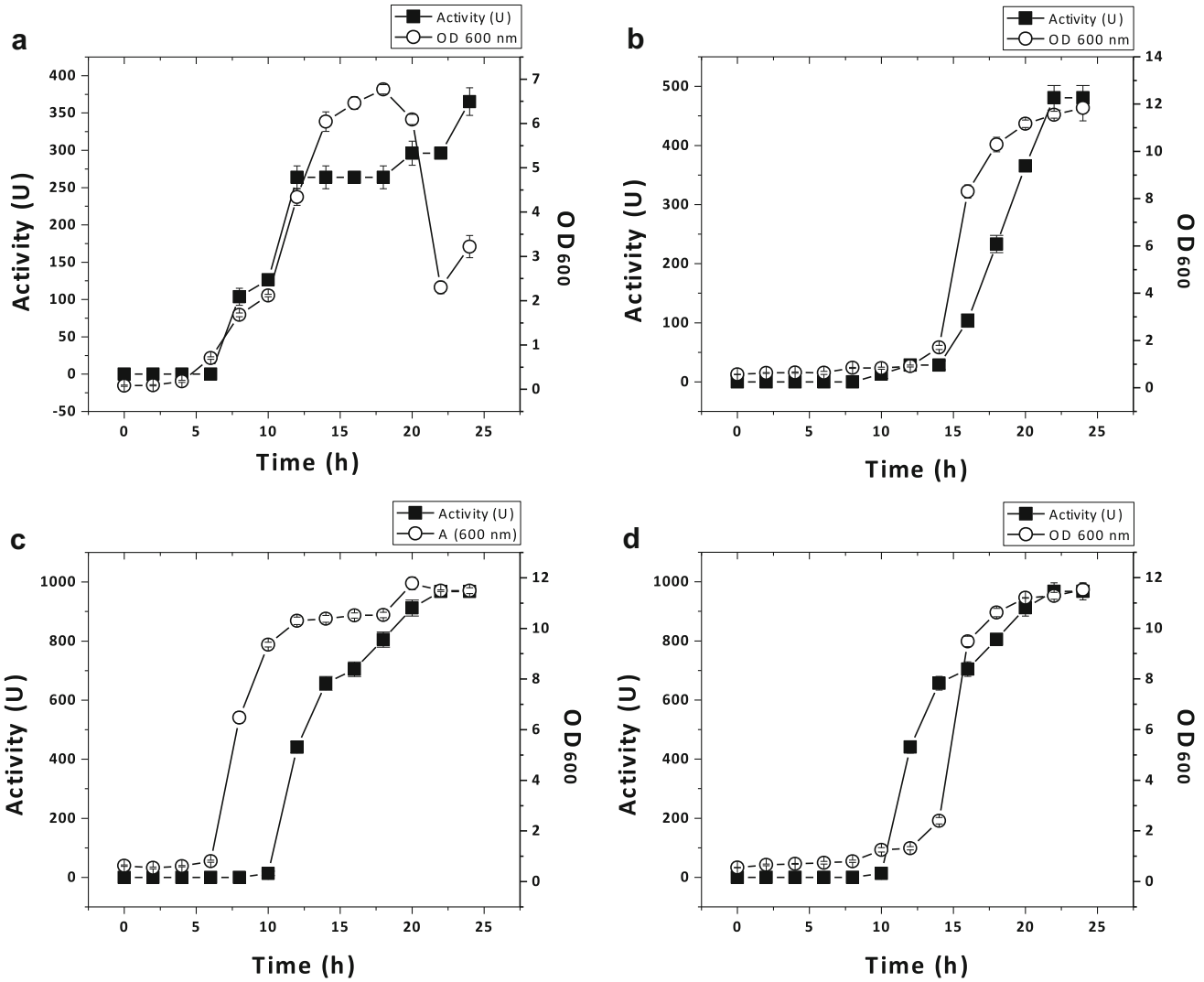

Fig. 1 Correlation between growth and the appearance of inhibitory activity in culture medium. Bacteria were grown in MRS broth and duplicate samples were collected at ~2 h intervals and the inhibitory activity was tested against *E. coli* ATCC 25922. **a** *M. petroleiphilum* MAe5, **b** *Lactobacillus helveticus* MN27, **c** *E. faecium* MN28, and **d** *L. plantarum* MN29

Discussion

In the present study we isolated an identified bacteria from goat milk and goat cheese produced in the center of Mexico which synthesize bacteriocins that inhibit a wide variety of clinically significant bacteria. From goat cheese, we identified lactic bacteria that synthesize enterocin A, whereas in goat milk we selected microorganisms that may not represent the common microbiota found in this product, and most probably were environmental contaminants. In our initial qualitative assay, we focused only on bacteria with the broadest and highest inhibitory effect against *B. cereus* 183, *L. monocytogenes*, *L. innocua*, *M. luteus*, *S. aureus* and *E. coli* ATCC 25922, without taking into consideration whether they belong to the normal microbiota or they were environmental contaminants. It was interesting that *E. coli* ATCC 25922 was one of the most susceptible bacterium in this work, as frequently Gram-negative bacteria are resistant to enterocins. However, the susceptibility of *E. coli* ATCC 25922 to enterocin has been reported previously [17].

From goat cheese, we isolated and identified lactic bacteria such as *Lactococcus rhamnosus*, *L. plantarum*, *L. helveticus* and *E. faecium*, microorganisms that frequently occur in goat milk [18]. All isolates putatively produced enterocin A, a bacteriocin commonly synthesized by *Enterococcus* strains [8], although other bacteria, such as *L. lactis* [19] are known to synthesize this peptide. Additionally, as the nisin gene is commonly found in lactic bacteria [8], we expected to find this gene in the *Lactococcus* species isolated in this study, but were unable to do so. It is possible that the corresponding nisin gene might be

Table 1 Comparative inhibitory activity of partially purified bacteriocins[a] against *E. coli* ATCC 25922 using different protein concentrations

Source	Strains	mg protein/mL[b]	Activity (U)[c]
Goat's milk	*A. fontiphilum* Mae1	15	126.4494 ± 7.6
	A. fontiphilum MAe2	8.32	44.7678 ± 5.5
	M. petroleiphilum MAe5	10	28.2744 ± 5
	S. xylosus LPAe1	15	28.2744 ± 5
	P. aquaticus LPAn2	15	82.467 ± 6.5
Goat's cheese	*L. rhamnosus* LC20	5	263.8944 ± 10.2
	L. plantarum LC22	5	204.204 ± 9.1
	L. pentosus MN24	5	296.0958 ± 8
	L. plantarum MN26	5	263.8944 ± 10.2
	L. helveticus MN27	5	263.8944 ± 7.6
	E. faecium MN28	5	204.204 ± 6.8
	L. plantarum MN29	5	204.204 ± 9.1

[a] Bacteria were cultivated for the time where the highest inhibitory activities were detected in growth curves and supernatants were concentrated with ammonium sulfate to obtain unpurified bacteriocins

[b] Protein concentration was determined by the Bradford method (BioRad)

[c] One unit is defined as 1 mm^2 of the zone of inhibition as determined by the well-diffusion method (see text)

Table 2 Inhibitory effect of bacteriocins produced by different isolates against *E. coli* ATCC 25922 after treatments with enzymes, temperature, pH and organic chemicals

Strains	Enzymes		Temp (°C)			pH				Methanol	Ethanol
	Proteinase K	Trypsin	60	80	100	2	4	6	8		
A. fontiphilum Mae1	(−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+/−)	(+)
A. fontiphilum MAe2	(−)	(−)	(+)	(+)	(+/−)	(+)	(+)	(−)	(−)	(−)	(+)
M. petroleiphilum MAe5	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+/−)	(+)
S. xylosus LPAe1	(−)	(−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+/−)	(+)
P. aquaticus LPAn2	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+)
L. rhamnosus LC20	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)
L. plantarum LC22	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)
L. pentosus MN24	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)
L. plantarum MN26	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+)	(+)
L. helveticus MN27	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)
E. faecium MN28	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)
L. plantarum MN29	(+/−)	(+/−)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)

Bacteriocin samples were subjected to different treatments and then tested against *E. coli*. (+) indicates that bacteriocins are resistant to the treatment and retained inhibitory activity against *E. coli*. (−) Signal shows that bacteriocins were susceptible to treatment and did not show inhibitory activity against *E. coli*. (+/−) indicates that bacteriocins activity was reduced significantly against *E. coli*. Activity was determined using the well-diffusion method

present but have diverged such that it was unable to be amplified with the primers used is the study. Previously it has been reported that bacteria isolated from goat milk of sample obtained in farms from Spain harbor nisin, lacticin and enterocin genes [8]. In general, we found that antimicrobial peptides synthesized by bacteria isolated from goat cheese showed a broader spectrum of inhibitory activity compared with peptides from bacteria obtained from goat milk. When we treated the partial purified bacteriocins at different pH and temperatures, we found that they were acid-stable and thermotolerant, characteristics similar to other enterocins previously reported [20].

Interestingly, we did not isolate lactic bacteria from goat milk, but isolated microorganisms not commonly encountered in milk, i.e. *A. fontiphilum*, *P. aquaticus* and *M. petroleiphilum*; we suggest that these were likely environmental contaminants. To our knowledge these bacteria have not been reported in goat milk. Indeed, this does not

mean that goat milk lacks lactic bacteria, as these bacteria are the common microbiota in this product [3–5]. More likely, they were eliminated in our initial screen. In addition, *A. fontiphilum* and *P. aquaticus* have been previously isolated from water [21, 22], and *M. petroleiphilum* has been implicated in metabolism of oxygenate methyl tert-butyl ether [23]. There are no reports on the pathogenicity of *A. fontiphilum* and *M. petroleiphilum*, so their impact on human and animal health and safety is not known. We also isolated *S. xylosus*, a coagulase-negative staphylococcus that has been previously isolated from goat milk and cheese and is able to induce spontaneous infections under special conditions [24]. We were unable to amplify nisin, enterocin A and pediocin genes, and it is probable that these isolates produce other types of antimicrobial peptides or bacteriocins not yet characterized.

In general, the isolates described in this study produced antimicrobial peptides that inhibited known pathogenic microbes, including *S. aureus*, *L. monocytogenes*, *L. innocua*, *B. cereus*, *Str. uberis*, *P. aeruginosa*, *E. cloacae* and *K. pneumonia*, among others. Although bacteriocins produced by uncommon and lactic bacteria isolated in this study could play a primary role in suppressing or eliminating potential harmful microbes and commensals present in these natural products, it is possible that other bacteriocins not detected here, and/or other metabolites including lactic, acetic, and formic acids, and 2,3-butadione, acetaldehyde, and hydrogen peroxide could contribute to the biochemical integrity and stability of these products [25].

Conclusion

We report the isolation and identification of cultivable microbiota present in goat milk and cheese produced in the center of México, and show that they are able to synthesize antimicrobial peptides with activity against pathogenic bacteria significant in human and animal health. Although it is obvious that bacteria obtained from goat milk are environmental contaminants, it would be interesting to identify in future studies what kind of bacteriocins are synthesize by these bacteria, probably they represent novel antimicrobial peptides not reported yet.

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