Milk microbiota: a source of antimicrobial-producing bacteria with potential application in food safety

Alexandre Lamas*, Laura Sanjulián, Alberto Cepeda, Cristina Fente, Patricia Regal

Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Veterinary of Lugo, University of Santiago de Compostela

* alexandre.lamas@usc.es

Background

The discovery and use of antibiotics was a major advance in human health. Bacterial infections that could end in death began to be easily treated with these substances. Antibiotics also revolutionized animal production. These substances are used to treat animal infections but also were and still are used in some countries as growth promoters. Indiscriminate use of antibiotics results in the spread of antimicrobial resistance. Thus, at present there is a global problem due to the appearance of multi-resistance in pathogenic bacteria. This results in a significant increase in the number of deaths associated with multi-resistant bacteria. In the field of food science there is also increasing concern among consumers about the use of synthetic preservatives in food as some of them such as sulphites and nitrites have been linked to possible adverse effects on human health. there has also been an increase in the resistance of food pathogens to biocides commonly used in the food industry. Therefore, one of the main challenges of actual society is the search for alternative substances to antibiotics and preservatives. In the last years, microbiota from different sources has emerged as a potential source of natural substances with antimicrobial activity. Many of the bacteria present in the microbiota synthesize bacteriocins, ribosomally produced antimicrobial peptides synthesized by both Gram-positive and Gram-negative bacteria with activity against closely related bacteria (narrow spectrum) or a diverse group of bacteria (broad spectrum). Although some bacteriocins such as nisin are already known, there is still a large number to be discovered and evaluated.

Objective

The aim of this study was to isolate bacterial strains with potential antimicrobial activity against different pathogens from human and cow milk samples

Material and Methods

Milk samples

A total of 40 samples were included in this study for the isolation of strains with potential antimicrobial activity against different common pathogens. From those, 30 milk samples were collected aseptically from healthy woman in different breastfeeding stages. The other 10 samples were collected individually from different dairy cows. After collection samples were kept at -20ºC until use.

Isolation of antimicrobial-producing milk isolates

To isolate antimicrobial-producing milk isolates, milk samples were ten-fold serially diluted and spread-plated in BHI agar incubated aerobically at 37ºC for 48h and Columbia agar incubated anaerobically at 37ºC for 48h. BHI and Columbia agar incubated plates were overlaid with MRS sloppy agar containing 0.25% of and overnight culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* LMG 6901 used as indicator of antimicrobial products production. Plates were incubated anaerobically 24h at 37ºC. Colonies from BHI that exhibited zones of inhibition on MRS agar were stocked.

Well diffusion assays (WDA)

Milk isolated strains were growth in 50mL polystyrene filled with 20mL of BHI medium for 48h at 37ºC. After incubation, the growth media were centrifuged at 5,000 x*g* for 15 minutes. The cell free supernatant (CFS) was collected and filtered using 0.22µm syringe filters and saved at -20ºC until use. To eliminate inhibition due to acids produced during bacterial growth, the pH of the CFS was determined and neutralized to pH 7.0-7.2 using 2M NaOH. The antimicrobial activity of CFS was determined against eight different indicator bacteria (Table 1). After agar solidified, 5mm diameter holes were made and filled with 75µL of the milk isolated strain WDAs. The plates were incubated for 24h at 37ºC ant the inhibition zones were measured.

Results

A total of 32 colonies with potential antimicrobial activity according the overlaid result with L. delbrueckii ssp. bulgaricus LMG 6901 were isolated. From those 28 colonies were isolated from breast milk and 4 colonies were isolated from cow milk. The neutralized CFS of 10 strains showed antimicrobial activity against at least one pathogen tested in well diffusion assays. Seven strains were isolated from breast milk and 3 strains were isolated from cow milk. Eight of the 10 CFS inhibited the growth of *S. aureus*. These CFS also showed activity against *S. epidermidis*, *S. agalactiae*, *P. aeruginosa* and *L. monocytogenes*, *C. perfringens* and one strain against *C. diffile* (Figure 1).

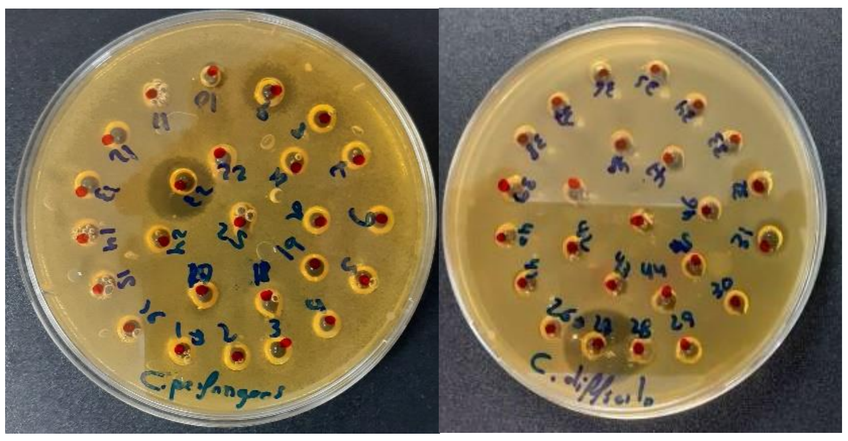

Figure 1. Well diffusion assays with *C. perfringes* and *C. difficile*.

Conclusions

Breast milk and cow milk microbiota is a source of antimicrobial-producing bacteria. In the next steps of the work, the strains isolated from breast milk and cow milk will be identified by 16s rRNA sequencing and mass spectrometry will be used to determine the antimicrobial product produced by isolated.

Table 1. Spectrum of inhibition of bacteriocin-producing milk isolates against indicator strains according well diffusion assays.. + <50mm2, ++50-150 mm2, +++150-249 mm2, ++++150-249 mm2.

Indicator/strain	LM18.1	LM16.1	LM61	LM95	LM27	LM12	LM88	L3.1	L3.2	L6.10
Staphylococcus epidermidis CECT 59	-	-	-	+	-	-	-	-	-	-
Staphylococcus aureus CECT 231	-	-	+	+	+	+++	+	++++	+	+
Streptococcus agalactiae CECT 183T	+	++	++++				+++	-	-	-
Listeria monocytogenes CECT 934	-	-	-	-	-	-	-	+++	-	-
Clostridium perfringens CECT 376T	-	-	+	-	-	-	-	-	-	-
Clostridioides difficile CECT 531T	++	+++	-	-	-	-	-	-	+++	++++
Salmonella Typhimurium CECT 4594	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa CECT 108	-	-	-	-	-	-	-	+	-	-

UNIVERSIDADE DE SANTIAGO DE COMPOSTELA
Facultade de Veterinaria, 27002 (Lugo)
Contact: alexandre.lamas@usc.es
T + 34 982 28 59 00
www.usc.es , www.lhica.org

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