Preliminary studies on the microbiological characterization of lactic acid bacteria in suero costeño, a Colombian traditional fermented milk product

Cueto C.,* García D.,* Garcés F.,* Cruz J.*

ABSTRACT. Suero costeño is a fermented milk product from the Colombian Atlantic coast, which is produced by the spontaneous acidification of raw milk due to the action of environmental microbes during traditional and semi-industrial processes. Eleven fermentations were carried out in experimental settings replicating traditional conditions and changes in concentration among microbial groups involved during the process (Aerobic Mesophilic bacteria, Yeasts, Enterobacteriaceae and Lactic Acid Bacteria (LAB)). LAB plays an important role in the fermentation process, especially during the final stage (24 hours). In addition, yeasts seem to have an effect on fermentation, showing an increase during the first hours of the process, while Enterobacterial counts decreased during fermentation. Thirty six LAB strains were isolated from commercial samples and thirty two were identified using the API 50 CH kit (BioMérieux). 41% of the strains identified belonged to the species Lactococcus lactis subsp. lactis and 19% were Lb. paracasei subsp. paracasei. Sugars fermented by LAB include milk carbohydrates such as D-Lactose, D-Glucose and D-Galactose. Because of their capacity to use other carbohydrates (manose, cellobiose, maltose, fructose, ribose, trehalose, salicin, gentiobiose), it would also be possible to use these strains as starter cultures for other fermentations.

Key words: Acid lactic bacteria, suero costeño, milk fermentation, isolation.

INTRODUCTION

Traditional food fermentation processes use raw materials available in the region where they are produced, such as several cereals, vegetables, milk and meat. Products like fermented milk, salami, and pozol offer nutritional benefits, apart from diversity in the diet (Díaz-Ruiz et al, 2003; Erdogrun and Erbilir, 2006; Savadogo et al, 2004; Sulie-man et al, 2006).

Searching for desirable microbial strains for the food industry, isolation of microorganisms from traditional fermented products and characterization of physiological properties are a constant effort of scientific communities around the world (Steinkraus, 2002). Therefore, analysis of lactic acid fermentation processes is a necessary step, especially in products where changes in microbiological composition have been detected (Parente and Ricciardi, 1999). These changes are evident not only in the increase in the population of fermentation-related bacteria, which are resistant to the resulting conditions, but also in a decrease in undesirable microorganisms as well, including Enterobacteriaceae.

Suero costeño is a traditional product from the Colombian Caribbean coast and is the result of spontaneous lactic acid fermentation of cow milk in calabashes (dry fruit of Lagenaria vulgaris) (Rodríguez, 1988). Although the final product resembles sour cream, milk is the main raw material, rather than cream, and it is commonly used as a food dressing. Its traditional process is a result of a combination of factors, including warm temperature conditions in the Caribbean region (av. temp. 28°C), as well as the indige-
nous microbial population that is fixed after successive stages in the calabashes (commonly-used old-fashion fermentators), which work as natural bioreactors.

Although data on the microbial diversity in several fermented dairy products are known (Erdogrul and Erbiliz 2006; Savadogo et al, 2004; Sulieman et al, 2006), there are no studies that provide a detailed microbial characterization of suero costeño.

In order to prepare the product, the calabashes are adapted and prepared and then filled with fresh cow milk, sealed and subsequently placed in a warm site, with an average temperature of 35°C. Fermentation is characterized by a liquid-solid two-phase system, where the liquid part is called lactosuero; the other one, known as suero, has a cream-like thickness, as well as the desired organoleptic properties. Time required for obtaining suero costeño depends on the desired viscosity; the whole process can take between 1 – 3 days. After this period, suero costeño (thickness phase) is retrieved from the calabashes and packed in traditional recipients. In contrast to other fermented dairy products, NaCl is added to suero costeño in order to reach concentrations near 1 to 3%. One of the most common ways to consume it is as food dressing.

Its process is possible because of a combination of factors, including warm temperature conditions in the Caribbean region (av. temp. 28ºC), as well as the indigenous microbial population, that has been fixed after successive stages in the calabashes (commonly-used old-fashion fermentators), which work as natural bioreactors.

The aim of this study was to quantify the population of the most representative bacteria in the suero costeño production process, and to identify the most important strains of LAB during fermentation. This effort could be an important contribution towards future standardization, industrialization and commercialization of suero costeño.

METHODS

Sample gathering

Five samples of suero costeño were obtained from traditional producers located within the surrounding area of Valledupar in the department of Cesar (814 km from Bogotá); these samples were delivered to the analysis site in refrigerated containers. The samples were used not only to identify the microbial populations, but also as inocula in laboratory fermentations.

Laboratory production of Suero costeño

Eleven fermentations were carried out under experimental conditions. Eight of them were performed with raw milk in calabashes with a previously formed inner bio-layer. The last three were cultivated in a sterile Erlenmeyer flask with UHT milk inoculated with an aliquot of suero costeño (0.5 ml inoculum / 500 ml milk). Fermentations were performed at 37°C for 24 hours.

Population counting

Plate counts were performed at 4 different time periods (0, 5, 8 and 24 hours) from specific plates for each bacterial group described in Table 1. Fermentations were carried out in aerobic conditions at the temperatures and periods of time recommended by the manufacturers. Three serial dilutions and manual counting were used for measuring the number of colonies present.

Isolation and characterization of lactic acid bacteria

Individual isolates from countable De Man Rogosa Sharpe agar MRS (De Man, et al, 1960) plates were randomly picked, and representatives from all morphologically different colonies were subcultured and purified using the streak plate isolation technique five or six times. Cultures were incubated at 37°C for 24 hours under aerobic conditions and were classified according to stage of fermentation, labeled as initial (0 hours), intermediate (5 – 8 hours) and final (24 hours). Pure strains, as judged by microscopic observation for homogeneity of cellular morphology, were analyzed using phase-contrast microscopy, and were recognized as LAB according to characteristics of coccus or bacillus, Gram stain (positive) and catalase tests (negative) (Sharpe, 1979). Catalase activity was de-
Cueto et al  
Characterization of lactic acid bacteria in suero costeño  
Rev Latinoam Microbiol 2007; 49 (1-2): 12-18

determined by transferring fresh colonies from MRS agar to a glass slide and adding 5% \( \text{H}_2\text{O}_2 \). Biochemical identification of LAB was performed using the API 50 CH kit (Biomérieux, France), (Guessas and Khial, 2004).

**Strains conservation**

Working cultures of isolated strains were stored at -70°C using a CRYOBANK® adapted-system in MRS broth with 20% of glycerol as a cryoprotectant medium (Díaz-Ruiz et al., 2003).

**RESULTS AND DISCUSSION**

**Microbial population of traditional Suero costeño**

Samples of suero costeño obtained from local producers in Valledupar had counts between \( 9 \times 10^1 \) and \( 1 \times 10^5 \) CFU/ml, (Fig. 1). Prevalence of LAB over other types of bacteria found in suero costeño was evident, while the population of yeast and aerobic mesophilic bacteria revealed similar results. In contrast, Enterobacteria showed a minor presence of viable cells (\( 1 \times 10^1 - 1 \times 10^3 \) CFU/ml) in the traditionally produced final product.

An important variation in the bacterial concentration of samples obtained from northern Colombia were observed, due to the non-standardized elaboration conditions of suero costeño, as well as changes in production processes from one producer to another.

**Microbiological profile of Suero costeño**

Figure 2 shows the population distribution of 3 groups of bacteria and yeast studied during fermentation of raw and UHT milk. Prevalence of LAB was evident in both milk fermentations, mainly at the final stages of fermentation (24 hours). Yeast counts were higher in raw than in UHT milk, because of the processing of the latter, as well as the higher number of initial colonies that may have affected the intermediate and final counts of yeast. The number of Enterobacteriaceae increased in the five initial hours, but the counts had declined at the end of the process. On the other hand, the aerobic mesophilic bacterial population was superior in UHT milk, possibly due the fact that lower counts of other bacteria (which represent the competition) in UHT milk may have favored the growth of this microbial group. However, they both decreased their percentage at 24 hours, possibly because of the effect of acidification of the broth due to lactic acid buildup. In contrast, the very low percentage of Enterobacteria in the final stage of fermentation in raw and UHT milk indicates that producing suero costeño could be a relatively successful approach to the preservation of milk in warm regions where economic and logistical problems...
do not allow safe commercialization of food and dairy products.

The profile is parallel to other fermented products such as salamis (Duffy et al., 1999) and pozol (Ben Omar and Ampe, 2000; Wacher et al., 2000), where yeast and enterobacterial counts showed an increase during the first hours of fermentation, followed by a predominance of LAB in the final stages.

Figure 3 shows the fermentation profile of the bacteria studied. Homogeneous growth during the first hours of fermentation of all bacterial groups was observed, and the prevalence of the LAB population was evident in both fermentations. Yeast and Aerobic Mesophilic Bacteria showed similar counts, although final populations of both bacterial groups were higher in raw milk.

Enterobacterial population increased in the first phase (5 – 8 hours), but it experienced a decrease in population at 24 hours, reaching values comparable to initial ones (0 hours), possibly due to an inhibition by competition and the antimicrobial effects produced as a result of LAB metabolism, including lactic acid and antimicrobial substances such as BLIS (Bacteriocin-Like Inhibitory Substances). Finally, the increase in the LAB population at the final stage (24 hours) was markedly evident.

Figure 3 also shows the differences when better-quality milk was used for suero costeño production. Counts in raw milk fermentations were larger than in UHT milk, especially in the final population of Enterobacteria. This phenomenon suggests that the population of undesirable bacteria in the final product can be controlled when their initial counts are low or nonexistent.

Isolation and identification of LAB

LAB was the predominant microbial group during fermentation, which is important because of the key role it plays in fermentation processes and its production of lactic acid and antimicrobial substances, including bacteriocins.
and BLIS (bacteriocin-liked inhibitory substances), as well as its potential use as a starter of suero costeño in standardized production. For these reasons, LAB strains were isolated and identified. 36 LAB bacterial isolates were obtained, of which 32 were identified using a sugar-fermentation profile API 50 CH (Biomérieux sa), as shown in Table 2. However, the last 4 strains isolated were not possible to identify with this method, because API is a computer program commercially available that discriminates between species on the basis of a pattern-matching principle; for 4 strains the ID percentages were under 70% and these results were not considered to be acceptable (not valid).

The diversity of LAB species during the whole process was observed, identifying 11 different species of LAB, most of them were species frequently found in dairy fermentations (Lactobacillus plantarum, Lactobacillus paracasei, Lactococcus lactis) and other species were reported in inferior amounts, such as Leuconostoc mesenteroides strains.

Based on API 50 CH identification, 15 biochemical profiles were registered as Lb. plantarum, 7 as Lb. paracasei subsp. paracasei and 3 as Lc. lactis subsp. lactis. The prevalence of Lb. plantarum and Lb. paracasei during the entire process is an indicator of the key role played by these bacteria in the suero costeño fermentation process; analogous behavior has been reported in a large variety of foods, such as fermented milk products (Savadogo et al., 2004), cereals (Togo et al., 2002) and vegetables (Salminen et al., 2004). Figure 4 shows the predominance of Lactobacillus genera (84% of the total LAB), as expected. Those types of bacteria are commonly associated with the warm climatic conditions of productive regions (Sava-
The fact that strains isolated were able to ferment a large number of sugars commonly found in food materials and products, including D-lactose, D-glucose, D-galactose D-fructose and D maltose, suggests that LAB found in suero costeño have the potential to adapt to media other than milk and to grow in a broad range of raw materials, including cereals, fruits and vegetables, providing the opportunity for new products and applications.

CONCLUSIONS

LAB was the most important group of bacteria found during the fermentation of suero costeño, as it dominated during fermentation, especially at the final stages.

The Enterobacterial population was reduced at the end of the fermentation process, as has also been reported other lactic acid fermentation processes which occur in meats and sausages, where reduction in cell population is driven by the initial amount of microbes, interaction between other groups of bacteria and the loss of moisture within the product.

*Lb. plantarum* and *Lb. paracasei paracasei* prevail over other species during suero costeño fermentation, possibly because of their acid resistance.

Differences in fermentation phases suggest a succession of fermentative strains, due to the acidification of medium. In addition, LAB have been adapting their metabolism, using the most important sugars in milk as a source of energy and carbon. LAB strains isolated from suero costeño have the possibility of growing in several types of foods, because they have the ability to use a broad range of sugars in their metabolic pathways.

ACKNOWLEDGEMENTS

We would like to thank Colciencias-SENA, the Colombian national research council (Project 1230-08-1211) and Universidad de La Sabana for their economic and logistical support.

REFERENCES


Correspondence to:

MSc Clementina Cueto
Facultad de Ingeniería,
Universidad de La Sabana,
Campus Puente del Común,
km 21 autopista Bogotá-Chía,
Cundinamarca, Colombia,
E-mail: maria.cueto@unisabana.edu.co