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Revista Latinoamericana de MICROBIOLOGÍA
ALAM

Vol. 49, Nos. 1-2
January - March. 2007
April - June. 2007
pp. 12 - 18

ORIGINAL ARTICLE

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 *Lb. plantarum*, 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, celobiose, 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.

RESUMEN. El suero costeño es un producto lácteo fermentado de la costa Atlántica colombiana, resultado de la acidificación espontánea de la leche por la acción de microorganismos naturales en procesos artesanales o semi-industriales. Se llevaron a cabo 11 fermentaciones replicando las condiciones artesanales, se determinó el cambio de concentración de bacterias ácido lácticas (BAL). Las BAL juegan un papel importante en los procesos de fermentación, especialmente en la fase final (24 horas). Adicionalmente las levaduras parecen tener un efecto en el desarrollo de la fermentación al presentar un incremento importante en las primeras etapas de fermentación y se observó una disminución significativa en la población final de Enterobacterias. Se aislaron 36 cepas de BAL de las muestras comerciales, de las cuales 32 pudieron ser identificadas por medio de la bacteria de fermentación de azúcares API 50CH (BioMérieux). La mayoría de las cepas encontradas pertenecen a las especies *Lactobacillus plantarum* y *Lactococcus lactis* subsp. *lactis*. Además, los azúcares fermentados por las BAL durante la fermentación corresponden a los encontrados en la leche, entre los cuales se incluyen D-lactosa, D-glucosa y D-galactosa. Debido a su capacidad de fermentar otros carbohidratos (manosa, celobiosa, maltosa, fructosa, ribosa, trehalosa, salicina, gentiobiosa) sería también posible usar estas cepas como iniciadores de otras fermentaciones.

Palabras clave: Bacterias ácido lácticas, suero costeño, fermentación leche, aislamiento.

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; Erdogrul and Erbilir, 2006; Savadogo *et al*, 2004; Sulieman *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 vigalis*) (Rodriguez, 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. 28ºC), as well as the indige-

* Facultad de Ingeniería, Universidad de La Sabana, Campus Puente del Común, km 21 autopista Bogotá - Chía, Cundinamarca, Colombia.

First version received: January 15, 2007; second version received in revised form: May 2, 2007: accepted: May 21, 2007

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 37ºC 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 subcultered and purified using the streak plate isolation technique five or six times. Cultures were incubated at 37ºC 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-

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Table 1. Plate counts and conditions for counting of microbial groups

Culture media	Microbial group	Conditions
Agar Plate Count agar (APC)	Aerobic mesophilic bacteria	24 a 48 h, 37 °C
Potatoe Dextrose agar (PDA)	Yeast and molds	2 a 5 days, 25 °C
Violet crystal-Red neutro-bilis-glucose Agar (VRBD)	Enterobacteriaceae	24 a 48h, 37 °C
Man Rogosa Sharp agar (MRS)	Lactic acid bacteria	24 a 48 h, 37 °C

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termined by transferring fresh colonies from MRS agar to a glass slide and adding 5% H_20_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 -70ºC 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 $9x10^1$ and $1x10^5$ 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 ($1x10^1$ -$1x10^3$ 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

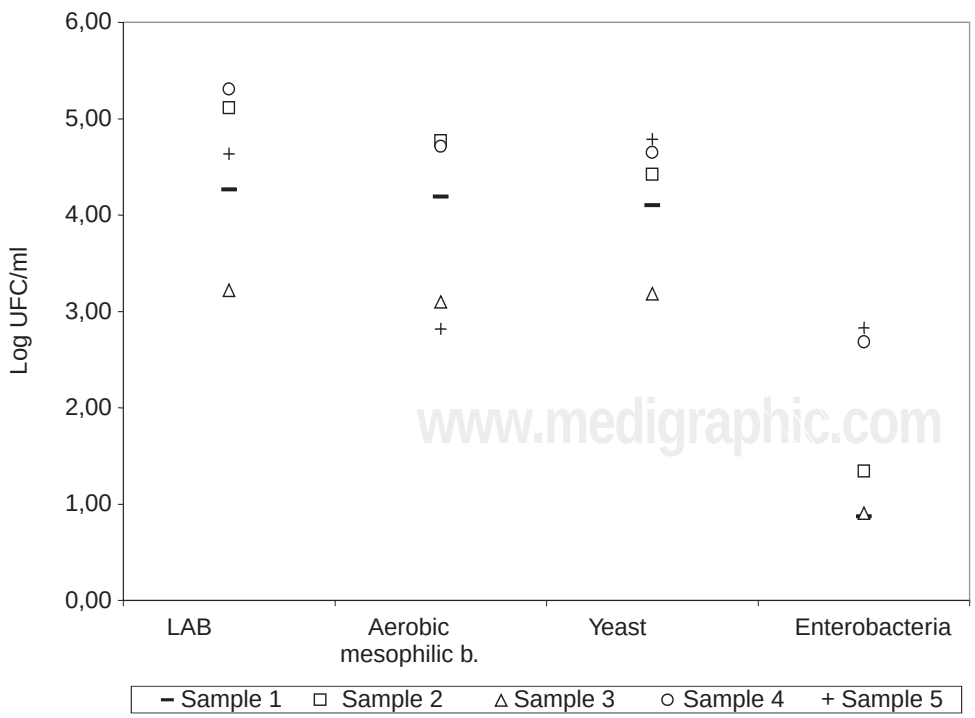

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Figure 1. *Microbial counts of suero costeño samples from traditional producers.*

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

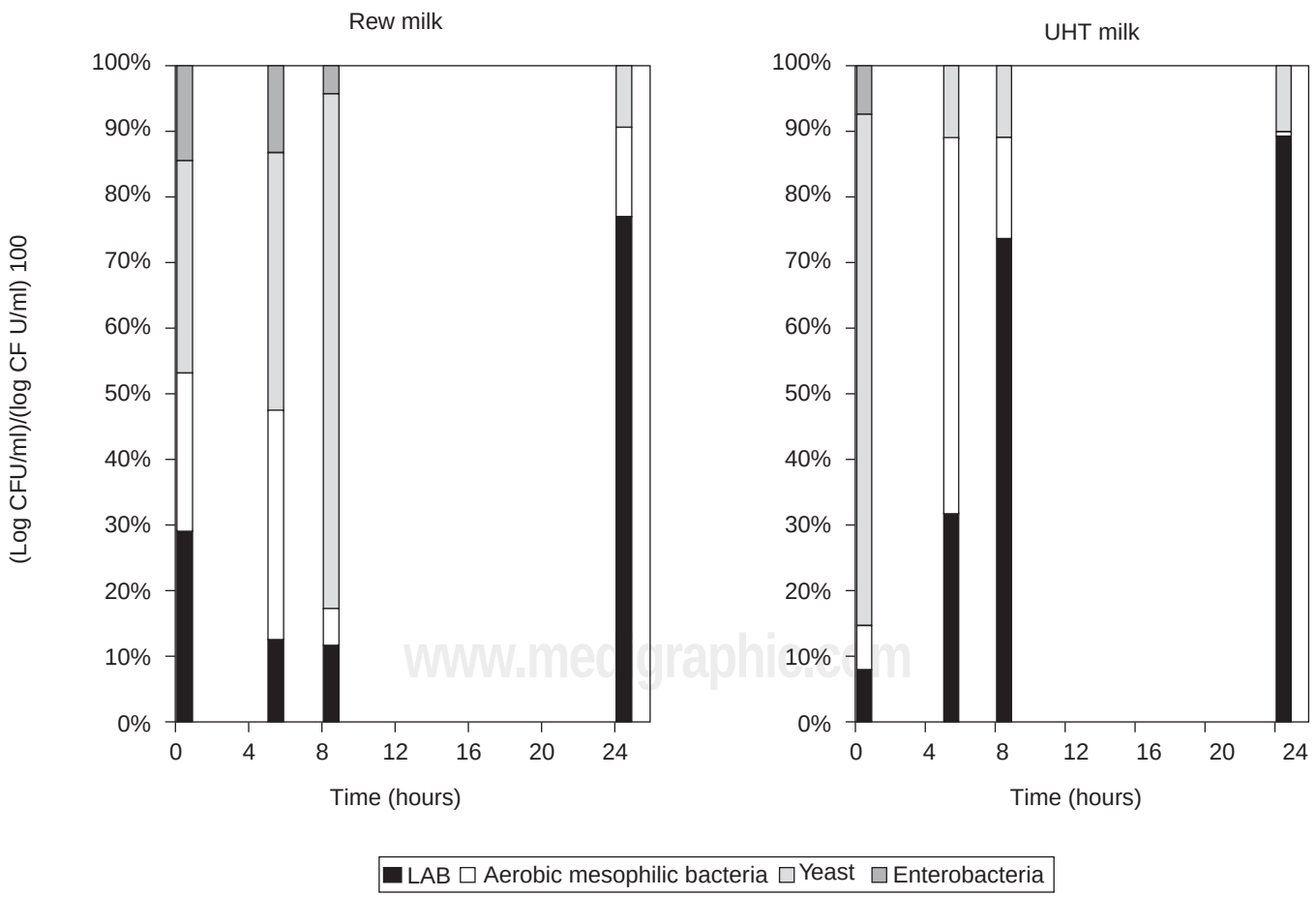

Figure 2. *Perceptual distribution of the microbial population of* suero costeño *fermentation under experimental conditions.*

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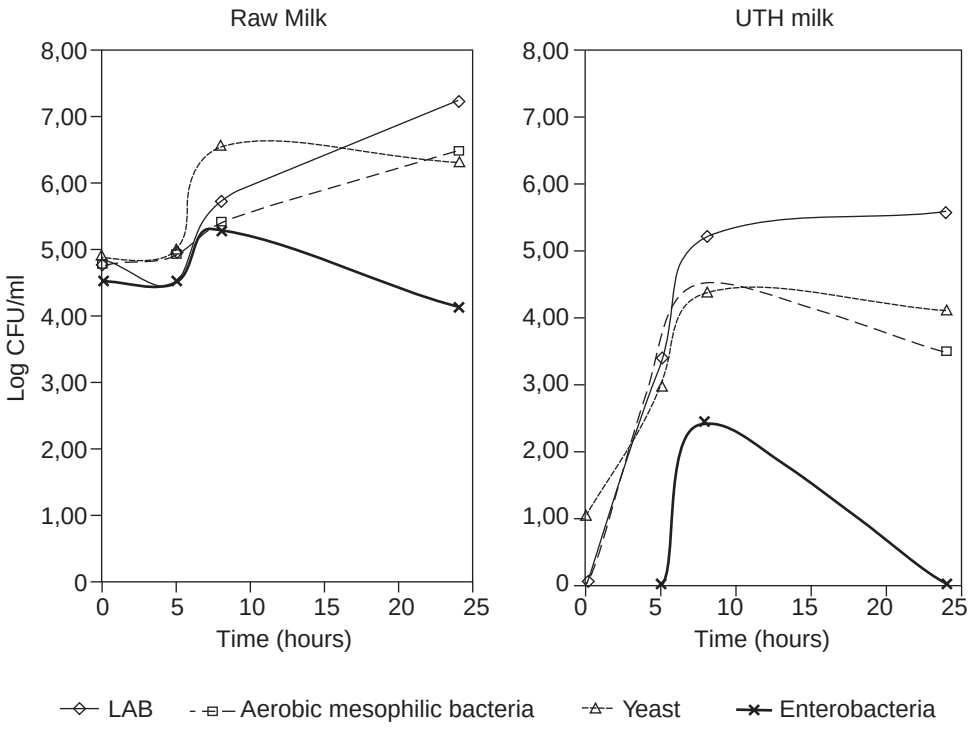

Figure 3. *Microbial fermentation profile of* suero costeño *under experimental conditions.*

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-

Table 2. LAB identified in *suero costeño*.

	Stage of fermentation		
	Initial (0 hours)	Intermediate (5-8 hours)	Final (24 hours)
Lactobacillus acidophilus			1
Lactobacillus brevis			1
Lactobacillus delbruekii delbruekii			1
Lactobacillus paracasei paracasei	2	3	2
Lactobacillus pentosus			1
Lactobacillus plantarum	3	4	8
Lactobacillus rhamnosus			1
Lactococcus lactis lactis			1
Lactococcus lactis lactis 1	1		
Lactococcus lactis lactis 2		1	
Leuconostoc lactis		1	
Leuconostoc mesenteroides cremoris		1	
Not identified	1		3

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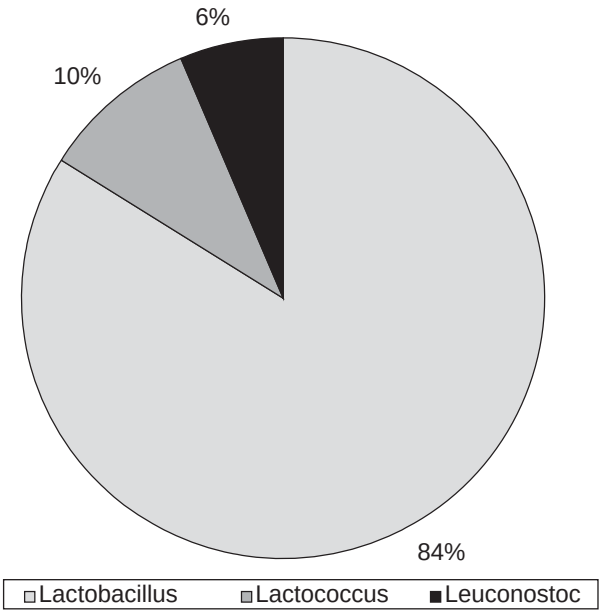

Figure 4. *Distribution of LAB genera identified in* suero costeño *fermentation.*

dogo *et al*., 2004), as found in the Colombian Atlantic coast, where the average year round temperature is approximately 28°C.

The succession of strains during the fermentation process was evident. While *Lactococcus* and *Leuconostoc* strains were found at initial and intermediate stages (0 to 8 h), *Lactobacillus,* especially *L. plantarum,* were predominant at 24 h. Certain strains that were found in the final phase were not identified in the first 8 hours of fermentation (van Beek and Priest, 2002) It is possible that certain strains more resistant to the low pH of the final product were selected.

The final stage of fermentation was characterized by a higher proportion of *Lb. plantarum* strains. *Lb. paracasei paracasei*, commonly found in fermented products, were found, but in lower numbers than *Lb. plantarum*.

Sugar-fermentation profile by lactic acid bacteria

Most of the bacteria identified by API 50 CH test were able to use D-lactose (97% of identified bacteria), the most important sugar in milk, as well as D-glucose, and D-galactose (97%), sugars produced by hydrolysis of lactose. More than 10 sugars were fermented by up to 80% of the bacteria isolated (manose, celobiose, maltose, N-acetilglucosamine, arbutin, fructose, ribose, trehalose, salicin, gentiobiose and D-sacarose), suggesting the adaptation of those types of microorganisms to a medium such as milk and the influence of the environment on their metabolism.

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.

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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

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