

## Research Article

### Isolation and identification of Autochthonous Lactic Bacteria for use in Cultures Starters in the Processing of Coastal Cheese

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**Abstract:** In this study, native lactic bacteria present in raw milk used for the elaboration of coastal cheese were isolated and identified. Aseptic milk samples were taken from three (3) companies, stored at temperatures between 0-4°C. Make dilutions were performed with peptone water and plaque (MRS agar), species were purified by morphology, reaction to oxidase case, catalase and gram staining. The Identification of the isolated species was done using the API 50 CH system (BioMerieux). The results of the first two (2) companies showed the species *Lactobacillus paracasei ssp paracasei* 3, with a percentage of identification above 99.5%. Third company showed growth of yeast colonies without the presence of lactic bacteria. There was no growth of other bacteria during the investigation, possibly due to the use of inhibitory substances by producers, such as hydrogen peroxide, during the transfer of milk to processing centers. It is concluded that one of the autochthonous bacteria of raw milk that affect the final characteristics of the coastal cheese is *Lactobacillus paracasei ssp paracasei* 3 since it showed a considerable growth rate, despite possible inhibition conditions. This species can be used in starter's cultures and inoculated in pasteurized milk, to obtain typical organoleptic characteristics of cheese made from raw milk.

**Keywords:** Bacteria, incubation, lactobacillus, milk, morphology, native

## INTRODUCTION

In Colombia, there are technical norms that regulate and oblige the production of dairy products from pasteurized milk, however, Is still made with raw milk through a non-standardized process with an uncontrolled coagulation that generates a product with non-homogeneous organoleptic characteristics and with a poor microbiological quality due to its high microbial load, with microbiological counts of molds and yeasts, coliforms, *Staphylococci aureus* and *Aerobic mesophiles* above the permissible limits in national regulations, evidencing contamination by hygienic-sanitary deficiencies during its process of transformation and manipulation (Hernández *et al.*, 2010) and even with presence of *Salmonella*, representing a danger to health of the consumer (Acosta and Naranjo, 2006). Same it, a coastal cheese frequently contaminated with *Listeria spp. L. ivanovii* and *L. innocua*, which is an indicator of the degree or quality of sanitation in many food industries, which shows that the conditions of production and sale are not the most adequate and that consumption of coastal cheese is not safe in this form.

According to the National Public Health Surveillance System (Sistema Nacional de Vigilancia

en Salud Pública-SIVIGILA), during the year 2014, the second department that reported the most for Foodborne Diseases-ETA was the department of Sucre (SIVIGILA, 2014). According to the National Institute of Health-INS (INS, 2014), 17.3% of outbreaks are due to consumption of milk, dairy products and their derivatives. This information has been published even in local media and according to the latest report for the year, 2015 occupied the fourth place in reports by ETA (SIVIGILA, 2015).

Decree 3075 of 1997 of the Ministry of Social Protection considers and includes milk and dairy products as high-risk foods in public health, with the entry into force of this decree and in accordance with Resolution No. 01804 of 1989 Ministry of Health, in the process of making cheese, the milk must undergo a pasteurization treatment to achieve said purposes of safety of this product. However, this thermal treatment does not eliminate only the pathogenic and altering bacteria, but also those beneficial bacteria of the milk (lactic acid bacteria-BAL), which are largely responsible for the final organoleptic quality of the cheese (Montel *et al.*, 2014). Because of the above, with the implementation of the pasteurization operation in the process of processing the coastal cheese, a

Table 1: Cultures conditions BAL counted

Microorganisms	Cultures type	Cultures form	Incubation
<i>Lactococcus, Lactobacillus y Leuconostoc</i>	Profundity	MRS	30-35°C/48-72 h

Authors

product will be generated that does not have the organoleptic characteristics of the traditional cheese made from raw milk.

The final alternative solution to this problem should seek to improve the microbiological quality but maintaining the organoleptic quality of the product. Given the need to implement the process of pasteurization in the elaboration of coastal cheese to comply with the regulations for the production this product and that there are studies that show the changes generated by the implementation of this operation in the organoleptic characteristics of the coastal cheese (De Oro *et al.*, 2014), it is necessary to mitigate this effect by returning the characteristics of the coastal cheese to the final product. This may be possible due to the inoculation of autochthonous lactic bacteria after the pasteurization process. There is very little research or studies related to the coastal cheese, whose results allow to know the types and proportions of lactic cultures to be used in its elaboration process, with the exception for some research work carried out on other Colombian cheeses (López *et al.*, 2010)

## MATERIALS AND METHODS

Milk samples were taken from three (3) cheese-producing companies in the municipality of Sincelejo registered in the Camara de Comercio and this receiving milk volume from different municipalities in the department of Sucre. In each selected company, samples consisting of 100 mL fresh raw milk were collected aseptically in glass vials and stored in iceboxes with abundant ice at temperatures between 0-4°C and transported to the microbiology laboratory at the University of Sucre (NTC 4092, 2009).

Each sample was labeled with the following information in a visible place: Sample name, date, place of sampling, destination and corresponding numbering. The label information was collected and formatted in the respective format (sample receiving format) and refrigerated.

For the count of the lactic acid bacteria present in the milk, 10 mL of raw milk plus 90 mL of the diluent, 2% peptone, was made to make the dilution 1:10 (dilution 1). Once the sample is diluted, another three decimal dilutions are performed by sowing 1 mL in 9 mL of 0.9% solution.

From the previous dilutions was plated in duplicate by sowing in depth. For mass seeding, 1 mL of inoculum was split and the culture medium was then added. The sowing conditions for the BAL count were synthesized in Table 1.

After the respective incubation period, colonies were counted and BAL populations were established numerically.

Table 2: BAL ID percentage

Code	Taxón Significativo	ID (%)
C3*	<i>Lactobacillusparacaseisspparacasei 3</i>	99.8
C6*	<i>Lactobacillusparacaseisspparacasei 3</i>	99.5
CQ**	<i>Lactobacillusparacaseisspparacasei 3</i>	99.8

Authors; \* Company 1; \*\* company 2

After colony counts, those with characteristic BAL morphologies were selected in the respective media. For this, they were taken with bacteriological round handle, inoculated in nutrient broth (MRS) and incubated at the optimum temperature. Once grown in the liquid medium they were seeded on nutrient agar and growth was observed after the incubation to verify that it was a pure culture.

The strains were selected based on their morphology, their reaction against oxidase (negative), catalase (negative) and their behavior on gram (positive) staining.

The identification of the isolated species was performed using a battery of biochemical tests with the API 50 CH system (BioMerieux), which provided the information regarding the name of the species involved in the fermentation process in fresh cheese.

## RESULTS AND DISCUSSION

The results of the first two (2) companies showed the species *Lactobacillus paracasei ssp paracasei 3*, with a percentage of identification above 99.5% in three (3) samples (Table 2). These high percentages indicate that the behavior of the isolated bacteria is very similar to the species mentioned and the isolation procedure at all stages was performed correctly. Low percentages indicate that there is not much security in setting the microorganism.

The third company showed no growth of lactic bacteria. However, yeast colonies that were not part of the study object grew. During the investigation there was no growth of other lactic bacteria, it is possible that some milk producers in the region may use inhibitory substances, such as hydrogen peroxide, during the transfer of milk to processing centers for milk conservation purposes. It is also likely that the bacterium identified has played an antimicrobial role against the other lactic acid bacteria since bacteriophage and probiotic capacity has been found in both pathogenic and beneficial microorganisms (Deng *et al.*, 2015)

## CONCLUSION

Although only one lactic bacterium was identified during this experiment, the importance of this bacterium in the process of elaboration of coastal

cheese is emphasized. When showing growth under possible inhibition conditions, it is concluded that one of the indigenous bacteria of raw milk that affect the final characteristics of the coastal cheese is *Lactobacillus paracasei ssp paracasei* 3. Therefore, it can be used in cultures starters and inoculated in pasteurized milk, to obtain organoleptic characteristics typical of cheese made from raw milk. In this way, a milk with low microbial load and with acceptable sensorial quality would be obtained.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this study.

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