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Characterization of lactic acid bacterial communities associated with a traditional Colombian cheese: Double cream cheese

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ABSTRACT

Culture-dependent and Independent methods such as temporal temperature gradient electrophoresis (TTGE) and evaluation of the technological properties of five strains representing isolated groups, were used to evaluate the bacterial composition of three traditional double cream cheese companies in Colombia-South America. The results showed that fat, protein, moisture, and titratable acidity were significantly different between cheeses. Genetic analysis of these bacterial communities revealed a low level of diversity, based on the TTGE analysis. The dominant phylogenetic affiliations were grouped into four genera *Enterococcus*, *Lactobacillus*, *Leuconostoc,* and *Lactococcus*, related to acidification processes and formation of aromas and flavours in the cheese. The characterization of lactic acid bacteria (LAB) isolates showed predominance of the genera *Leuconostoc* and *Weissella*. These bacteria are related to the development of certain features such as texture and production of antimicrobial metabolites. In the evaluation of technological properties *Weissella viridescens* and *Weissella paramesenteroides* displayed greater pH reduction at 24 h, *Pediococcus acidilactici*, *Leuconostoc mesenteroides* and *Leuconostoc citreum* presented diacetyl production. The combination of different approaches allowed inferring the presence of a dominant population of LAB present in this cheese, this results complements the information needed for the establishment of a product protected under the status of Designation of Origin.

1. Introduction

Double cream cheese is a dairy product made through artisanal practices in Colombia, following the technology developed in Ubaté and Chiquinquirá Valleys, located on the "cundiboyacense" plateau in the Andean region of the country. It is recognized as one of the most traded cheeses in Colombia and its production has shown a steady growth in recent years (Novoa & Lopez, 2008). For the preparation of this cheese, a mixture of fresh and acidified cow milk is required. It has been characterized as a non-matured acid cheese, with a semi-cooked and stringy paste (Osorio, Novoa, & Gutierrez, 2012). During the process of milk acidification, which occurs naturally, a native microbiota encompassing lactic acid bacteria (LAB) which promote the organoleptic, physico-chemical and microbiological characteristics to the finished product (Ramírez-Navas, 2010).

Identification of LAB in cheese has been carried out taking into account two types of approaches. The first one is based on culture-dependent methods, which involves the isolation of microorganisms on selective agars medias (MRS, M17, Elliker), morphological, biochemistry, and molecular identification using Ribosomal Intergenic Spacer Analysis (RISA), Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Pogačić et al., 2013) and 16S rDNA sequencing for assignation of isolates to species; followed by the characterization of the technological properties of the isolates (Carraro et al., 2011; Neviani, Bottari, Lazzi, & Gatti, 2013). The second approach focuses on culture-independent methods in which the DNA total obtained from the sample is extracted and analysed to characterize a bacterial community (richness and

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relative abundance) with molecular techniques how Temperature/Denaturing gradient gel electrophoresis (T/DGGE) (Cocolin, Alessandria, Dolci, Gorra, & Rantsiou, 2013), Fluorescence In Situ Hybridization (FISH) (Bottari, Santarelli, Neviani, & Gatti, 2010) and Next-Generation Sequencing (NGS) (Guidone et al., 2016), combined with the polymerase chain reaction (PCR), to allow the identification of microbial groups not detected by conventional methods (Aponte, Fusco, Andolfi, & Coppola, 2008).

The TGGE is a culture-independent technique that provides an ecological view of the predominant species in complex microbial communities (Ahrne, Pettersson, Molin, & Va, 2001). It is also used in many fields as a tool for the identification of microorganisms present in food, the impact assessment of probiotic bacteria in human native gastrointestinal microbiota and evaluation of microbial diversity during food fermentation (Monnet & Bogovic Matijasic, 2012; Walter, 2008). Different studies focused on microbial diversity in traditional cheeses how mozzarella (Guidone et al., 2016), manchego (Nieto-Arribas, Sesena, Poveda, Palop, & Cabezas, 2010), cotija (Chombo-Morales, Kirchmayr, Gschaedler, Lugo-Cervantes, & Villanueva-Rodríguez, 2016), have had a great impact on the producers, providing them with tools for understanding all the processes in which microorganisms are involved, taking into account the secondary metabolites involved in the consolidation of the organoleptic properties and in the health benefits of consumers. Prevention of certain diseases and high nutritional value by the presence of conjugated linoleic acids (CLAs) and amino acids derived from metabolic activities during cheeses elaboration (Kongo, 2013; Montel et al., 2014; Quigley et al., 2013).

Traditional cheeses produced through artisanal practices in some parts of the world are recognized with the distinction "Designation of Origin" (DO), because of unique features developed in their processing environment and involvement of native microbiota (Gobbetti, De Angelis, Di Cagno, Mancini, & Fox, 2015; Scatassa et al., 2015). Given the limited amount of information available on the microbiological characteristics of double cream cheese, the aim of this work was to study the diversity of lactic acid bacteria indigenous communities associated with artisanal double cream cheese from Ubaté Valley region in Cundinamarca, Colombia. This was achieved using molecular and conventional microbiology methods and will lead to major advances in the understanding of this ecosystem and improve the elaboration and quality of the cheese in this region.

2. Materials and methods

2.1. Double cream cheese samples

Samples were collected in the municipality of Ubaté, Cundinamarca-Colombia (5°18′26″N 73°48′52″O) (Fig. 1). Three cheeses, from three independent different production batches (L1, L2 and L3) were collected from three representative companies (Q1-Q2-Q3) in the traditional artisanal way.

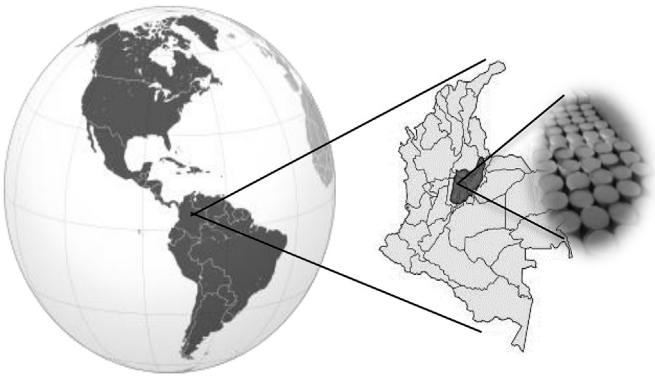

Fig. 1. Map of the location of the studied area in Colombia-South America.

2.2. Physicochemical analysis

The following parameters were measured: pH, measured according to AOAC Official Methods 981.12 Ed.18 using a WTW pH meter (Measurement Systems, Inc. Model 330i-SET), 10–20 ml of distilled water (50–60 °C) were added per 100 g of product to form a uniform paste. Titratable acidity according to AOAC Official Methods 942.15 Ed.18. About the 9–10 g of the sample were weighed, 100 ml of water at 50° C was added in an Erlenmeyer flask and were vigorously shaken, and the mixture is filtered with a dry filter. Total fat was determined using the Röse-Gottlieb principle (AOAC Official Methods 933.05 Ed. 18), aapproximately 10 g of cheese were weighed and mashed, then mixed with water at 50 °C. Ashes, Moisture and Protein content, according to AOAC Official Methods (Ed.18 923.03, 926.08, Ed 18 and 988.05 Ed.18, respectively).

2.3. Microbiological analysis of artisanal double cream cheese

Ten grams of each cheese were added to 90 ml of 0.1% peptone water (Merck® Darmstadt-Germany) and homogenized for three minutes in a Stomacher (AES Laboratories, France). Serial decimal dilutions were plated in MRS agar (Man, Rogosa and Sharpe) (Merck® Darmstadt-Germany) and M17 agar (Merck® Darmstadt-Germany) (Franciosi, Settanni, Cavazza, & Poznanski, 2009). Finally, dilutions were incubated at 30 °C for 48 h under anaerobiosis, using an indicator and AnaeroGen (Oxoid). Assays were performed in triplicate.

2.4. Quantification of bacteria

The total bacterial cell count was performed by epifluorescence microscopy with acridine orange (AMRESCO®) following the methodology of Bottari et al. (2010), with some modifications: 100 μl from the 10^{-2} dilution were extended and stained with 0.1% acridine orange solution. Plates were observed at 100X on an epifluorescence microscope (Nikon 80i) with the filter B-2E/C. Each count was performed in duplicate. The Quantification of LAB viable cells were made from dilutions with growth between 30 and 300 colonies were selected. Results were expressed as $\log_{10}$ of colony forming units (CFU) per gram of sample. Counts were made in triplicate.

2.5. Ribosomal intergenic spacer analysis (RISA) of the isolates

Pure colonies were re-isolated MRS agar media at least three times and the pure isolates were characterized by gram stain and ribosomal intergenic spacer analysis (RISA) to choose representative ones for sequencing. DNA of each bacterial isolate was obtained by lysing a loop-full of the culture in 50 μL of TE 0.1X at 100 °C for 10 min and centrifuging at 5000 rpm for 5 min. The crude lysates supernatant was diluted to a 1:10 dilution with Milli Q water. For the PCR reaction, 2 μl of the bacterial lysate of the isolate were used with primers L1 (5-CAAGGCATCCACCGT-3) and G1 (5-GAAGTCGTAACAACG-3) (Silva-Bedoya, Sanchez-Pinzon, Cadavid-Restrepo, & Moreno-Herrera, 2016). The PCR Mix and reaction program used is described in Moreno, Romero, and Espejo (2002). The IGS patterns were resolved with polyacrylamide gel electrophoresis 8% (PAGE), stained with AgNO3 (AMRESCO, OH, USA) (Sanguinetti, Dias Neto, & Simpson, 1994) and analysed with the