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IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ARTISANAL MINAS CHEESE FROM THE \"ENTRE SERRAS\" REGION: CULTURE-DEPENDENT METHOD

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Minas Gerais is a Brazilian state renowned for being the largest producer of cheese in the country, particularly noted for its Artisanal Minas Cheese (QMA). This type of cheese is produced in various regions of the state, following basic procedures that involve the addition of rennet and a natural starter culture to raw milk, known as \"pingo\". The process includes coagulation, cutting, stirring, molding, salting, and maturation at room temperature. Among the producing regions, \"Entre Serras da Piedade ao Caraça\" stands out, a mountainous area in Minas Gerais with a cheese production history dating back to the 18th century. Production nearly disappeared in the 1950s but was recently revitalized and recognized as a QMA producer in 2021. However, the lack of scientific data on QMA from this region necessitates studies to characterize the production process, microbial identification and characterization of the cheeses, and their changes during maturation. Different types of QMA can present distinct sensory characteristics due to specific production methods and geoclimatic properties, known as terroir. The cheese microbiome has been studied using culture-dependent and culture-independent approaches, with 16S rRNA gene sequencing revealing the dominance of lactic acid bacteria (LAB) in the product. Therefore, the study aimed to isolate and molecularly characterize LAB from QMA from a farm in the “Entre Serras” region. For this purpose, the culture bank of the INOVALEITE laboratory (Laboratory of Milk and Dairy Products, Federal University of Viçosa) was used, where the isolates were stored. In total, 760 isolates were obtained after purification, being morphologically Gram-positive and catalase-negative. All isolates underwent DNA extraction using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA). After that, the DNAs were subjected to PCR reaction and stored in 96-well plates and finally sent for 16S rRNA sequencing at Macrogen Inc. (Seoul, South Korea). The electropherograms were analyzed using MEGA software and compared through the BLAST tool (National Center for Biotechnology - NCBI). Of these, 739 showed results for LAB. The results demonstrated that 6 genera of LAB were found: Lactiplantibacillus (n=513), Lacticaseibacillus (n=205), Pediococcus (n=7), Enterococcus (n=12), Weissella (n=1), and Lactococcus (n=1), indicating considerable microbiological diversity in the studied QMA. This diversity is crucial for the development of sensory and safety characteristics of the cheese. Each genus and species within these groups can contribute different fermentation profiles and production of bioactive compounds. The genus Lactiplantibacillus was the most frequent in this group, demonstrating that it is highly adapted to the QMA environment in the region. Therefore, it is concluded that the results indicate a predominance of the Lactiplantibacillus genus and a considerable diversity of LAB in the QMA from the “Entre Serras” region. Although the culture-dependent method has its limitations, it is valuable for the initial isolation and characterization of microorganisms, being essential for understanding the microbiological diversity of the farm in the region.

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