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Technological characterization of *Lactobacillus* in semihard artisanal goat cheeses from different Mediterranean areas for potential use as nonstarter lactic acid bacteria

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ABSTRACT

The potential of 25 *Lactobacillus* isolates from 8 semihard artisanal goat cheeses manufactured in 4 different Mediterranean areas was examined for use as nonstarter lactic acid bacteria. The isolates were identified using 16S rDNA sequence analysis. Sixteen strains belonged to *Lactobacillus paracasei* and 9 to *Lactobacillus rhamnosus*. The isolates were first screened for salt tolerance, exopolysaccharide and diacetyl production, proteolytic and lipolytic activity, and acidification and autolyzing capacities. Most of the lactobacilli displayed strong salt tolerance [20 strains, including 13 of *Lb. paracasei* and 7 of *Lb. rhamnosus*, could grow at 6% (wt/vol) salt], low acidification activity (16 strains, including 9 of *Lb. paracasei* and 7 of *Lb. rhamnosus*, presented change in pH $\leq$0.4 U after 6 h of growth), and high autolytic activity (14 strains, including 9 of *Lb. paracasei* and 5 of *Lb. rhamnosus*, showed autolysis values ranging between 25 and 65%). Eleven *Lb. paracasei* and 6 *Lb. rhamnosus* produced exopolysaccharide, whereas 8 *Lb. paracasei* and 4 *Lb. rhamnosus* produced diacetyl. Moreover, 9 *Lb. paracasei* and 6 *Lb. rhamnosus* showed proteolytic activity; none of the isolates showed lipolytic activity. Based on the above characteristics, 8 strains were further evaluated for peptidase activity, including aminopeptidase, dipeptidyl aminopeptidase, and dipeptidase activities. The results indicated that all strains showed peptidase activity toward selected substrates. The substrate specificity and extent of peptidase activities were strain-dependent. Four strains (A-3, B-4, D-3, and D-8) presented the best characteristics and represented the most promising nonstarter lactic acid bacteria candidates for use in industrial manufacturing of goat cheese.

Key words: goat cheese, *Lactobacillus*, NSLAB

INTRODUCTION

Goat milk distinguishes itself from cow milk by higher digestibility, distinct alkalinity, higher buffering capacity, and presence of certain medicinal and therapeutic values (de Almeida Júnior et al., 2015); therefore, the production and fabrication of goat cheese has shown a growing trend in recent years. Cheese, a dairy product, has been part of the human diet for centuries and greatly affects human nutrition. Artisanal cheeses are manufactured in farmhouses following traditional techniques without the deliberate addition of selected starter cultures. They are typically characterized by a unique taste due to the spontaneous fermentation of unpasteurized milk and are greatly appreciated by consumers around the world. Reports show that their organoleptic characteristics correlate strongly with nutritional characteristics and the environmental contamination level of milk used for cheese production, the manufacturing process, and the presence of appropriate lactic acid bacteria (**LAB**) for fermentation (Carafa et al., 2015). In general, cheese production comprises 2 different microbiological steps, in which different LAB are involved. The first step, namely the manufacturing of cheese, requires starter LAB; the second step, ripening of the cheese, takes advantages of secondary microbiota nonstarter LAB (**NSLAB**; Di Grigoli et al., 2015), which are typically used in raw milk cheeses (Carafa et al., 2015). Fermentation properties of the NSLAB strongly affect the sensorial characteristics of the finished cheese.

The family of NSLAB consists of mostly of facultative heterofermentative mesophilic lactobacilli species such as *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum*. It also contains of other genera such as *Pediococcus*, *Leuconostoc*, and *Micrococcus* (Franciosi et al., 2009; Piras et al., 2013; Bozoudi et al., 2016). Typically NSLAB grow after cheese brining, as they are resistant to heat and acid treatment that occur during cheese manufacturing and maturation. Bacterial autolysis pro-

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vokes the release of enzymes during the final months of a long maturation process (Martínez et al., 2011; Lazzi et al., 2016). Although industrial cheese production is now well established, the NSLAB composition remains an uncontrolled factor and is suspected to be the cause of inconsistent quality of hard and semihard cheeses (Briggiler-Marcó et al., 2007; Burns et al., 2012). In fact, reports show that alterations in the dominant strains of NSLAB can lead to the formation of off-flavors and biogenic amines in addition to the possibility of gas blowing (Di Cagno et al., 2012). Therefore, to ensure consistency in cheese manufacturing and improve its sensory properties, potent NSLAB strains should be carefully selected when used as adjuncts for controlling the adventitious growth of undesirable NSLAB.

Mediterranean countries are producers of goat milk that is mostly used for cheese production (Juan et al., 2016). Indeed, traditional dairy products derived from goat milk represent a viable sector in the national economy of many Mediterranean countries, such as Spain, Greece, and Turkey, among others (Navarro-Alarcón et al., 2011; Pacheco Da Silva et al., 2016). In most Mediterranean countries, cheeses of raw goat milk represent a significant proportion of ripened cheeses (Serhan et al., 2010).

The most promising bacteria for adjunct cultures are those isolated from indigenous microflora of traditional products. In the present study, we isolated and characterized LAB from artisanal semihard goat cheeses of different Mediterranean countries for developing specific NSLAB. We then evaluated salt tolerance, acidification, autolytic activity, and enzymatic activity of the NSLAB.

MATERIALS AND METHODS

Cheese Samples

Eight semihard raw goat milk cheeses were purchased from local stores of 4 Mediterranean areas, namely, Ibores (sample A), Tenerife (sample B), and Babia (sample C) from Spain, Batzos (sample D) and Xinotyri (sample E) from Greece, Sepet (sample F) and Tulumn (sample G) from Turkey, and Darfiyeh (sample H) from Lebanon.

Microbiological Analysis

To obtain abundant NSLAB from these cheeses, 2 portions (about 10 g) per sample were collected using a sterile knife, specifically from the rind (sampled from the first 5 mm on each side of the cheese, including the edges) and the core (sampled from the center of cheese). The samples were then mixed for continuous microbiological analysis. Cheese samples were homogenized following the procedure described by Tsafrakidou et al. (2016). Briefly, cheese samples were homogenized with 90 mL of sterile 20 g/L sodium citrate solution at 45°C in a Stomacher 400 laboratory blender (Seward, London, UK) for 4 min at maximum speed. Dilutions (1/10) of the homogenates were prepared with a sterile solution of 0.85% (wt/vol) sodium chloride and then plated on de Man, Rogosa, and Sharpe (**MRS**; pH 5.7) agar plates that were incubated under anaerobic conditions at 30°C for 5 d before presumptive lactobacilli examination. Fifteen colonies per sample were randomly picked from the MRS agar plates, totaling to 120 colonies. Twenty-five colonies, which were gram-positive, catalase-negative, and able to grow at 15 and 45°C, were selected for the next test. Finally, pure cultures were frozen (−80°C) in MRS broth containing 20% (vol/vol) glycerol for storage. Isolates were activated by successive transfer in their respective medium and incubation at 37°C for production.

Identification of Isolates

Isolates were identified using the 16S rDNA sequencing method. Briefly, genomic DNA was extracted using the E.Z.N.A. bacterial DNA kit (Omega Bio-Tek, Norcross, GA) and amplified by the 16S rDNA universal pair of primers 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′, and 1492R: 5′-TACGGTTACCTTGTTACGACTT-3′. The amplified products were sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). Sequence similarity searches were performed by comparing the isolated sequences with the ones collected in the GenBank using the BLAST search program of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Technological Characterization

Salt Tolerance. The ability of the strains to grow in increasing saline solutions of 2, 6, and 10% (wt/vol) NaCl was evaluated according to the method described by Ferrari et al. (2016). After incubation at 37°C for 48 h, a color change of the culture from purple to yellow due to acidification of the substrates was considered as positive growth.

Exopolysaccharide and Diacetyl Production. For screening of exopolysaccharide (**EPS**) production, strains were grown in modified MRS agar medium in which the glucose present in the original formulation was replaced by 10% sucrose (Fluka, Buchs, Switzerland). Cultures were then streaked on plates that were