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CHARACTERIZATION OF *LACTOBACILLUS* FROM ALGERIAN GOAT'S MILK BASED ON PHENOTYPIC, 16S rDNA SEQUENCING AND THEIR TECHNOLOGICAL PROPERTIES

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ABSTRACT

Nineteen strains of *Lactobacillus* isolated from goat's milk from farms in north-west of Algeria were characterized. Isolates were identified by phenotypic, physiological and genotypic methods and some of their important technological properties were studied. Phenotypic characterization was carried out by studying physiological, morphological characteristics and carbohydrate fermentation patterns using API 50 CHL system. Isolates were also characterized by partial 16S rDNA sequencing. Results obtained with phenotypic methods were correlated with the genotypic characterization and 13 isolates were identified as *L. plantarum*, two isolates as *L. rhamnosus* and one isolate as *L. fermentum*. Three isolates identified as *L. plantarum* by phenotypic characterization were found to be *L. pentosus* by the genotypic method. A large diversity in technological properties (acid production in skim milk, exopolysaccharide production, aminopeptidase activity, antibacterial activity and antibiotic susceptibility) was observed. Based on these results, two strains of *L. plantarum* (LbMS16 and LbMS21) and one strain of *L. rhamnosus* (LbMF25) have been tentatively selected for use as starter cultures in the manufacture of artisanal fermented dairy products in Algeria.

Key words*:* Lactic acid bacteria; *Lactobacillus*; identification; goat's milk; technological properties; Algeria.

INTRODUCTION

The identification of lactobacilli has been based mainly on fermentation of carbohydrates, morphology, and Gram staining and these methods are still being used. However, the characterization of some *Lactobacillus* to species level by biochemical methods alone is not reliable (27, 40), because of the considerable variations in biochemical attributes between strains currently considered to belong to the same species. In fact, some species are not readily distinguishable in terms of phenotypic characteristics (12). In recent years, the taxonomy has changed considerably with the increasing knowledge of the genomic structure and phylogenetic relationships between *Lactobacillus* spp. (27, 43, 47). This novel taxonomy based on

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DNA analysis offers a variety of advantages over other more conventional typing procedures, such as the stability of the genomic DNA analysis, the capacity to discriminate bacteria at the strain level, and the amenability to automation and statistical analysis (21). These methods have been employed for differentiation or identification and typing of different species of *Lactobacillus*. The species of lactobacilli most commonly found in milk, and dairy product, especially in goat's milk are *L. plantarum, L. rhamnosus*, *L. casei* or *L. paracasei.* For this reason, the selection of *Lactobacillus* strains from goat's milk has been considered in the search for new industrially important cultures, in order to select those with the highest potential for industrial applications. In Algeria, goat's milk plays a vital role in human consumption, most being consumed by the rural community, while little is available on the market (5). Algerian people make various fermented dairy products using goat's milk. The transformation of goat's milk into traditional Algerian dairy products, such as El – Klila, a traditional cheese which is popular in the country side and is made from unpasteurised cow or goat surplus milk (7), Jben (local traditional fresh cheese), Raïb, and Lben (local traditional fermented milks), is achieved through spontaneous fermentation without the addition of any selected starter. Such products generally present irregular sensorial qualities. The aim of the present study was to characterize *Lactobacillus* isolated from goat's milk from north-west of Algeria, using physiological, phenotypic and genotypic methods. There are no previous reports concerning the genetic identification of *Lactobacillus* or studies that combined the phenotypic and the genotypic identification of *Lactobacillus* isolated from goat's milk in Algeria. Additionally, in order to select adequate strains susceptible to be used as starter cultures for the manufacture of artisanal fermented dairy products in Algeria, some important technological properties, including the capacity of acidification/coagulation of skim milk, exopolysaccharide production, aminopeptidase, antibacterial activity, and antibiotics resistance, were also studied.

MATERIAL AND METHODS

Milk samples

Five samples of goat's milk collected from farms located in the region north-west of Algeria were used in this study. The samples were collected aseptically in sterile bottles kept in an ice-box, and transported immediately to the laboratory.

Phenotypic characterization

One milliliter of each milk sample was homogenized with 9 ml of sterile Ringer's solution 1:4 and mixed thoroughly for 60s. Serial dilutions were made and aliquots (100 µl) of each dilution were streaked on MRS agar (Oxoid, UK) (17). The MRS plates were incubated at 30 °C and 45°C for 24 to 48h under anaerobic conditions (Anaerogen, Oxoid). Ten colonies from plates corresponding to the highest dilutions were randomly selected and purified by subculturing. Gram-positive, catalase negative cultures were stored at -80 °C in MRS supplemented with 20% glycerol. Isolates were phenotypically assigned to the genus level on the basis of: cell morphology, Gram-positive and catalase-negative, according to the methods and criteria described by Sharpe (42) and Kandler and Weiss (26); CO_2 production from glucose in MRS broth containing inverted Durham tubes (32); hydrolysis of arginine, growth at 15 °C and 45 °C, tolerance to 20, 40, 65 g L^{-1} NaCl. The acid production from carbohydrates (fructose, glucose, mannitol, lactose, mannose, rhamnose, glycerol, arabinose, sorbose, dulcitol, amygdalin, melibiose, melezitose, starch, tagatose, arabitol, ribose, maltose, galactose, and xylose) was evaluated by using a miniaturized assay in microtiter plates, as described by Jayne-Williams (25). Ability to ferment carbohydrate substrates was studied, using the API 50 CHL system (BioMérieux, Lyon, France), following the manufacturer recommendations.

DNA extraction and 16S rDNA sequencing

Isolates were grown in MRS broth at 30 °C until OD of

1.6 - 1.8 at 600 nm. A 1.5 mL aliquot of each overnight culture was centrifuged at 10000 × *g* for 30 s at room temperature in order to pellet cells. Bacterial DNA was isolated by using the UltraClean™ Microbial DNA isolation Kit (MoBio Laboratories Inc., Solana Beach, CA, USA), following the instructions of the manufacturer.

The 5′ end variable region of the 16S rDNA was PCR-amplified with primers 27F (5′-AGAGTTTGATCCTGGCTC AG-3′) and 558R (5′-GTATTCCGCGGCTG-3) or with the primers 27F and 1525R (5′-AAGGAGGTGWTCCARCCG CA-3′) using a total volume of 50 µl containing 50 ng of DNA, 25 pmol of each primer, 1.6 mM of dNTPs, 2 mM $MgCl_2$ and 1U of Taq DNA polymerase (Biotools Lab, Spain), using the reaction buffer supplied by the manufacturer.

Amplifications were carried out in a Thermal Cycler (PTC-100 Peltier Themal Cycler, MJR), using the following program: for primers 27F and 558R, the 16S rDNA was amplified as described by Linaje *et al*. (30); for the primers 27F and 1525R, the PCR mixtures were subjected to an initial denaturing step of 95 °C for 5min, followed by 30 PCR cycles (94 °C, 15s; 52 °C, 30s; 72 °C, 2min) and final cycle at 72 °C for 5min. The PCR products were subjected to gel electrophoresis in 1% agarose gel, followed by staining with ethidium bromide and visualization under UV light. A Lambda DNA (Biorad) digested with PstI ladder was used as a molecular mass marker.

Polymerase chain reaction products were purified by using the GFX PCR DNA and a gel band purification Kit (General Electric Healthcare, Spain), following the manufacturer's instructions. DNA sequencing was carried out by the Central Service of Research Support of the University of Valencia (Spain), by using the dideoxynucleotide DNA chain termination method.

Technological properties

Acidifying activity in skim milk was assayed as described by Psono *et al*. (35). Sterile skim milk samples (100 mL; 1.0%) were inoculated with overnight cultures which had been previously activated by two successive transfers in milk. The pH changes were measured with a pH meter (glass electrode, Crison, Spain) after 6, 12, and 24 h of incubation at 30°C. Acidification activity was measured by following the change in the pH during time. Coagulation of milk was determined after 24 h of incubation at 30°C. Screening of exopolysaccharide (EPS) was carried out in ruthenium red milk plates, as described by Stingele *et al.* (44).

Aminopeptidase activity of the strains was determined using the synthetic substrates L-alanine ρ-nitroaniline (Ala-ρ-NA) (Sigma,USA), and L-leucine ρ-nitroaniline (Leu-ρ-NA) (Sigma,USA) as described by Zotta *et al.* (51). Stationary phase cells grown overnight in MRS broth were harvested by centrifugation at 10000 × *g* for 5 min, washed twice with sterile 50 mM potassium phosphate buffer, pH 7.0, and re-suspended in the same buffer to obtain cell suspensions of (A_{650} = 1.0). Aminopeptidase activity was measured, according to Macedo *et al.*(31). The assay mixture contained: 30µl of 20 mM aminoacyl ρ-nitroanilide substrates dissolved in methanol, 195 µL of 50 mM potassium phosphate buffer (pH 7.0), 95 µL of 0.05% (w/v) sodium azide solution, and 75 µL of cell suspension. After incubation at 30 °C for 1 to 4 h, the reaction was stopped by the addition of 900 µL of 1% (v/v) acetic acid. The release of ρ-nitroaniline (ρ-NA) (Sigma, USA) was measured spectrophotometrically (Hewlett Packaro, Diod Array Spectrophotometer, Germany) at 410 nm after centrifugation of the mixture at 10000 × *g* for 5min. Data obtained were compared to a calibration curve prepared using ρ-NA (Sigma, USA) dilutions ranging from 0.1 to 20.0 mM. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of ρ-NA min^{-1} under the assay conditions.

Antibacterial activity of *Lactobacillus* strains was tested with the well diffusion method described by Linaje *et al.* (30). Cells were grown overnight in MRS broth. Cell-free supernatants were obtained by centrifugation at 10000 × *g* for 10 min at 4 °C, adjusted to pH 6.5 with 1N NaOH, and then filtered through 0.22µm Durapore membrane filters (Millipore). The supernatants were adjusted to pH 6.5, in order to eliminate the eventual antimicrobial activity linked to

organic acids. A 100 µL aliquot of an overnight culture of the indicator bacteria *Listeria monocytogenes* CECT 932^T, *Bacillus cereus* INRA AVZ 421, *Staphylococcus aureus* CECT 86^T and *Staphylococcus aureus* UT 602, was mixed with 5 mL of soft agar (Trypticase Soya Broth for *Bacillus cereus* and *Staphylococcus aureus* and Brain Heart Infusion for *Listeria monocytogenes*, supplemented with 0.8% agar). Aliquots (50 µl) of supernatant of overnight cultures were poured in the wells digged in the soft agar. After 24 h of incubation at 37 °C, inhibition zones were read. A clear zone of inhibition >1 mm around a well was scored as positive. In order to check the thermoresistance of the bacteriocins, cell free supernatant samples were heated at 100°C for 10 min, prior the antibacterial assay. The proteinaceous nature of the inhibitory activity was tested by the addition of 0.5 µg of proteinase K (Roche Molecular Biochemicals) to the concentrated culture supernatants (50 µL) distributed among the wells of the assay plates.

Antibiotic susceptibility testing

Susceptibility testing was based on the agar overlay disc diffusion test described by Charteris *et al*. (10), as modified by Aymerich *et al*. (4). Briefly, *Lactobacillus* strains were grown overnight in MRS broth at 30°C under anaerobic conditions (Anaerogen, Oxoid). Eight ml of MRS soft agar kept at 50°C were inoculated with 200 µL of the grown culture. Petri dishes containing 15 mL of MRS were overlaid with 8.2 mL of the inoculated MRS and allowed to solidify at room temperature. Antibiotic discs were placed onto the overlaid plates and all plates were incubated at 30°C for 24 h under anaerobic conditions. All isolates were screened for their susceptibility to penicillin G (10 µg), ampicillin (10 µg), vancomycin (30 µg), tetracycline (30 µg), erythromycin (15 µg), kanamycin (30µg) gentamicin (10 µg), and chloramphenicol (30 µg). Inhibition zones diameters of antibiotics were compared to those defined by Charteris *et al*. (10) for lactobacilli.

RESULTS AND DISCUSSION

Phenotypic identification of isolates

Isolated strains were identified based on their physiological and biochemical characteristics given by Kandler and Weiss (26), Dellaglio *et al*. (15) and Stiles and Holzapfel (43), to the species level, and also by using API 50 CHL test strips (BioMérieux, Lyon, France) for confirmation of species of selected strains.

All isolates (19 *Lactobacillus* strains) were rod shaped cells, Gram-positive, catalase-negative, non motile and facultative anaerobic bacteria. Isolates were classified as belonging to the genus *Lactobacillus*. All isolates were able to grow at 15°C, 2%, and 4% NaCl. They were divided into two preliminary groups (I and II), according to the results for CO_2 production from glucose, and NH_3 production from arginine: Group I, facultatively heterofermentative, and arginine-negative lactobacilli (94.73%); group II strictly heterofermentative, and arginine-positive strain (one *Lactobacillus* strain).

Table 1 shows the carbohydrate utilization patterns and other physiological and biochemical characteristics of the lactobacilli isolates. The analysis of data compared with those of the criteria given by several authors, resulted in four subgroups (A-D). Group I was subdivided into three subgroups (A-C) and group II, comprised only one subgroup (D). Isolates belonging to the four subgroups were able to ferment fructose, glucose, mannitol, lactose, mannose, ribose, maltose, and galactose and unable to ferment xylose.

- Subgroup A was the largest one, with 11 strains (58%) identified as *L. plantarum*. The strains were able to ferment amygdalin, melibiose, melezitose, and arabitol, but unable to ferment rhamnose, glycerol, sorbose, dulcitol, and tagatose. However, variations in fermentation patterns were observed for some sugars: arabinose was fermented by 36%, starch by 18%, sorbose by 9%, and dulcitol by 9% of the strains. Most of the

lactobacilli reported as being able to produce amylase are strictly homofermentative (26), although some *L. plantarum* have been reported as starch fermenting strains (33). Eighteen percent of isolates from this subgroup were able to grow at 45°C. Some of the *L. plantarum* strains were capable of growing at 45°C, in contrast to the characteristics given in Bergey's Manual (26). Other studies have also reported the isolation of *L. plantarum* strains capable of growing at this temperature (20, 39).

- Subgroup B, comprised 5 strains (26%) identified as belonging to *L .plantarum/L. pentosus* species. The isolates only differed from those of subgroup A in the inability to ferment the starch and arabitol. The ability of some strains (40%) to ferment glycerol resulted in their classification either as *L. plantarum* or as *L. pentosus* (8, 50). Growth at 45°C was observed for 40% of isolates.
- Subgroup C, included two isolates able to ferment rhamnose, arabinose, amygdalin, and melezitose. However, glycerol, sorbose, dulcitol, melibiose, starch, tagatose, and arabitol were not fermented. All the isolates were able to grow at 45°C and identified as *L. rhamnosus*.
- Subgroup D, with one isolate able to ferment rhamnose, arabinose amygdalin, melibiose and tagatose. In contrast, this strain was incapable of fermenting glycerol, sorbose, dulcitol, melezitose, starch and arabitol. This isolate was able to grow at 45°C and was classified as *L. fermentum*.

Table 1. Biochemical and physiological characteristics of *Lactobacillus* strains isolated from Algerian goat's milk.

Group	**I**			**II**
Subgroup	**A**	**B**	**C**	**D**
Number of isolates	**11**	**5**	**2**	**1**
CO_2 from glucose	-	-	-	+
Arginine Hydrolysis	-	-	-	+
Growth at				
15°C	+	+	+	+
45°C	18	40	+	+
Growth in				
2 % NaCl	+	+	+	+
4 % NaCl	+	+	+	+
6.5 % NaCl	-	-	-	-
Sugar fermentation				
Fructose	+	+	+	+
Glucose	+	+	+	+
Mannitol	+	+	+	+
Lactose	+	+	+	+
Mannose	+	+	+	+
Rhamnose	-	-	+	+
Glycerol	-	40	-	-
Arabinose	36	+	+	+
Sorbose	9	-	-	-
Dulcitol	9	20	-	-
amygdalin	+	20	+	+
Melibiose	+	+	-	+
Melezitose	+	+	+	-
Starch	18	-	-	-
Tagatose	-	-	-	+
Arabitol	+	-	-	-
Ribose	+	+	+	+
Maltose	+	+	+	+
Galactose	+	+	+	+
Xylose	-	-	-	-

+: Positive reaction; -: Negative reaction; 20: 20% of isolates showed a positive result; Subgroup A: LbMA9, LbMF13, LbMF33, LbMS4, LbMS9, LbMS14, LbMS16, LbMS20, LbMS21, LbMS24, LbMO16; Subgroup B: LbMO27, LbMO42, LbMS40, LbMT9, LbMT10; Subgroup C: LbMF24, LbMF25; Subgroup D: LbMA47.

To establish the final phenotypic identification, all strains tested for their biochemical and physiological characteristics (Table 1), were submitted to further biochemical characterization, using API 50 CHL galleries (BioMérieux, Lyon, France). The programme of identification (Cox and Thomson, Biochemistry institute, Odense University) plus database was used for the interpretation of the strains fermentation profiles (Table 2). The strains of the subgroups A and B were classified as *L. plantarum* and those of subgroups C and D as *L. rhamnosus* and *L. fermentum*, respectively.

A clear identification of species, especially within the genus *Lactobacillus*, based on phenotypic methods, such as fermentation patterns, may sometimes be difficult, due to an increasing number of lactic acid bacteria species which vary on a small number of biochemical traits (36). Commercially available systems based on carbohydrate fermentation should be combined with conventional phenotypic properties other than carbohydrate fermentation or with genotypic techniques.

Table 2. Fermentation of carbohydrates by *Lactobacillus* strains from Algerian goat's milk, tested by the API 50 CHL system

Groups	**A**											**B**					**C**		D
Isolates	**LbMA9**	**LbMF13**	**LbMF33**	**LbMS4**	**LbMS9**	**LbMS20**	**LbMS14**	**LbMS16**	**LbMS24**	**LbMS21**	**LbMO16**	**LbMO27**	**LbMO42**	**LbMS40**	**LbMT9**	**LbMT10**	**LbMF24**	LbMF25	LbMA47
Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
L-Arabinose	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Mathyl- D-Mannopyranoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
Mathyle-D-Glucopyrannoside	+	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-	+	+	+
N-Acetylglucosamine	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+
D-Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
D-Melezitose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Starch	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
D-Tagatose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
D-Arabitol	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
L-Arabitol	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

+: Positive reaction; -: Negative reaction.
All isolates were able to ferment D-ribose, D-galactose, D-glucose,D-fructose,D-mannose, D-mannitol, D-sorbitol, arbutin, esculin, salicin, D-celibiose, D-maltose, D-lactose, D-sucrose, D-trehalose, D-raffinose, gentibiose, D-turanose and gluconate. None fermented erythriol, D-arabinose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, inositol, inulin, glycogen, xylitol, D-rhamnose, D-fucose, L-fucose, 2-ketogluconate and 5-ketogluconate.

Genotypic identification of the isolates by 16S rDNA sequencing

The 16S rDNA of the 11 strains of subgroup A and 5 isolates of the subgroup B was amplified by 27F and 558R primers, as reported by Acedo-Félix and Pérez-Martinez (1). By partial sequencing of 16S rDNA all strains belonging to the subgroup A were classified as *L. plantarum*. Of the 5 isolates forming subgroup B, 60% were identified as *L. pentosus*, (LbMS40, LbMT9 and LbMT10) and 40% as *L. plantarum* (LbMO42, and LbMO27). The 16S rDNA of two isolates of subgroup C were amplified with primers 27F and 1525R and identified as *L. rhamnosus* (LbMF24 and LbMF25). The 16S rDNA of the isolate of subgroup D was amplified by the primers used for subgroup C and identified as *L. fermentum* (LbMA 47).

Results of the PCR assay correlated with those obtained using the API 50 CHL system for 13 isolates identified as *L. plantarum*, 2 isolates as *L. rhamnosus*, and one isolate as *L. fermentum*. However, three isolates identified as *L. plantarum* by the API system were found to be *L. pentosus* by sequencing 16S rDNA (Table 3). This result is not surprising, given that the two species themselves have very similar 16S rDNA sequences that differ only by 2pb (19). In fact, it is widely acknowledged that *L. plantarum* and *L. pentosus* belong to the same 16S rRNA phylogenetic group and could only be distinguished using phylogenetic analysis of sequences of the 16S-23S large spacer region (22).

The comparative evaluation of phenotypic and genotypic results confirmed that the phenotypic test, in spite of giving information on the biochemical and metabolic traits of LAB, are not reliable enough for the identification of these microorganisms, although it is a useful tool for presumptive classification. *Lactobacillus* UFV H2B20, a probiotic strain, for example, which was first identified as *L. acidophilus* based on its sugar fermentation profile (38), was afterwards classified as *L. delbrueckii* using molecular methods (16). One major reason for the mismatch between phenotypic and genotypic data might be ascribed to loosing or acquiring plasmids, which leads to metabolite inconsistencies, as some carbohydrate fermentation capacities are plasmid encoded (2). Genotypic techniques are doubtlessly rapid and accurate tools for the identification of LAB. The advantages of genotyping include the stability of genomic DNA, its composition being independent of cultural conditions or preparation methods, and amenability to automation and statistical data analysis (21).

Table 3. Phenotypic and genotypic identification of *Lactobacillus* isolated from Algerian goat's milk.

Phenotypic dentification	Isolates	Genotypic identification (16S rDNA)	Most similar sequence (Acc. Nº)
Lactobacillus plantarum	LbMA9, LbMO16, LbMO27	*Lactobacillus plantarum*	AB362768.1
L. plantarum	LbMF13	*L. plantarum*	EF185922.1
L. plantarum	LbMF33	*L. plantarum*	AB362758.1
L. plantarum	LbMS4, LbMS9, LbMS14, LbMS16, LbMS20, LbMS21,LbMS24	*L. plantarum*	EU257480.1
L. plantarum	LbMO42	*L. plantarum*	AB362625.1
L. plantarum	LbMS40, LbMT9	*L. pentosus*	AB362758.1
L. plantarum	LbMT10	*L. pentosus*	AB362712.1
L. rhamnosus	LbMF24,LbMF25	*L. rhamnosus*	AB008211.1
L. fermentum	LbMA47	*L. fermentum*	AB362626.1

Technological characteristics of strains

Results on acidifying activity of *Lactobacillus* strains isolated from goat's milk, after 6, 12 and 24 h of growth in skim milk are shown in Table 4. Milk pH after 24 h of incubation varied between 4.40 and 5.54 for all cultures. All *Lactobacillus* isolates tested reduced the pH of milk to 6.43

(6.12 - 6.43) after 6 h of growth, except for strains LbMA47,LbMS21 and LbMS16 which, respectively, were able to decrease milk pH to values 5.31, 5.78 and 5.97. After 12 h, the pH values ranged from 5.24 (LbMS21) to 5.94 (LbMT10), while after 24 h, 47.37% of the strains had lowered milk pH to 4.40 - 4.96. According to their ability to reduce the pH more or less rapidly, three clusters of *L. plantarum* isolates were observed. (i) Slow acidifying strains (cluster I, 23% of *L. plantarum* strains) showed a slow rate of acidifying ability during the first 6 h of incubation (ΔpH ranging between 0.27-0.40 pH units). These *L. plantarum* strains lowered the ΔpH values between 0.86-1.01 pH units and 1.33-1.60 pH units after 12 h and 24 h of incubation, respectively. (ii) Medium acidifying *L. plantarum* strains (cluster II) showed a faster rate of acidifying ability after 6 h of incubation, with a ΔpH ranging between 0.50 and 0.57 pH units. In this cluster, two subgroups were observed with different behaviours in their acidifying capacity. The first subgroup A (31% of *L. plantarum* strains) in which the ΔpH values was achieved, respectively, 0.78-0.98 and 1.16-1.74 pH units, after 12 h and 24 h of incubation. On the other hand, the second subgroup B (31% of *L. plantarum* strains) showed a faster acidification rate. This subgroup included *L. plantarum* strains showing a similar acidifying activity until 6 h but capable of achieving ΔpH values ranging between 1.05-1.13 and 1.96-2.14 pH units after 12 h and 24 h of incubation.(iii) Fast acidifying *L. plantarum* strains (15% of *L. plantarum* strains) showed the highest acidifying capacity. This two *L. plantarum* strains, LbMS16 and LbMS21, showed a fast rate of acidifying ability until 6 h (0.73 and 0.92 pH units respectively). This high capacity of acidifying milk by strains of this cluster was also shown after 12 h and 24 h of incubation: ΔpH values were achieved, respectively, 1.31 to 2.20 pH units for LbMS16 and 1.46 to 2.30 pH units for LbMS21. Result of acidifying capacity of *L. plantarum* strains after 6 h and 12 h of incubation is in agreement with several authors (14, 23). In contrast, the acidifying rate of isolates after 24 h of incubation was lower than those reported in the former studies. The possibility to find groups of strains characterized by different acidifying ability is frequent in *L. plantarum* species (49).

L. pentosus and *L. rhamnosus* strains showed a more homogenous acidifying behaviour. Strains of the first species had a medium acidifying capacity with ΔpH ranging between 0.43 and 0.58 pH units, after 6 h of incubation and achieved 1.18-1.30 pH units until 24 h. Furthermore, the two *L. rhamnosus* strains showed a fast acidifying activity, with values of ΔpH 0.63 and of 0.69 pH units after 6 h. After 24 h of incubation the ΔpH reached values of 1.87 and 1.96 pH units. *L. fermentum* strain showed the lowest rate of acidifying: ΔpH 0.25 pH units after 6 h and resulted in ΔpH 1.35 pH units until 24 h of incubation. Generally, *L. plantarum* strains produce acid more rapidly, when compared to other lactobacilli (41, 14). The fast acidifying strains (mostly *L. plantarum* and *L. rhamnosus* isolates) should be selected as part of a starter preparation.

Seven isolates of *L. plantarum* strains and two *L. rhamnosus* were able to coagulate skim milk, when inoculated in skim milk after 24 h at 30 °C (Table 4). None of the *L. pentosus* and *L. fermentum* strains were able to coagulate milk. Coagulation of milk by some strains of *L. plantarum* and two strains of *L. rhamnosus* revealed their potential as starters or adjunct cultures in production of fermented dairy food products (34).

Many strains of LAB produce exopolysaccharide (EPS), which might be a capsule, closely attached as slim (9). The production of EPS by *Lactobacillus* strains was examined in milk culture and on ruthenium red milk plates. Ruthenium red stains the bacterial cell wall, thus producing red colonies for nonropy strains. Production of EPS prevents this staining, and hence ropy colonies appear white on the same plates (44). The results revealed EPS-production by isolated *Lactobacillus* strains (Table 4). Among the *Lactobacillus* strains tested, eight showed EPS-production. Four *L. plantarum* (LbMS14, LbMS16, LbMO16 and LbMO27 strains), two *L. rharmnosus* (LbMF24 and LbMF25 strains), one *L. pentosus* (LbMT10 strain) and one *L. fermentum* (LbMA47 strain) produced EPS. Exopolysaccharides play a major industrial role in the production of fermented products, in particular for the

production of yoghurt, drinking yoghurt, cheese, fermented cream and milk-based desserts (18).

Results on aminopeptidase (AP) activity of 19 strains of *Lactobacillus* strains tested using (Ala-ρNa and Leu-ρNa) are shown in Figure 1. Amino acids released from peptides derived from hydrolysis of casein may contribute directly or indirectly for the development of flavour during ripening of cheese (48). The proteolytic activity of dairy LAB is essential for the bacterial growth in milk and it is involved in the development of sensory properties of different fermented milk products (11). Tested strains exhibiting aminopeptidase activity ranging between 1.02 and 14.10 U for L-alanine-ρNA and 0.95 to 11.37 U for L-leucine-ρNA. The AP was divided, according to the activity of each strain to high, medium and low activity. Eleven percent of strains presented high activity to release Ala-ρNA (13.12-14.10 U) and 16% of strains showed high ability to release Leu-ρNA (10.10-11.37 U). Twenty six percent of tested strains presented medium Ala-aminopeptidase activity (6.14-8.35 U) and 26% Leu-aminopeptidase (5.15-9.70 U). However, 63% of isolates revealed low activity to degrade Ala-ρNA (1.02-4.93 U) and 58% to release Leu-ρNA (0.95-4.32 U). All tested strains of *L. pentosus, L. rhamnosus*, and *L. fermentum* exhibited low AP activity, except for *L. pentosus* LbMT10 strain, which had medium Leu-aminopeptidase activity. Concerning the results obtained for *L. plantarum* strains, two strains (LbMF13 and LbMO16) had high Ala-aminopeptidase activity and four strains (LbMS14, LbMS16, LbMS24 and LbMO16) had high Leu-aminopeptidase activity.

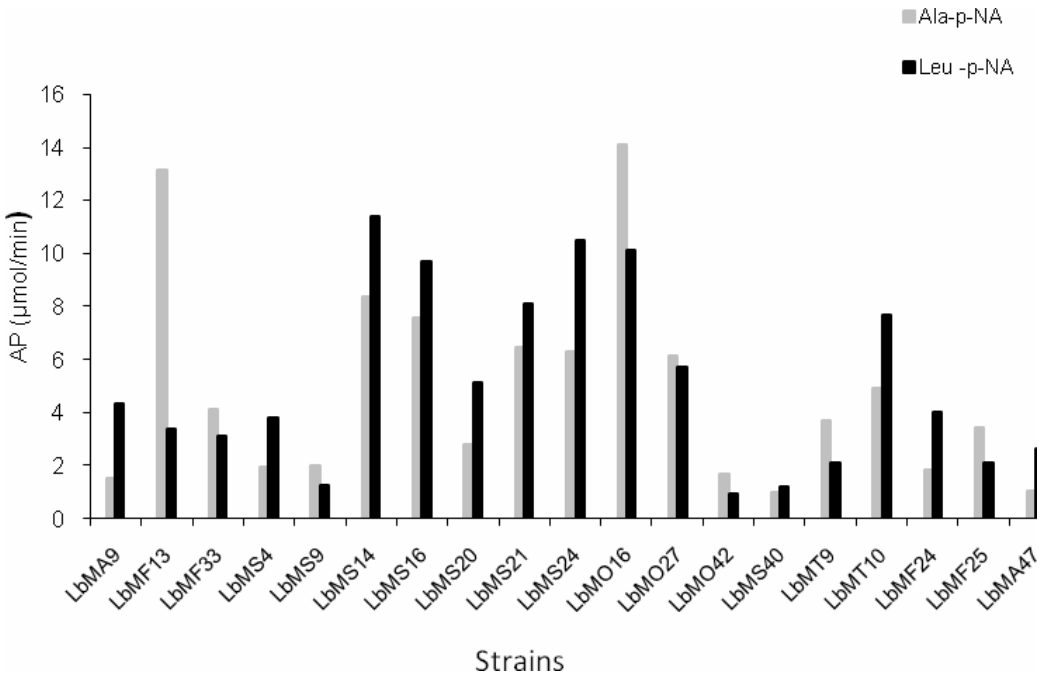

Figure1. Aminopeptidase activity of strains of the *Lactobacillus* tested in this study.

Well diffusion assay was used to screen 19 *Lactobacillus* strains isolated from goat's milk for their antibacterial activities against several pathogenic indicator bacteria (*Staphylococcus aureus* CECT 86^T, *Staphylococcus aureus* UT 602, *Bacillus cereus* INRA AVZ421 and *Listeria monocytogenes* CECT 932^T) (Table 4). Bacteriocin like antimicrobial compound production was indicated by a zone of clearing of more than 1 mm against at least one of the indicator bacteria tested. The cell-free supernatants of five *L. plantarum* strains (38.46 %, LbMA9, LbMF13, LbMS14, LbMS24 and LbMO16) produced

an inhibition zone on agar against *S. aureus* CECT 86^T and *S. aureus* UT 602 and three *L. plantarum* strains (23,07%, LbMF13, LbMS24, and LbMO16) against *Bacillus cereus* AVZ 421. Antibacterial activity is a relatively frequent feature of *L. plantarum* natural isolates (46). However, one strain of *L. rhamnosus* strain (LbMF25) showed antimicrobial activity against *S. aureus* CECT 86^T and *S. aureus* UT 602. Nevertheless, none of the tested strains of *L. pentostus* and *L. fermentum* displayed inhibitory activity against any of the indicator bacteria. In addition, all strains tested were inactive against *Listeria monocytogenes* CECT 932^T. Complete inactivation of the antimicrobial activity was observed after treatment with proteinase K, indicating the proteinaceous nature of the antimicrobial compound, whereas treatment with heat did not affect the inhibitory activity. Bacteriocins produced by LAB are of particular interest because of their potential use as natural food preservatives. The antibacterial activity potential of some *L. plantarum* strains against *Staphylococcus aureus* (37, 6) and *Bacillus cereus* (6) has been previously reported.

Our results confirm the high incidence of bacteriocin-producing lactic acid bacteria in milk samples, with inhibitory activity against both pathogenic and spoilage microorganisms. Goat's milk may represent a source of new *Lactobacillus* strains with the potential to inhibit undesirable and pathogenic microorganisms for use in the biopreservation of dairy products. The resistance to high temperature, the proteinaceous nature, and the spectrum of activity of these antimicrobial compound are advantageous for their use as biopreservatives in food (37).

Antibiotic resistance

A key requirement for probiotic strains is that they should not carry transferable antibiotic resistance genes. Transferable resistance genes may pose a risk, as they can be transferred to pathogenic bacteria (4). In this study, antibacterial susceptibility testing of *Lactobacillus* strains was made according to Charteris *et al.* (10). All *Lactobacillus* strains isolated from goat's milk were assayed for their susceptibility to eight antibiotics, using the disk diffusion method. Zone diameters were measured, and strains were classified as, susceptible (S), moderately susceptible (MS), and resistant (R) (Table 4). No strains of *Lactobacillus* were totally susceptible to all antibiotics tested and multiple resistances to most antibiotics were observed. Most strains were susceptible to β-lactam, inhibitors of cell wall synthesis (penicillin G and ampicillin). Some tested strains are moderately susceptible to the former antibiotics. However, two *L. plantarum* strains, LbMO42 and LbMS24, were resistant to penicillin G or ampicillin, respectively, and one *L. pentosus* strain (LbMT9) was resistant to these two antibiotics simultaneously. Several studies report that species of lactobacilli exhibited susceptibility to almost all penicillins (10). Nevertheless, the resistance of *Lactobacillus* strains to penicillin G and ampicillin has been described in other studies (45, 13, 24).

Our result show that all tested strains were resistant to vancomycin, which is equally a cell wall synthesis inhibitor (non-β-lactam). Resistance of *Lactobacillus* species to vancomycin is due to the presence of D-Ala-D-lactate in their peptidoglycan, rather than the D-ala-D-ala dipeptide (28). Such resistance is usually intrinsic, that is, chromosomally encoded and nontransmissible (27). Concerning the protein synthesis inhibitors, all strains tested were susceptible to tetracyclin, erythromycin and resistant to kanamycin and gentamycin. Lactobacilli are generally susceptible to antibiotics which inhibit the synthesis of proteins, such as erythromycin and tetracycline and more resistant to aminoglycosides (kanamycin and gentamicin) (10). Chloramphenicol inhibited most tested strains. Three *Lactobacillus* strains showed a moderate susceptibility and one *L. plantarum* (LbMO42) strain was resistant to this antibiotic. The high natural susceptibility of lactobacilli to chloramphenicol (protein synthesis inhibitor) is well known (10, 45).

Table 4. Technological characteristics and antibiotic resistance of *Lactobacillus* strains isolated from Algerian goat's milk

	ΔpH			Coagulation	EPS	Antibacterial activity				Antibiotics							
Strains	**6h**	**12h**	**24h**	**24h**		***S. aureus* CECT 86[a]**	***S. aureus* UT 602[b]**	***Bacillus cereus* INRA AVZ 421[c]**	***Listeria monocytogenes* CECT 932[a]**	**P**	**A**	**V**	**T**	**E**	**K**	**G**	**C**
L. plantarum																	
LbMA9	0.27	1.01	1.60	+	-	+	+	-	-	MS	S	R	S	S	R	R	S
LbMF13	0.50	0.97	1.46	-	-	+	+	+	-	S	MS	R	S	S	R	R	S
LbMF33	0.52	0.78	1.16	-	-	-	-	-	-	S	MS	R	S	S	R	R	S
LbMS4	0.53	1.05	2.14	-	-	-	-	-	-	S	S	R	S	S	R	R	S
LbMS9	0.51	1.13	1.97	+	-	-	-	-	-	S	S	R	S	S	R	R	S
LbMS14	0.56	1.11	2.07	+	+	+	+	-	-	S	S	R	S	S	R	R	S
LbMS16	0.73	1.31	2.20	+	+	-	-	-	-	S	S	R	S	S	R	R	S
LbMS20	0.57	1.07	1.96	+	-	-	-	-	-	MS	S	R	S	S	R	R	S
LbMS21	0.92	1.46	2.30	+	-	-	-	-	-	S	S	R	S	S	R	R	S
LbMS24	0.56	0.91	1.49	-	-	+	+	+	-	S	R	R	S	S	R	R	S
LbMO16	0.27	0.89	1.43	-	+	+	+	+	-	S	MS	R	S	S	R	R	MS
LbMO27	0.56	0.98	1.74	+	+	-	-	-	-	S	S	R	S	S	R	R	S
LbMO42	0.40	0.86	1.33	-	-	-	-	-	-	R	S	R	S	S	R	R	R
L. pentosus																	
LbMS40	0.43	0.89	1.29	-	-	-	-	-	-	S	S	R	S	S	R	R	MS
LbMT9	0.56	1.19	1.3	-	-	-	-	-	-	R	R	R	S	S	R	R	S
LbMT10	0.53	0.76	1.18	-	+	-	-	-	-	S	MS	R	S	S	R	R	S
L. rhamnosus																	
LbMF24	0.63	1.10	1.87	+	+	-	-	-	-	S	S	R	S	S	R	R	S
LbMF25	0.69	1.40	1.96	+	+	+	+	-	-	S	S	R	S	S	R	R	S
L. fermentum																	
LbMA47	0.25	0.83	1.35	-	+	-	-	-	-	S	MS	R	S	S	R	R	MS

+: Positive reaction; -: Negative reaction.
EPS: Exopolysaccharide production
(a) CECT: Colección Española de Cultivos Tipo, Valencia, Spain
(b) U.T: University of Tlemcen laboratory collection, Algeria
(c) INRA AVZ : Station de Technologie des Produits Végétaux, Institut national de la Recherche Agronomique, Avignon, France (INRA)
PG: penicillin (10µg); A: ampicillin (10µg); V: vancomycin (30µg); T: tetracycline (30µg); E: erythromycin (15µg);
K: kanamycin (30µg); G: gentamicin (10µg); C: chloramphenicol (30µg).
S: sensitive strain; MS: moderately resistant strains; R: resistant strain.

With regard to antibiotic susceptibility profiles, *L. plantarum* strain (LbMO42) and *L. pentosus* strain (LbMT9) showed the highest resistance to 5 of the 8 antibiotics tested. At present, multiresistance seems to be uncommon among LAB species, but an increasing number of strains displaying atypical resistance levels to some antibiotics are being isolated (3). Development of antibiotic resistance in bacteria is mainly based on two factors, the presence of resistance genes and the selective pressure through the use of antibiotics (29). The overuse of antibiotics in veterinary medicine as therapeutic agents, prophylactics and animal growth promoters on farms may favour the development of resistance to antibiotics among LAB. Therefore, strains intended to be used in feed and food systems should be systematically monitored for resistances, in order to avoid their inclusion as starters and probiotics (3).

In conclusion, as far as we know, this is the first report on the genetic identification of *Lactobacillus* strains using 16S rDNA sequence of Algeria goat's milk isolates. This molecular method distinguishes the closely related species *L. plantarum* and *L. pentosus* identified using physiological features and API 50 CHL system as *L. plantarum*. Results on technological properties suggest the pontential of some of *L. plantarum* (LbMS16 and LbMS21) *and L. rhamnosus* (LbMF25) strains as starter cultures in the manufacture of artisanal fermented dairy product in Algeria. Moreover, these strains should be tested in mixed cultures, in order to obtain fermented dairy product with sensorial characteristics similar to those of artisanal products.

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REFERENCES

1. Acedo-Félix, E.; Pérez-Martínez, G. (2003). Significant differences between *Lactobacillus casei* subsp. *casei* ATTCC 393^T and a commonly used plasmid-cured derivative revealed by a polyphasic study. *Int. J. Syst. Evol. Microbiol*., 53: 67-75.
2. Ahrné, S.; Molin, G.; Stahl, S. (1989). Plasmids in *Lactobacillus* strains isolated from meat and meat products. *Syst. Appl. Microbiol*., 11: 320-325.
3. Ammor, M.S.; Flóre, A.B.; Mayo, B. (2007). Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacterium. *Food Microbiol*., 24: 559-570.
4. Aymerich, T.; Martin, B.; Garriga, M.; Vidal-Carou, M.C.; Bover-Cid, S.; Hugas, M. (2006). Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *J. Appl. Microbiol*., 100: 40-49.
5. Badis, A.; Guetarni, D.; Moussa-Boudjema, B.; Henni, D.E.; Tornadijo, M.E.; Kihal, M. (2004). Identification and technological properties of lactic acid bacteria isolated from raw goat's milk of four Algerian races. *Food Microbiol*., 2: 579-588.
6. Ben Omar, N.; Abriouel, H.; Lucas, R.; Martínez-Cañamero, M.; Guyot, J-P.; Gálvez, A. (2006). Isolation of bacteriocinogenic *Lactobacillus plantarum* strains from ben saalga, a traditional fermented gruel from Burkina Faso. *Int. J. Food Microbiol*., 112: 44-50.
7. Boubekri, K.; Ohta, Y. (1996). Identification of lactic acid bacteria from Algerian traditional cheese, El-Klila. *J. Sci. Food Agric.,* 70: 501-505.
8. Bringel, F.;Curk, M-C.; Huert, J-C. (1996). Characterization of *Lactobacillus* by Southern-Type hybridation with a *Lactobacillus plantarum pyr DFE* probe. *Int. J. Syst. Bacteriol*., 46: 588-594.
9. Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiol. Rev*., 87: 113-130.
10. Charteris, W.P.; Kelly, P.M.; Morelli, L.; Collins, J.K. (1998). Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *J. Food. Prot.,* 61: 1636-1643.
11. Christensen, J.E.; Dudley, E.G.; Pederson, J.A.; Steele, J.L. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek*., 76: 217-246.
12. Coeuret, V.; Dubernet, S.; Bernardeau, M.; Gueguen, M.; Vernoux, J.P. (2003). Isolation, characterisation angbd identification of lactobacilli focusing mainly on cheeses and other dairy products. *Lait*., 83: 269-306.
13. Coppola, R.; Succi, M.; Tremonte, P.; Reale, A.; Salzano, G.; Sorrentino, E. (2005). Antibiotic suscebtibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *Lait.,* 85: 193-204.
14. Dagdemir, E.; Ozdemir, S. (2008). Technological characterization of the natural lactic acid bacteria of artisanal Turkish white pickled cheese. *Int. J. Dairy Technol*., 61: 133-140.
15. Dellaglio, H.; De Roissart; H.; Torriani, S.; Curk, M.C.; Janssens, D.

(1994). Caractéristiques générales des bactéries lactiques. *In* : de Roissart, H.; Luquet, F.M. (eds) *Les bactéries lactiques : aspects fondamentaux et technologiques*. Lorica, Uriage, p. 25-116.

16. De Magalhães, J.T.; Uetanabaro, A.P.T.; de Moraes, C.A. (2008). Identification of *Lactobacillus* UFV H2B2 (Probiotic strain) using DNA-DNA hybridation. *Braz. J. Microbiol*., 39: 524-546.
17. De Man, J.C.; Rogosa, M.; Sharp, M.E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol*., 23: 130-135.
18. De Vuyst, L.; Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol. Rev.,* 23: 153-177.
19. Ennahar, S.; Cai, Y.; Fujita, Y. (2003). Phylogenetic diversity of lactic acid bacteria associated with paddy rice silage as determined by 16S ribosomal DNA analysis. *Appl. Environ. Microbiol*., 69: 444-451.
20. Estepar, J.; Del Mar Sánchez, M.; Alonso, L.; Mayo, B. (1999). Biochemical and microbiological characterization of artisanal '*Peñamellera'* cheese: analysis of its indigenous lactic acid bacteria. *Int. Dairy J.*, 9: 737-746.
21. Fitzsimons, N.A.; Cogan, T.M.; Condon, S.; Beresford, T. (1999). Phenotypic and genotypic characterization of non-starter lactic acid bacteria in mature Cheddar cheese. *Appl. Environ. Microbiol*., 65: 3418-3426.
22. Hammes, W.P.; Vogel, R.F. (1995). The genus *Lactobacillus*. p. 19-54. *In*: Wood, B.J.B.; Holzapfel, W.H (eds), the genera of lactic acid bacteria, vol. 2. The genera of lactic acid bacteria. Blackie Academic and Professional. London, United Kingdom.
23. Herreros, M.A.; Fresno, J.M.; González Prieto, M.J.; Tornadijo, M.E. (2003). Technological characterization of lactic acid bacteria from Armada cheese (a Spanish goat's milk cheese). *Int. Dairy. J*., 13: 469-479.
24. Herreros, M.A.; Sandoval, H.; González, L.; Castro, J.M.; Fresno, J.M.; Tornadijo, M.E. (2005). Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *Food Microbiol*., 22: 455-459.
25. Jayne-Williams, D.J. (1976). The application of miniaturized methods for characterization of various organisms isolated from the animal gut. *J. Appl. Microbiol*., 40: 189-200.
26. Kandler, O.; Weiss, N. (1986). Genus *Lactobacillus* Beijerinck 1901, 212 AL. *In*: Sneath, P.H.A.; Mair, N.S.; Sharpe, M.E.; Holt, J.G. (eds.) *Bergey's Manual of Systematic Bacteriology*. vol 2, Baltimore, Williams & Wilkins , p. 1209-1234.
27. Klein, G.; Pack, A.; Bonapartes, C.; Reuter, G. (1998). Taxonomy and physiology of probiotic lactic acid. *Int. J. Food Microbiol.*, 41: 103-125.
28. Klein, G.; Halmann, C.; Casas, I.A.; Abad, J.; Louwers, J.; Reuter, G. (2000). Exclusion of vanA, vanB and vanC type glycopeptides resistance in starters of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by polymerase chain reaction and hybridation methods. *J. Appl. Microbiol*., 89: 815-824.
29. Levy, S.B. (1992). *The antibiotic paradox: How Miracle Drugs are Destroying the Miracle*. Plenum Press, New York, p 279.
30. Linaje, R.; Coloma, M.D.; Pérez-Martinez, G.; Zúñiga, M. (2004). Characterization of faecal enterococci from rabbits for the selection of probiotic strains. *J. Appl. Microbiol*., 96: 161-771.
31. Macedo, A.C.; Viera, M.; Poças, R.; Malcata, F.X. (2000). Peptide hydrolase system of lactic acid bacteria isolated from Serra de Estrela cheese. *Int. Dairy J*., 10: 769-774.
32. Müller, T. (1990). Comparaison of methods for differentiation between homofermentative and heterofermentative lactic acid bacteria. *Zentralbl. Mikrobiol*., 145: 363-3666.
33. Nigatu, A. (2000). Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate *Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact sake, Lact. parabuchneri, Lact. gallinarum, Lact. Gallinarum, Lact. casei, Weissella* minor and related taxa isolated from kocho and tef. *J. Appl. Microbiol*., 89: 969-978.
34. Olasupo, N.A.; Shillinger, U.; Holzapfel, W.H. (2001). Studies on some technological properties of predominant lactic acid bacteria isolated from Nigerian fermented food. *Food Microbiol*., 15: 157-167.
35. Psono, L.; Kotzamanides, C.; Andrighetto, C.; Lombardi, A.; Tzanetakis, N.; Litopoulou-Tzanetaki, E. (2006). Genotypic and phenotypic heterogeneity in *Enterococcus* isolated from Batzos, a raw goat milk cheese. *Int. J. Food Microbiol*., 109: 109-120.
36. Quere, F.; Deschamps, A.; Urdaci, M.C. (1997). DNA probe and PCR-specific reaction for *Lactobacillus plantarum*. *J. Appl. Microbiol*., 82: 783-790.
37. Rodríguez, E.; Gonzalez, B.; Gaya, P.; Nuñez, M.; Medina, M. (2000). Diversity of bacteriocins produced by lactic acid bacteria isolated from raw milk. *Int. Dairy Journal*., 10: 7-15
38. Sabioni, N.S.S.; Pinheiro, A.J.R.; Teixeira, M.A. (1998). Acidophilus milk: Isolation and characterization of *Lactobacillus acidophilus* from breast-fed children and from calves' feces. *Braz. J. Microbiol*., 19: 393-398.
39. Sánchez, I.; Palop, L.; Ballesteros, C. (2000). Biochemical characterization of lactic acid bacteria isolated from spontaneous fermentation of 'Almagro' eggplantes. *Int. J. Food Microbiol*., 59: 9-17.
40. Schleifer, K.H.; Ehrmann, M.; Beimfohr, C.; Brokmann, E.; Ludwig, W.; Amann, R. (1995). Application of molecular methods for classification and identification of lactic acid bacteria. *Int. Dairy J*., 5: 1081-1094.
41. Seseña, S.; Sánchez, I.; Palop, L. (2005). Characterization of *Lactobacillus* strains and monitoring by RAPD-PCR in controlled fermentations of "Almagro" eggplants. *Int. J. Food Microbiol*., 104: 325-335.
42. Sharpe, M.E. (1979). Identification of the lactic acid bacteria. *In*: Skinner, F.A.; Lovelock, D.W. (eds.) *Identification Methods for Microbiologists*, Academic Press, London, p. 233-259.
43. Stiles, M.E.; Holzapfel, W.H. (1997). Lactic acid bacteria of food and their current taxonomy. *Int. J. Food Microbiol*., 36: 1-29.
44. Stingler, F.; Neeser, J.R.; Mollet, B. (1996). Identification and characterization of the *eps* (Exopolysaccharide) gene cluster from *Streptococcus thermophilus* Sfi6. *J. Bacteriol.,* 178: 1680-1690.

45. Temmerman, R.; Pot, B.; Huys, G.; Swings. J. (2003). Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int. J. Food Microbiol*., 81: 1-10.
46. Todorov, D.S. (2009). Bacteriocins from *Lactobacillus plantarum*-production, genetic organization and mode of action. *Braz. J. Microbiol*., 40: 209-221.
47. Vandamme, P.; Pot, B.; Gillis, M.; De Vos, P.; Kersters, K.; Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematic. *Microbiol. Rev*., 60: 407-438.
48. Williams, A.G.; Felipe, X.; Banks, J.M. (1998). Aminopeptidase and dipeptidyl peptidase activity of *Lactobacillus* spp. And non-starter lactic acid bacteria (NSLAB) isolated from Chaddar cheese. *Int. Dairy J*., 8: 255-266.
49. Xanthopoulos, V.; Hatzikamari, M.; Adamidis, T.; Tzaneetakis, N.; Tsakalidou, E.; Litopoulou-Tzanetaki, E. (2000). Heterogeneity of *Lactobacillus plantarum* isolated from Feta cheese throughout ripening. *J. Appl. Microbiol*., 88: 1056-1064.
50. Zanoni, P.; Farrow, J.A.E.; Phillips, B.A.; Collins, M.D. (1987). *Lactobacillus pentosus* (Fred, Peterson, and Anderson) sp. nov., nom. rev. *Int. J. Syst. Bacteriol*., 37: 339-341.
51. Zotta, T.; Ricciardi, A.; Parente, E. (2007). Enzymatic activities of lactic acid bacteria isolated from Cornetto di Matera sourdoughs. *Int. J. Food Microbiol*., 115: 165-172.