Antibiotic susceptibility and heterogeneity in technological traits of lactobacilli isolated from Algerian goat's milk

Leila Bousmaha-Marroki · Ahmed Marroki

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Abstract The objective of this study was to identify and study the heterogeneity of technological traits of lactobacilli from goat's milk of Algeria and to evaluate in vitro their safety aspect. Using API50 CHL system and 16S rDNA sequencing, 51 % of strains were assigned as *Lactobacillus plantarum*, 34 % as *L. pentosus*, 7 % as *L. rhamnosus* and 8 % as *L. fermentum*. A large variability was noted for the acidifying capacity in skim milk after 6, 12 and 24 h of incubation. All strains expressed aminopeptidase activity against alanine- ρ -NA and leucine- ρ -NA at different levels. All strains were resistant to vancomycin and most of strains showed more susceptibility to β -lactam antibiotic. High susceptibility toward the inhibitors of protein synthesis was also observed. Minimum inhibitory concentrations data obtained revealed that isolates were susceptible to penicillin and chloramphenicol, and resistant to gentamicin and vancomycin. Minimum inhibitory concentrations distribution of other antibiotics showed variability. The analysis of graphical representation of principal component analysis of technological properties of *L. plantarum* and *L. pentosus* strains showed diversity among the isolates. Finally, eight *L. plantarum* (LAM1, LAM3, LAM21, LAM25, LAM35, LF15, LAM34, and LAM35), four *L. pentosus* (LAM38, LAM39, LF9 and LF16) and two *L. rhamnosus* (LF3 and LF10) strains, could be good candidates as adjunct culture in dairy product in Algeria.

Keywords Lactobacilli · Goat's milk · Heterogeneity · Technological properties · Antibiotic susceptibility · Algeria

Introduction

Lactobacilli are rods belonging to the Lactic Acid Bacteria (LAB) which contains a diverse assemblage of 140 species (Euzéby 1997), and includes : Gram-positive, catalase negative, non-motile, non-sporulating, facultative anaerobes, growing under microaerophilic to strictly anaerobic conditions (Klein et al. 1998). For many years, lactobacilli have been widely found in raw milk and fermented dairy products such as cheese, yoghurt and fermented milk (Bernardeau et al. 2008; Coeuret et al. 2003). In many cases, these lactobacilli are widely used as starter cultures in industrial and artisanal food fermentation and as tools for food safety (Adams and Mitchell 2002; De Vries et al. 2006; Leroy and de Vuyst 2004; Ross et al. 2002). Traditionally, lactic acid bacteria such as *Lactobacillus* species have been classified on the basis of phenotypic properties, eg., morphology, mode of glucose fermentation, growth at different temperatures, and carbohydrates fermentation. However, identification of the *Lactobacillus* to species level by only phenotypic methods is not reliable and can be difficult because of considerable variations in biochemical attributes that seem to occur between strains currently considered to belong to the same species. Some species are not readily distinguishable in terms of phenotypic characteristics contrary to the PCR- based method, such as sequencing of 16S rRNA gene, extensively used for estimation of the genotypic diversity among *Lactobacillus* (Coeuret et al. 2003; Vásques et al. 2005). However, a comprehensive characterization of these microorganisms cannot ignore the evaluation of some technological traits (Vandamme et al. 1996). Recent attention was paid to the possibility that lactobacilli strains may play a role as being health promoters,

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L. Bousmaha-Marroki · A. Marroki
Department of Biology, Faculty of Natural and Life Sciences,
University Djillali Liabes, Sidi Bel Abbès 22000, Algeria

A. Marroki (✉)
Laboratory of Microbial Genetic - Faculty of Natural and Life
Sciences, University of Oran, Oran 31100, Algeria
e-mail: a_marroki@yahoo.fr

beyond their technological functions (Milesi et al. 2009; Settanni and Moschetti 2010). The addition of selected non-starter lactobacilli as adjunct cultures could also play a probiotic role in cheese and dairy products (Bertazzoni et al. 2004; Bude-Ugarte et al. 2006; Maragkoudakis et al. 2006). Raw and fermented milk as *Laben* and *Rayeb* (local traditional fermented milks), from cow, sheep, camel and goat's have been known in different region of Algeria. These products are frequently consumed and prepared by people in Algeria. These products were manufactured and stocked under traditional and artisanal condition without addition of commercial starters. On the other hand, the quality of these products is dependent on diversity of microflora of these products. Recently, some research have been focused for selected strains from goat's milk with a technological potential and safety, susceptible to be used as adjunct cultures in dairy products (Ayad et al. 2004; Badis et al. 2004a, b; Marroki et al. 2011). Strains intended for the use in food systems as starters or probiotics adjunct culture should therefore be carefully examined following important criteria: identification at the species by different methods, evaluation of some technological traits and safety aspect (Vandamme et al. 1996). Regarding the safety of probiotic strains, antibiotic resistance is one of the aspects that needs to be analyzed because of the serious concerns about the growing level of resistance to antibiotics in regular use in human medicine (Malek et al. 2012). This study is a widespread of our research that has of objective to identify and study the technological traits heterogeneity of lactobacilli strains isolated from goat's milk samples collected in different regions of Algeria based on their acidifying capacity, skim milk coagulation and aminopeptidase activity. The safety of isolates was also evaluated by studying their antibiotic susceptibility and bile tolerance in order to select interesting strains for an appropriate adjunct culture in fermented dairy products in Algeria.

Materials and methods

Isolation of Lactobacillus strains

Fourteen samples of raw goat's milk were collected in farms located in rural areas of North West of Algeria, samples were collected aseptically in sterile bottles kept in an ice-box and transported to the laboratory for analyses. Ten millilitres of each sample were aseptically mixed with 90 mL of Ringer's solution 1:4 and mixed thoroughly for 60 seconds. Serial dilutions (10^{-1} to 10^{-7}) were made and 1 mL aliquot of appropriated dilutions were pour-plated on MRS agar adjusted to pH 5.5 with hydrochloric acid (1 N HCL) and incubated anaerobically for 24 to 48 h at 30 °C, in anaerobic jars containing gas generating sachets (Anaerogen, Oxoid). Ten colonies were randomly picked from the plates of each sample, purified

by successive streaking on MRS agar plates and stored at −80 °C in MRS supplemented with 20 % glycerol.

Sugar fermentation and general growth features

Isolates were initially examined for Gram reaction, cell morphology and catalase reaction according to the methods and criteria to Kandler and Weiss (1986). Only rod-like, Gram-positive and catalase-negative isolates were selected, for further studies. Initially, a simplified sugar fermentation test was applied to differentiate strains that would be further characterized. Isolates were subjected to the following phenotypic tests: growth at 15, 45 °C, growth at acidic pH adjusted to 4.5 with (1 N HCL) and 6.5, growth at 15 and 45 °C, and in the presence of NaCl (2 and 4 %) was determined in MRS broth (Oxoid, UK). Results were recorded after 24 to 48 h at 30 °C. All the growth analyses were done at least in duplicate. In addition, production of gas from glucose (heterofermenters) was determined in MRS basal broth (Veyrat et al. 1994) supplemented with 1 % D-glucose in tubes with Durham chambers and covered with liquid paraffin. Arginine degradation was determined as described by Schillinger and Lücke (1987). Acid production from carbohydrates (fructose, glucose, mannitol, lactose, mannose, rhamnose, glycerol, sorbose, sorbitol, dulcitol, amygdalin, melibiose, melezitose, starch, tagatose, arabinol, ribose, maltose, galactose, and xylose) was evaluated by using a miniaturized assay in microtiter plates (Jayne-Williams 1976). Carbohydrate fermentation patterns were determined by using the API 50 CHL system (BioMérieux, Lyon, France) according to the supplier instructions. Results were recorded after 24 and 48 h at 30 °C. The programme of identification (Cox and Thomson, Biochemistry institute, Odense University, Denmark) plus database identification software was used to analyse the fermentation profiles obtained.

Genotypic characterization

Isolates were grown in MRS broth at 30 °C until Optic Density (OD) of 1.6–1.8 at 600 nm. A 1.5 mL aliquot of each overnight culture was centrifuged at $10000 \times g$ 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 or using the method described by Martin-Platero et al. (2007). The 16S rRNA encoding genes were amplified by using the universal primers 27 F (5'-AGAG TT TG AT CC TG GC TC AG -3') and 558R (5'-GTAT TC CG CG GC TG -3') or with the primers 27 F and 1525R (5'-AAGG AG GT GWTCCA RCCGCA-3'). PCR reactions were performed in a total volume of 50 µL containing 50 ng of DNA, 25 pmol of each primer, 1.6 mM of dNTPs 2 mM MgCl₂ and 1U of taq DNA polymerase (Biotools Lab,

Spain) with reaction buffer supplied by the manufacturer. Amplifications were carried out in a Thermal Cycler (PTC-100 Peltier Thermal Cycler, MJR) using the following program: for primers 27 F and 558R, the 16S rDNA was amplified as described previously (Linaje et al. 2004); for the primers 27 F and 1525R, the PCR mixtures were subjected to an initial with the following program: PCR mixtures were subjected to an initial denaturing step of 95 °C for 5 min, followed by 30 cycles of 94 °C, 15 s; 52 °C, 30 s; 72 °C, 2 min, and a final cycle at 72 °C for 5 min. PCR products were examined by electrophoresis on 0.8 % agarose gel in 1× TBE buffer containing 0.5 g/mL ethidium bromide and visualised under UV light. A Lambda DNA (Biorad) digested with PstI ladder was used as a molecular mass marker. PCR products were purified by using the Illustra GFX PCR DNA and gel band purification kit (General Electric Healthcare, Spain) according to the manufacturer's instructions. Purified PCR products were stored at −20 °C. 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. For genes encoding 16S rRNA were edited with Chromas version 1.43 (Griffith University, Brisbane, Queensland, Australia), and compared with the Ribosomal Database Project II (<http://rdp.cme.msu.edu/>) and from the GeneBank database using the BLAST algorithm. The sequences were aligned using ClustalW (Thompson et al. 1994) and manually correction where necessary. Taxonomic strain identification was performed by comparing the partial 16S rRNA gene sequence obtained of each isolate with those reported in the Basic BLAST database (Altschul et al. 1997).

Acidification and coagulation of skim milk

Growth in skim milk of the isolates was determined as described by Psono et al. (2006). Sterile reconstituted skim milk with initial pH 6.65 (100 mL) was inoculated with 1.0 % of overnight *Lactobacillus* cultures. 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 the change in the pH during time. Milk coagulation was determined after 24 and 48 h of incubation at 30 °C.

Aminopeptidase activity

The aminopeptidase activity of the isolates was tested on the substrates L-alanine ρ -nitroanilide (Ala- ρ NA) (Sigma, USA) and L-leucine ρ -nitroanilide (Leu- ρ NA) (Sigma, USA) as described by Zotta et al. (2007). Stationary phase cells grown overnight in MRS broth were harvested by centrifugation at $10.000\times g$ 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. (2000). The assay mixture contained: 30 μ L of 20 mM methanol aminoacyl ρ -nitroanilide substrates, 195 μ L of 50 mM potassium phosphate buffer, 95 μ L of 0.05 % (w/v) sodium azide solution and 75 μ L of cell suspension. After incubation at 30 °C for 1 to 4 h, the reaction was stopped by addition of 900 μ L of 1 % (v/v) acetic acid. The release of ρ -nitroaniline (ρ -NA) (Sigma, USA) was measured spectrophotometrically at 410 nm after centrifugation of the mixture at $10.000\times g$ for 5 min. 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 μ mol of ρ -NA. min^{-1} under the assay conditions.

Bile tolerance determination

Bile tolerance was determined by the streaking single colony on MRS agar plates containing oxgall bile (0.3 % w/v, Sigma, Germany) as described by Linaje et al. (2004). Control plate without oxgall bile was prepared as well. The plates were incubated at 30 °C for 24 h and visually examined for the growth.

Antibiotic susceptibility by disc diffusion method

Susceptibility testing was based on the agar overlay disc diffusion as described by Charteris et al. (1998) modified by Aymerich et al. (2006). Antibiotic discs produced by Bioanalyse (Turkey) were employed to determine the pattern of antibiotic resistance of 61 lactobacilli tested strains. The following antibiotics were tested: penicillin G (10 μ g), ampicillin (10 μ g), amoxicillin (25 μ g), oxacillin (5 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), ceftazidime (30 μ g), erythromycin (15 μ g), tetracycline (30 μ g), chloramphenicol (30 μ g), imipinen (10 μ g), vancomycin (30 μ g), trimethoprim-sulfamethoxazole (1.25/23.75 μ g) and fusidic acid (10 μ g). Inhibition zones diameters of antibiotics were compared to those defined by Vlkova et al. (2006).

Determination of the Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) of 11 antimicrobial agents were determined by microdilution using the newly developed and standardized (LSM: LAB susceptibility test medium) broth, consisting of a mixture of Iso-Sensitest (IST; Oxoid) broth (90 %) and MRS broth (10 %) adjusted to pH 6.7 as described by Klare et al. (2005) for 45 strains of *Lactobacillus*. The following antimicrobials were tested in the concentration ranges (μ g/mL) given in parentheses: erythromycin (0.016–32), clindamycin (0.032–32), ampicillin (0.032–64), penicillin G (0.032–64), oxytetracycline (0.063–

128), chloramphenicol (0.125–256), vancomycin (0.125–256), kanamycin (1–2048), gentamicin (1–2048), streptomycin (2–2048), neomycin (1–2048). Each isolate, grown in antibiotic-free LSM broth, the inoculum was adjusted to a turbidity equivalent to 0.5 McFarland standard (10^5 CFU/mL). Aliquot of 100 μ L of the inoculum was inoculated into each well. As positive control, bacteria were inoculated in LSM without added antibiotic, and an inoculum-free well was used as negative control. Plates were incubated under aerobic conditions at the appropriate growth temperature for 24 h. MIC values of each antibiotic were visually evaluated as the lowest concentrations where no growth was observed. Interpretation for susceptibility status was based on EFSA (2008) and values of breakpoint were determined as described Ammor et al. (2007).

Statistical analysis Hierarchical cluster analysis was used to classify the strains according to their different properties. The computer software used was XLSTAT (Addinsoft, Paris, France).

Results

Phenotypic identification

Seventy one strains isolated and randomly selected from fourteen samples of Algerian goat's milk were presumed belonging to the genus *Lactobacillus* based on cell morphology, Gram reaction, catalase test and physiological and biochemical characteristics. Based on results of production of CO_2 from glucose, and NH_3 from arginine, the seventy one isolates tested in this study were classified into two groups (G 1 and G 2). Because none of the isolates in G 1 produced CO_2 from the fermentation of glucose, they were tentatively classified as facultatively heterofermentative rods (group 1 *Lactobacillus*; 91.54 % of isolates). Isolates of G 2 produced CO_2 from fermentation of glucose and NH_3 from arginine, they were classified as obligately heterofermentative rods (group 2 *Lactobacillus*; 8.84 % of isolates).

Table 1 shows the phenotypic characterization of the isolates, including carbohydrate utilization patterns, physiological and biochemical characteristics of the isolates. Result of sugar fermentation reactions subdivided the 65 isolates of G 1 into four sub-groups (1.1–1.4) and G 2 with six isolates into three sub-groups (2.1–2.3). All strains of the first group were able to ferment, fructose, glucose, mannitol, lactose, mannose, sorbitol, melibiose, ribose, maltose and galactose and unable to ferment xylose. Small differences in sugar fermentation reactions separated the subgroups. Sub-group 1.1 was the largest with 41 isolates (57.74 %), strains of this sub-group were able to ferment melezitose. However, acid production

Table 1 Phenotypic traits of 71 *Lactobacillus* strains characterized in the present study.

Group	1				2		
Sub-group	1.1	1.2	1.3	1.4	2.1	2.2	2.3
Number of isolates	41	17	4	3	2	2	2
CO_2 from glucose	–	–	–	–	+	+	+
Arginine hydrolysis	–	–	–	–	+	+	+
Growth at 15 °C	+	+	+	+	+	+	+
Growth at 45 °C	–	+	–	+	–	+	+
Growth at pH 4.5	+	+	+	+	+	+	+
Growth at pH 6.5	+	+	+	+	+	+	+
Growth in 2 % NaCl	+	+	+	+	+	+	+
Growth in 4 % NaCl	+	+	+	+	+	+	+
Growth in 0.3 % Oxgal	+	+	+	+	+	+	+
Sugar fermentation:							
Fructose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Rhamnose	22*	14	–	33	+	–	–
glycerol	10	6	+	–	–	–	–
Sorbose	10	–	–	+	+	+	–
Sorbitol	+	+	+	+	+	+	+
Dulcitol	12	–	–	33	+	–	–
Amygdalin	95	+	+	+	+	+	+
Melibiose	+	+	+	+	–	–	+
Melezitose	+	+	+	33	–	–	–
Starch	37	–	–	–	–	+	–
Tagatose	10	–	–	33	+	–	+
Arabitol	7	–	–	–	–	+	–
Ribose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Xylose	–	–	–	–	–	–	–

*: percentage of isolates showed a positive result; +: positive reaction; –: negative reaction

by these strains from rhamnose, glycerol, sorbose, dulcitol, amygdalin, starch, tagatose and arabitol is variable. The isolates of sub-group (1.1) were unable to grow at 45 °C and identified as *L. plantarum*. Sub-group 1.2, comprised 17 strains (23.94 %) identified as *L. plantarum*, and distinguished from those of sub-group 1. 1. by their ability to ferment amygdalin and inability to ferment sorbose, dulcitol, starch, tagatose and arabitol. In addition they were grown at 45 °C. Strains belonging to sub-group 1.3 (5.63 %), were assigned as *L. plantarum* /*L. pentosus*. These strains fermented glycerol and amygdalin and were incapable to assimilate rhamnose, sorbose, dulcitol, starch and tagatose and to grow at 45 °C.

Subgroup 1.4 include three strains (4.22 %) able to ferment sorbose and amygdalin and not able to ferment glycerol, starch and arabitol. This isolates are able to grow at 45 °C and were assigned to *L. rhamnosus* species. The second group included six strains (8.45 %) and sub-divided in three sub-groups (2.1–2.3), all strains were able to ferment fructose, glucose, mannitol, lactose, mannose, sorbitol, amygdalin, ribose, maltose and galactose but not able to ferment glycerol, melizitose and xylose. Sub-group 2.1, with two isolates, able to ferment rhamnose, sorbose, dulcitol and tagatose. These strains were not able to assimilate melibiose, starch, arabitol and to grow at 45 °C. They were identified as *L. fermentum*. Sub-group 2.2 included also two strains assigned as *L. fermentum* and were able to grow at 45 °C. These isolates were characterized by their ability to ferment sorbose, starch and arabitol, and inability to produce acids from rhamnose, dulcitol, melibiose and tagatose. Sub-group 2.3 comprised two strains identified as *L. fermentum*. These isolates differed from those of former sub-groups by their ability to ferment melibiose and incapacity to ferment sorbose. To establish the final phenotypic identification, all 71 strains tested for their biochemical and physiological characteristics, were inoculated to API 50 CHL galleries to determine their sugar fermentation patterns. Using API 50 CHL system, strains of sub-groups 1.1, 1.2 and 1.3 were assigned as *L. plantarum* and those of sub-group 1.4 as *L. rhamnosus*. Concerning the second group (sub-groups 2.1–2.3) strains were identified as *L. fermentum* (Table 2). Results of bile tolerance obtained exhibited that all *Lactobacillus* strains tested are able to grow in 0.3 % of ogall

Molecular identification of lactobaccili by sequencing of 16S rRNA gene

Sequencing of the 16S rRNA gene is the technique used most commonly for bacterial identification. Table 2, shown result of 16S rRNA gene sequencing of 71 strains isolated from Algerian goat's milk identified as belonging to the genera of *Lactobacillus* sub-divided into four species. Comparison of our result with previously known sequence in the databases showed that strains belonging to first group were assigned as *L. plantarum* (33 strains), *L. pentosus* (27 strains) and *L. rhamnosus* (5 strains). Twenty-nine strains of sub-group 1.1 were identified as *L. plantarum* and 12 strains as *L. pentosus*. Among the 17 strains, forming the subgroup 2.2, four strains were identified as *L. plantarum*, eleven strains as *L. pentosus* and two strains as *L. rhamnosus*. All strains belonging to subgroups 1.3 and 1.4 were assigned respectively to *L. pentosus* (4 strains) and *L. rhamnosus* (3 strains). Finally, result obtained for all strains of group 2 (sub-groups 2.1–2.3) showed that they were identified as *L. fermentum* by sequencing of 16S rDNA.

Acidifying activity

Test for acidifying activity of 71 *Lactobacillus* strains isolated from goat's milk was determined after 6, 12 and 24 h of growth in sterile skim milk (Table 3). Milk pH varied between 4.36 and 5.65 (mean value: 5.24 ± 0.33) after 24 h of incubation in all cultures. All *Lactobacillus* isolates tested reduced the pH of milk between 5.43 and 6.50 (mean value: 6.21 ± 0.21) after 6 h of growth. After 12 h of incubation, pH values ranged from 5.24 to 6.02 (mean value: 5.78 ± 0.16). According to their ability to reduce pH more or less rapidly three clusters of *L. plantarum* isolates were observed: (i) slow acidifying strains (cluster 1; 15 strains; 42 % of *L. plantarum* strains), which showed a slow rate of acidifying ability during the first 6 h of incubation (pH ranging between 6.28 and 6.48). Three sub-groups were observed, sub-group 1.1 (5 strains, 14 %) which showed the lowers acidifying capacity: pH values observed after 12 and 24 h of incubation were respectively 5.92–6.00 and 5.41–5.65; sub-group 1.2 (with 8 strains; 22 %), these *L. plantarum* strains lowed pH values between 5.80 and 5.88 and 4.97–5.25 after 12 and 24 h of incubation respectively; sub-group 1.3 (two strains; 6 %) which showed the faster rate of acidifying capacity: pH values achieved between 5.69 and 5.72 after 12 h of incubation and between 5.10 and 5.30 after 24 h of incubation. (ii) Medium acidifying *L. plantarum* strains (cluster 2 with 17 strains; 47 %), which showed a faster rate of acidifying ability after 6 h of incubation comparing to the first cluster, with a pH ranging between 6.03 and 6.25. In this cluster, three sub-groups were observed with different behaviours in their acidifying capacity after 12 and 24 h of incubation. In the first sub-group 2.1 (4 strains; 11 %) pH values achieved were respectively 5.90–5.95 and 5.18–5.54 after 12 and 24 h of incubation. In contrast, the second subgroup 2.2 (with 7 strains; 19 %) included *L. plantarum* strains showing a similar acidifying activity until 6 h but capable to achieve pH values ranging between 5.72 and 5.84 and 4.94–5.63 after 12 and 24 h of incubation. Comparing to previous subgroups, the third sub-group (sub-group 2.3 with 6 strains; 17 %) showed a faster acidification rate after 12 and 24 h of incubation (5.37–5.63) and 4.36–4.74 respectively). (iii) Fast acidifying *L. plantarum* strains (cluster 3 with 4 strains; 11 %), which showed the highest acidifying capacity. This four *L. plantarum* strains, showed a fast rate of acidifying ability until 6 h (pH between 5.43 and 5.97). This cluster was sub-divided into two sub-groups according to their acidifying ability until 12 and 24 h of incubation. Sub-group 3.1 (with two strains), in which pH values achieved after 12 and 24 h of incubation were respectively 5.81–5.82 and 5.13–5.27. Concerning strains of the second sub-group (sub-group 3.2 including 2 strains), the high capacity to acidifying milk is also showed after 12 and 24 h of incubation: pH values achieved are respectively 5.24 to 5.39 and 4.40 to 4.50. According to their ability to reduce the pH more or less

Table 2 Conventional and molecular identification of *Lactobacillus* from Algerian goat's milk

Isolats			Phenotypic Identification		Genotypic identification Sequencing of 16S rDNA)	Most similar sequence Acc. No. ^a	
			Bergy's manual, 1986	API 50CHL			
Group 1	S.G ^b 1.1	LAM1, LAM2, LAM3, LAM4, LAM5, LAM6,LAM7, LAM8, LAM9, LAM10, LAM13, LAM15, LAM17, LAM29, LAM30,LAM31, LAM32, LAM36, LAM37, LAM41	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	AB362768	
		LAM12, LAM14, LAM16, LAM19, LAM20, LAM22, LAM23,	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. penosus</i>	AB362758	
		LAM18, LAM21, LAM24, LAM25, LAM26, LAM27, LAM28	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	EU257480	
		LAM33, LAM34, LAM40	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	AB362625	
		LAM11, LAM35	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	EF185922	
		LAM38, LAM39	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. penosus</i>	AB362712	
		S.G1.2	LF1, LF2, LF4, LF5, LF9, LF16	<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. pentosus</i>	AB362758
	LF6, LF7, LF8, LF14, LF17		<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. pentosus</i>	AB362712	
	LF11, LF12, LF15		<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	AB362768	
	LF3		<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. plantarum</i>	EU 257480	
	LF10		<i>L. plantarum</i>	<i>L. plantarum</i>	<i>L. rhamnosus</i>	EF 533991	
	S.G1.3	LS1, LS2, LS3, LS4	<i>L.plantarum/L.pentosus</i>	<i>L. plantarum</i>	<i>L. pentosus</i>	AB 362758	
		SG1.4	LT1	<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	EF 533991
	LT2		<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	AB 008211	
	LT3		<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	<i>L. rhamnosus</i>	AB 288235	
	Group 2	S.G.2 (2.1–3)	LA1, LA2, LA3, LA6	<i>L. fermentum</i>	<i>L. fermentum</i>	<i>L. fermentum</i>	AB 362628
			LA4. LA5	<i>L. fermentum</i>	<i>L. fermentum</i>	<i>L. fermentum</i>	AB 362626

^a Acc. No. Accession number in GenBank^b S.G Sous-group

rapidly, two clusters of *L. pentosus* isolates were observed: (i) slow acidifying strains (cluster 1, 10 strains 42 % of *L. pentosus* strains), which showed a slow acidifying ability during the first 6 h of incubation (pH ranging between 6.20 and 6.48 pH). These *L. pentosus* strains lowered the pH values between 6.00 and 5.69 and 5.65–5.23 after 12 and 24 h of incubation respectively with two strains LAM39 and LF16, showed a fast acidifying ability after 24 h of incubation. (ii) faster acidifying *L. pentosus* strains (cluster 2, 58 strains), which showed a faster rate of acidifying ability after 6 h of incubation, with a pH ranging between 6.01 and 6.17. pH values after 12 and 24 h of incubation was achieved between 5.95 and 5.30 and 5.62–5.31 respectively. All strains of *L. rhamnosus* reduced the pH of milk after 6 h of incubation between 6.00 and 6.41, the mean acidifying activity values unregistered is 6.17 ± 0.21 . After 12 h of incubation, the pH values ranged from 5.30 to 5.83 (mean value: 5.62 ± 0.22), and after 24 h of incubation from 4.38 to 5.39 (mean value: 4.98 ± 0.40). *L. rhamnosus* strains were divided in two clusters: cluster 1, including low acidifying activities strains (LF10

and LT3) reducing pH after 6 h of incubation at 6.40–6.41, after 12 h at 5.30–5.83 and after 24 h of incubation at 4.84–5.03 respectively. Cluster 2, with three strains (LF3, LT1 and LT2 strains) which showed fast acidifying activity. pH values after 6, 12 and 24 h of incubation ranged respectively 6.00–6.03, 5.51–5.79 and 4.38–5.30. *L. fermentum* strains showed the less acidifying capacity. Milk pH varied between 6.19 and 6.50 (mean value: 6.320 ± 0.10) after 6 h of incubation; between 5.76 and 6.02 (mean value: 5.86 ± 0.09) after 12 h of incubation and between 5.25 and 5.47 (mean value is 5.36 ± 0.09) after 24 h of incubation. Figure 1 showed acidification profiles of the faster acidifying *Lactobacillus* strains belonging to each species. Result of coagulation of skim milk by *Lactobacillus* strains tested after 24 and 48 h of incubation were shown in Table 3. In total 20 strains (28.16 %) were able to coagulate the skim milk after 24 h at 30 °C. Twenty four strains (33.80 %) showed capacity to coagulate skim milk after 48 h of incubation. Moreover, ten (14 %) *L. plantarum* strains, three *L. pentosus* and all *L. rhamnosus* were able to coagulate

Table 3 Properties related to acidifying and aminopeptidase activity of 71 strains of *Lactobacillus* studies

Strains	pH (h)			Δ pH *(h)			Coagulation (h)		Aminopeptidase activity	
	6	12	24	6	12	24	24	48	Leucine- ρ -NA**	Alanine- ρ -NA
<i>L. plantarum</i>										
LAM1	6.33	5.87	5.28	0.36	0.82	1.41	–	+	13.40	11.80
LAM2	6.43	5.69	5.10	0.26	1.00	1.59	+	+	1.56	4.32
LAM3	6.17	5.37	4.36	0.52	1.32	2.33	+	+	5.53	11.80
LAM4	6.48	6.00	5.41	0.21	0.69	1.28	–	–	2.12	3.86
LAM5	6.19	5.82	5.52	0.50	0.87	1.17	–	–	0.63	1.53
LAM6	6.28	5.92	5.65	0.41	0.77	1.04	–	–	0.72	4.51
LAM7	6.48	5.88	5.45	0.21	0.81	1.24	–	–	1.98	5.79
LAM8	6.28	5.86	5.59	0.41	0.83	1.10	–	–	0.32	0.38
LAM9	6.15	5.84	5.63	0.54	0.85	1.06	–	–	0.25	1.22
LAM10	6.25	5.95	5.42	0.44	0.74	1.27	–	–	0.03	0.95
LAM11	6.44	5.84	5.26	0.25	0.85	1.43	–	–	1.01	1.20
LAM13	6.21	5.83	5.23	0.48	0.86	1.46	–	–	2.13	1.10
LAM15	6.24	5.78	5.34	0.45	0.91	1.35	–	–	1.95	1.09
LAM17	6.22	5.90	5.18	0.47	0.79	1.51	–	–	1.65	2.00
LAM18	6.17	5.65	4.56	0.52	1.04	2.13	+	–	1.98	3.96
LAM21	6.14	5.79	5.21	0.55	0.90	1.48	–	–	6.28	10.47
LAM24	6.19	5.57	4.73	0.50	1.12	1.96	+	+	2.01	1.27
LAM25	6.14	5.59	4.63	0.55	1.10	2.06	+	+	8.35	11.37
LAM26	5.97	5.39	4.50	0.72	1.30	2.19	+	+	7.56	9.70
LAM27	6.13	5.63	4.74	0.56	1.06	1.95	+	+	2.82	5.15
LAM28	5.78	5.24	4.40	0.91	1.45	2.29	+	+	6.45	8.10
LAM29	6.23	5.96	5.52	0.46	0.73	1.17	–	–	0.35	1.07
LAM30	5.43	5.81	5.27	1.26	0.88	1.42	–	–	2.52	2.97
LAM31	5.47	5.82	5.13	1.22	0.87	1.56	–	–	1.33	1.13
LAM32	6.14	5.72	4.96	0.55	0.97	1.73	+	+	2.18	3.76
LAM33	6.33	5.92	5.53	0.36	0.77	1.16	–	–	1.71	0.95
LAM34	6.48	5.72	5.34	0.21	0.97	1.35	–	+	7.22	7.79
LAM35	6.20	5.73	5.24	0.49	0.96	1.45	–	+	13.12	10.37
LAM36	6.03	5.51	4.38	0.66	1.18	2.31	+	+	7.20	7.58
LAM37	6.18	5.92	5.54	0.51	0.77	1.15	–	–	4.13	3.15
LAM40	6.50	5.81	5.25	0.19	0.88	1.44	–	–	1.85	5.59
LAM41	6.30	5.94	5.47	0.39	0.75	1.22	–	–	0.77	1.32
LF11	6.42	5.80	4.97	0.27	0.89	1.72	+	+	5.04	5.33
LF12	6.33	5.96	5.41	0.36	0.73	1.28	–	–	2.20	1.13
LF13	6.31	5.84	5.46	0.38	0.85	1.23	–	–	0.26	1.11
LF15	6.48	5.83	5.37	0.21	0.86	1.32	–	–	6.77	8.74
LAM12	6.31	5.78	5.23	0.38	0.91	1.46	–	–	0.35	1.11
LAM14	6.01	5.82	5.32	0.68	0.87	1.37	–	+	2.22	1.90
LAM16	6.12	5.30	5.36	0.57	1.39	1.29	–	–	3.72	2.10
LAM19	6.12	5.84	5.35	0.57	0.85	1.34	–	–	0.24	0.20
LAM20	6.08	5.85	5.36	0.61	0.84	1.33	–	–	2.37	1.10
LAM22	6.08	5.85	5.40	0.61	0.84	1.29	–	–	2.37	1.13
LAM23	6.26	5.8	5.40	0.43	0.89	1.29	–	–	1.02	1.20
LAM38	6.44	6.00	5.23	0.25	0.69	1.46	+	+	17.87	10.33
LAM39	6.25	5.86	4.76	0.44	0.83	1.93	+	+	18.78	13.47
LF1	6.24	5.79	5.50	0.45	0.90	1.19	–	–	2.51	4.22

Table 3 (continued)

Strains	pH (h)			Δ pH *(h)			Coagulation (h)		Aminopeptidase activity	
	6	12	24	6	12	24	24	48	Leucine- ρ -NA**	Alanine- ρ -NA
LF2	6.22	5.93	5.65	0.47	0.76	1.04	–	–	5.95	4.41
LF4	6.13	5.80	5.39	0.56	0.89	1.30	–	+	2.92	4.79
LF5	6.01	5.73	5.31	0.68	0.96	1.38	–	–	4.11	3.17
LF6	6.14	5.89	5.62	0.55	0.80	1.07	–	–	2.01	1.75
LF7	6.05	5.72	5.34	0.64	0.97	1.35	–	–	5.05	5.40
LF8	6.13	5.80	5.39	0.55	0.90	1.32	–	+	3.11	4.78
LF9	6.48	5.83	5.37	0.21	0.86	1.32	+	–	17.16	12.32
LF14	6.17	5.94	5.52	0.52	0.75	1.17	–	–	6.93	7.66
LF16	6.27	5.87	4.94	0.41	0.82	1.75	+	+	14.46	9.17
LF17	6.04	5.88	5.41	0.65	0.81	1.28	–	–	4.12	3.22
LS1	6.14	5.89	5.62	0.55	0.80	1.07	–	–	6.30	6.06
LS2	6.20	5.69	5.39	0.49	1.00	1.30	–	–	2.55	4.33
LS3	6.25	5.81	5.63	0.44	0.88	1.06	–	–	1.95	1.22
LS4	6.15	5.90	5.60	0.54	0.79	1.09	–	–	2.30	1.20
<i>L. rhamnosus</i>										
LF3	6.03	5.51	4.38	0.66	1.18	2.31	+	+	14.88	16.47
LF10	6.40	5.83	5.03	0.29	0.86	1.66	+	+	20.70	12.73
LT1	6.00	5.71	5.39	0.69	0.98	1.30	+	+	2.85	5.59
LT2	6.01	5.79	5.30	0.68	0.90	1.39	+	+	1.84	4.01
LT3	6.41	5.30	4.84	0.28	1.39	1.85	+	+	3.45	2.11
<i>L. fermentum</i>										
LA1	6.26	6.02	5.47	0.43	0.67	1.22	–	–	1.95	1.20
LA2	6.19	5.84	5.39	0.50	0.85	1.30	–	–	1.65	3.01
LA3	6.50	5.81	5.25	0.19	0.88	1.44	–	–	1.84	5.60
LA4	6.23	5.76	5.26	0.46	0.93	1.43	–	–	1.66	3.02
LA5	6.31	5.87	5.35	0.38	0.82	1.34	–	–	1.05	2.63
LA6	6.32	5.91	5.44	0.37	0.78	1.25	–	–	0.91	5.01

*Values of Δ pH was calculated as Δ pH = pH at time - pH zero time; + : Positive reaction; – : Negative reaction; ** : ρ - nitroanilide

skim milk after 24 h of incubation. After 48 h of incubation, 18 % of *L. plantarum* strains, 8 % of *L. pentosus* and all *L. rhamnosus* strains were able to coagulate the skim milk. In contrast, none of

Fig. 1 Acid production of the faster acidifying lactobacilli strains of each species

L. fermentum tested strains were able to coagulate the skim milk after 24 and 48 h of incubation.

Aminopeptidase activity

The values for aminopeptidase (AP) activities of whole cells of the 71 of *Lactobacillus* tested strains using (Ala- ρ Na and Leu- ρ Na) are presented in Table 3. All tested strains expressed aminopeptidase activity against the two substrates tested, however, at a somewhat different activity levels. Tested *L. plantarum* strains exhibited heterogeneous aminopeptidase activity ranging between 0.03 and 13.12 U for L-leucine- ρ NA and 0.38 to 13.40 U for L-alanine- ρ NA. The AP was divided in accordance to the activity of each strain to high, medium and low activity. Cluster 1, (26 strains; 72 %) with low aminopeptidase activity against both L-leucine- ρ NA and L-alanine- ρ NA. In the second cluster, eight strains (22 %) exhibited a medium leu-aminopeptidase (5.53–8.35 U) and ala-aminopeptidase (7.58–9.70 U) and three strains (LAM3, LAM21 and LAM25) releases L-alanine- ρ NA higher than L-leucine- ρ NA. The cluster 3, (2 strains; 6 %: LAM1 and LAM35) showed high ability to release both L-leucine- ρ NA (13.12–13.40 U) and L-alanine- ρ NA (10.37–11.80 U). According to the result obtained for *L. pentosus* strains for AP activity, three clusters were observed. Cluster 1, (18 strains; 75 %), with low aminopeptidase activity against L-leucine- ρ NA and L-alanine- ρ NA. In the second cluster, (two strains LF14 and LS1; 8 %) exhibit a medium leu-aminopeptidase (6.30–6.93 U) and ala-aminopeptidase (6.06–7.66 U). The cluster 3, (four strains LAM38, LAM39, LF9 and LF16; 17 %) showed high ability to release both L-leucine- ρ NA (14.46–18.78 U) and L-alanine- ρ NA (9.17–13.47 U). Two *L. rhamnosus* strains (LF3 and LF10) had a high activity to release Leu- aminopeptidase (14.88–20.70 U) and Ala-aminopeptidase (12.73–16.47 U) and three strains had low activity to degrade L-leucine- ρ NA and L-alanine- ρ NA. *L. fermentum* tested strains showed a more homogeneous aminopeptidase activity. All strains exhibited low AP activity against Leu- ρ NA (0.91–1.95 U) and Ala- ρ NA (1.20–5.60 U).

Antibiotic resistance

From 71 lactobacilli isolates, 61 strains belonging to *L. plantarum* (n=28), *L. pentosus* (n=22), *L. fermentum* (n=6) and *L. rhamnosus* (n=5), were subjected to antibiotic susceptibility testing against 14 antibiotics by using the agar diffusion method. Results of the sensitivity studies of the lactobacilli isolates tested against different types of antimicrobial agents are shown in Fig. 2. Zone diameters were measured, and strains were classified as, susceptible (S), intermediate (I), and resistant (R). Most of strains showed more susceptibility to β -lactam antibiotic (penicillin G, 52 %;

ampicillin, 82 % and amoxicillin, 80 %. Susceptibility was widespread also against cephalosporin's group (cefotaxime (39 %) and imipinen (56 %)). In addition, high susceptibility toward the inhibitors of protein synthesis was also observed such as erythromycin (48 %), tetracycline (49 %), chloramphenicol (80 %) and fusidic acid (26 %). However, all isolates were resistant to oxacillin, ceftazidime, ceftriaxone, vancomycin, and trimetoprim-sulfamide. With regard to antibiotic susceptibility profiles of *L. plantarum* tested strains results obtained showed a high susceptibility to penicillin (54 %), ampicillin (82 %), amoxicillin (89 %), imipinen (64 %) and cefotaxime (21 %). In addition, highest prevalence of sensitive among the isolates was shown to inhibitors of protein synthesis, chloramphenicol (89 %), erythromycin (43 %), tetracycline (43 %) and fusidic acid (36 %). For 22 *L. pentosus* strains result showed a high sensitivity to β -lactam groups; penicillin (41 %), ampicillin (91 %), amoxicillin (86 %), tetracycline (55 %), chloramphenicol (73 %), erythromycin (55 %) and imipinen (64 %). Regarding the five *L. rhamnosus* strains results showed that most of strains were sensitive to penicillin, ampicillin, cefotaxime, tetracycline, chloramphenicol, erythromycin and fusidic acid. The six *L. fermentum* tested strains showed a high susceptibility to penicillin, ampicillin, amoxicillin, cefotaxime, tetracycline, chloramphenicol and erythromycin. After a preliminary investigation by using an agar diffusion assay, the minimum inhibitory concentrations (MICs) of 11 different antibiotics were determined by the microdilution method for 45 of lactobacilli strains. MICs were determined by microdilution method after 24 h of incubation at 30 °C using the newly developed and standardised 'lactic acid bacteria susceptibility test medium' (LSM) broth, consisting of a mixture of Iso-Sensitest and MRS broth adjusted to pH 6.7. The MIC data obtained in vitro for the 11 antibiotic substances (Table 4; Table 1S, Supplementary data) revealed that all lactobacilli strains assayed were susceptible to penicillin (MIC lower than 2 μ g/mL) and chloramphenicol (MICs lower than 8 μ g/mL), and resistant to gentamicin (MIC $\geq$ 64 μ g/ mL) and vancomycin (MIC $\geq$ 256 μ g/ mL). MICs distribution of other tested antibiotics showed variability. In general, anarrow unimodal MIC distribution at low concentration were observed for erythromycin ($\leq$ 0.016–2 μ g/ mL), clindamycin (0.25–2 μ g/ mL), ampicillin (0.5–2 μ g/ mL) and penicillin (0.5–2 μ g/ mL). However, strains displayed broader unimodal MIC distribution at high concentration rang for kanamycin and gentamycin (4–64 μ g/ mL), streptomycin (2–8 μ g/ mL) and neomycin (4–8 μ g/ mL).

In order to classify *L. plantarum* and *L. pentosus* strains isolated from Algerian goat's milk principal component analysis was carried out. The PCA was conducted on the basis of the variables related to acid production (Δ pH variation after 6, 12 and 24 h of incubation in skim milk), aminopeptidase activity (Leucine- ρ -NA and Alanine- ρ -NA) and skim milk coagulation after 24 h of incubation. On the basis of Fig. 3,

Fig. 2 Distribution of lactobacilli strains sensitivity toward different antibiotics agents tested by disc diffusion method. Penicillin (a), Ampicillin (b), Amoxicillin (c), Cefotaxime (d), Kanamycin (e), Erythromycin (f), Tetracycline (g), Chloramphenicol (h), Imipinen (i), and Fusidic acid (j)

the technological parameters for *L. plantarum* strains showed three principal axes able to explain 93.44 % of the total

variation. The first axis F1 (57.50 % of the total variation) distinguished the strains according to the aminopeptidase

Table 4 Minimum inhibitory concentrations (MICs) of 11 antibiotics for 45 *Lactobacillus* strains of isolated from Algerian goat's milk

Antibiotics	Species	Number of strains**	Number of strains for which the MIC*(µg/mL) was as follows :															
			≤ 0,016	0,031	0,063	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	≥512
Erythromycin	<i>L.plantarum</i>	14	1			1		2	3	2	3		2					
	<i>L. pentosus</i>	21						4	5	2	10							
	<i>L. rhamnosus</i>	4						1	2		1							
	<i>L. fermentum</i>	6						1		3	1	1						
Clindamycin	<i>L.plantarum</i>	14							3	8	3							
	<i>L. pentosus</i>	21					1	3	7	6	4							
	<i>L. rhamnosus</i>	4							1	2	1							
	<i>L. fermentum</i>	6							3		3							
Ampicillin	<i>L.plantarum</i>	14		≤0,031	0,063	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	≥512
	<i>L. pentosus</i>	21						3	2	5	1	2						
	<i>L. rhamnosus</i>	4						1	5	3	9	3						
	<i>L. fermentum</i>	6							3		1							
Penicillin G	<i>L.plantarum</i>	14						1	1	1	3							
	<i>L. pentosus</i>	21						5	6	3								
	<i>L. rhamnosus</i>	4						4	10	7								
	<i>L. fermentum</i>	6						2	1	1								
Oxytetracyclin	<i>L.plantarum</i>	14			≤0,063	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	≥512
	<i>L. pentosus</i>	21			2		1			1	3	3	3		1	2		
	<i>L. rhamnosus</i>	4						1		3					6	5	4	
	<i>L. fermentum</i>	6									1	5						
Chloramphenicol	<i>L.plantarum</i>	14				≤0,125	0,25	0,5	1	2	4	8	16	32	64	128	256	≥512
	<i>L. pentosus</i>	21								8	2	4						
	<i>L. rhamnosus</i>	4						1		3	13	4						
	<i>L. fermentum</i>	6								4	2							
Vancomycin	<i>L.plantarum</i>	12																14
	<i>L. pentosus</i>	21																21
	<i>L. rhamnosus</i>	4																3
	<i>L. fermentum</i>	6																6
Antibiotics	Species	Number of strains**	Number of strains for which the MIC * (µg/mL) was as follows :															
			≤1	2	4	8	16	32	64	128	256	512	1024	≥2048				
Kanamycin	<i>L.plantarum</i>	14			1	5	1	2	2	2					1			
	<i>L. pentosus</i>	21			1	4	9	1	4	2								
	<i>L. rhamnosus</i>	4				1	3											
	<i>L. fermentum</i>	6			2	1		1	2									
Gentamycin	<i>L.plantarum</i>	14			1	3	5		1									
	<i>L. pentosus</i>	21			4	10	4	1	1	1								
	<i>L. rhamnosus</i>	4					1	3										
	<i>L. fermentum</i>	6				3		3										
Streptomycin	<i>L.plantarum</i>	14			1	5	4		1	2				1				
	<i>L. pentosus</i>	21		1	1	6	5		3	5								
	<i>L. rhamnosus</i>	4				1	2			1								
	<i>L. fermentum</i>	6				3	2			1								
Neomycin	<i>L.plantarum</i>	14						3	6	2	3							
	<i>L. pentosus</i>	21			1	1		6	9	4								
	<i>L. rhamnosus</i>	4				2			2									
	<i>L. fermentum</i>	6						1	4		1							

*MICs of *Lactobacillus* strains determined according to EFSA (2008) and Ammor et al. (2007) breakpoints

**Number of tested strains

Fields shaded in grey indicate the number of sensitive strains tested for each antibiotic

Number in bold represent the number of resistance strains tested for each antibiotic

activity and acid production after 24 h and ability to coagulate the skim milk. The F2 axis (22.20 % of the total variation) separated the strains according acid production after 6 and 12 h of incubation. According to axes F1 and F2, the 36 *L. plantarum* strains were subdivided into three clusters. Cluster 1 included 5 strains were characterized by high aminopeptidase activity (leucine- ρ NA and alanine- ρ NA), high capacity to produce acid after 24 h of incubation and unable to coagulate skim milk after 24 h of incubation. Strains of this group were assembled in the upper right part of the graphic. Cluster 2, including 6 strains showed moderate leucine- ρ NA and alanine- ρ NA release, fast acid production after 6, 12 and

24 h and able to coagulate skim milk after 24 h of incubation. They were gathered in the right part of the graph. Cluster 3, with 25 strains characterized by slow leucine- ρ NA, alanine- ρ NA releasing activity of and moderate or low acidifying capacity after 6 h, 12 and 24 h. Strains of this cluster were localised in the lower part and the upper left of the graph.

For *L. pentosus* strains (Fig. 4), the three axes explained 92.22 % of the relationship between the isolates and the technological parameters. The axis F1, which accounted 63.22 % of the data variance, describing leucine- ρ NA, alanine- ρ NA degradation ability, skim milk coagulation and acid production after 24 h of incubation. The F2 axis (17.86 %

Fig. 3 Graphical representation of the principal component analysis of the technological properties of *L. plantarum* strains showing axis F1 versus F2: **a** factor loading, **b** factor scor Leu- ρ -NA: L-leucine- ρ -nitroaniline; Ala- ρ -NA: L-alanine- ρ -nitroaniline; Δ pH6: pH variation after 6 h of incubation; Δ pH12: pH variation after 12 h of incubation; Δ pH24: pH variation after 24 h of incubation; Coag 24 h: coagulation 24 h: coagulation of skim milk after 24 h of incubation

of the total variation) and was defined by acid production after 6 and 12 h of incubation. The result of the first two axes was used to separate the strains into three distinct clusters. Four of the 24 *L. pentosus* strains were classified in a first cluster. They were characterized by high values of leucine- ρ NA, alanine- ρ NA releasing and able to coagulate skim milk and were located in the right part of graph. Cluster 2 included three strains they were located in the lower part of graph and they showed moderated releasing values of leucine- ρ NA, alanine- ρ NA, enable to coagulate skim milk, slow acid production after 6 h and moderate acidifying ability after 24 h of incubation. Finally, for the cluster 3, the seventeen strains of this group displayed low values of aminopeptidase activity excepted the strain LF7 which showed moderate value for leucine- ρ NA, alanine- ρ NA releasing. Concerning other parameters strains exhibited moderate values and no strains were able to coagulate skim milk. Strains of this group were located in the left part of graph.

Discussion

Classically, for the identification of *Lactobacillus* to species level the conventional phenotypic tests such as cell morphology, carbohydrate fermentation, substrate assimilation and products of metabolism were used. However, identification using this method is notoriously difficult largely due to the need for plenty of cumbersome biochemical tests along with the problems of highly resembling large number of species groups that are prone to transfer of plasmids among them. In order to make identification and characterization of a range of *Lactobacillus* species easier, clear and comprehensive, various schemes have been proposed by several authors, based on distinguishable features/characteristics, such as phenotypic, physiological, biochemical characteristics and genotypic method such as sequence comparisons of 16S rDNA gene (Kandler and Weiss 1986; Stiles and Holzapfel 1997). Acidification is an important functional logical property in

Fig. 4 Graphical representation of the principal component analysis of the technological properties of *L. pentosus* strains showing axis F1 versus F2 : (a) factor loading, (b) factor scor Leu- ρ -NA : L-leucine- ρ -nitroaniline; Ala- ρ -NA : L-alanine- ρ -nitroaniline; Δ pH6 : pH variation after 6 h of incubation; Δ pH12 : pH variation after 12 h of incubation; Δ pH24 : pH variation after 24 h of incubation; Coag 24 h : coagulation 24 h : coagulation of skim milk after 24 h of incubation

relevance with selection of starter culture among the LAB. The results of high acidifying capacity of *L. plantarum* strains after 6 and 12 h of incubation is in agreement with the work of Dagdemir and Ozdemir (2008). In contrast, the acidifying rate of isolates after 24 h of incubation is less than those reported in the former study. Also it seems there were marked variability within *L. plantarum* strains with respect to their acidifying activities. The possibility to find groups of strains characterized by different acidifying ability is frequent in *L. plantarum* species (Xanthopoulos et al. 2000). Results of acidifying activities of *L. rhamnosus* strains tested showed more homogeneous behaviour compared to *L. plantarum* strains. Finally, by comparing the ΔpH observed for tested *Lactobacillus* strains after 24 h of incubation we conclude that 18 strains (25.35 %) of the isolates have ΔpH more than 1.50 units (Thirty *L. plantarum*, two *L. pentosus* and three *L. rhamnosus* strains), and 74.65 % of strains, showed $\Delta\text{pH}<1.50$ units (Table 3). Generally, *L. plantarum* strains produced acid more rapidly when compared to other lactobacilli strains (Dagdemir and Ozdemir 2008). The fast acidifying strains (mostly *L. plantarum* and *L. rhamnosus* isolates) should be selected as part of a starter preparation. In fact, a further important characteristic for potential starter strains is their ability to acidify their environment rapidly, as the acid production and the accompanying pH decrease are well-known to extend the lag phase of sensitive organisms including foodborne pathogens (Smulders et al. 1986). Coagulation of milk by some strains of *L. plantarum* and *L. rhamnosus* strains reveals their potential as starters or adjunct cultures in production of fermented dairy food. The peptides are degraded intracellularly by a variety of peptidases, which have been extensively studied in lactobacilli species. This peptidase activity may be related to the cell wall or to the outer surface of the cell membrane or to a possible intracellular enzyme leakage (Requena et al. 1991). Another possibility might be the transfer of oligopeptides into the cell and their degradation by intracellular aminopeptidases. The proteolytic activity of dairy lactic acid bacteria is essential for the bacterial growth in milk and it is involved in the development of organoleptic properties of different fermented milk product (Christensen et al. 1999). Concerning *L. rhamnosus* strains two isolates (LF3 and LF10) had a high activity to release L-leucine- ρNA and L-alanine- ρNA and three strains had low activity to degrade L-leucine- ρNA and L-alanine- ρNA . *L. fermentum* tested strains showed a more homogeneous aminopeptidase activity. All strains exhibited low AP activity against Leu- ρNA and Ala- ρNA . Variability between strains of the same species has been also reported by other studies (Nieto-Arribas et al. 2009; Jenson and Ardö 2010). The selection of novel probiotic strains should be based on the knowledge of strain characteristics such as tolerance to gastric acidity and bile toxicity and ability to exclude pathogens (Monteagudo-Mera et al. 2012). The resistance of bile by strains is an important parameter and good

indication for their acceptability and for selecting potential strains to be used as probiotic in food as a dietary adjunct. It has been recommended to use 0.3 % bile as a suitable concentration for selection of probiotics (Dunne et al. 2001). Principal component analysis (PCA) is an exploratory method of data analysis, which through the calculation of linear combinations of original variables allows the number of dimensions in the data set to be considerably reduced while maintaining most of the original information of the data set, expressed as percent variance (Massart et al. 1988). By using PCA in our study, three clusters with different combinations of technological properties of *L. plantarum* and *L. pentosus* strains were obtained and showed diversity among the isolates. PCA technique has been successfully used for study the diversity of properties of several species of lactic acid bacteria isolated from dairy product by several authors (Gatti et al. 1999; Giraffa et al. 2001, 2004; Malek et al. 2012; Piraino et al. 2008).

Results of antibiotics susceptibility obtained in this study showed that most of strains were sensitive to penicillins and β -lactam inhibitors, and a high susceptibility to the inhibitors of protein synthesis was also observed. These results are in concordance with Danielsen and Wind (2003), but in contrast with result observed by Zarazaga et al. (1999). Results showed that tested strains using the disk diffusion method are resistant to vancomycin, oxacillin, cephalosporine (ceftazidime) and most strains were susceptible to erythromycin, tetracycline, chloramphenicol, fusidic acid and imipenem. These results were in accordance with various reports indicating that LAB are normally resistant to β -lactam, cephalosporins, aminoglycosides (Ammor et al. 2007; Charteris et al. 1998; Coppola et al. 2005; Danielsen and Wind 2003; Halami et al. 2000; Matur and Singh 2005). Comparison of the MICs distribution obtained in this study with recent research showed relatively similar. Resistance to gentamicin and vancomycin has been observed in *Lactobacillus* species, and it has been shown to have a wide range of MICs (Ammor et al. 2007; Charteris et al. 1998; Coppola et al. 2005; Zhou et al. 2005), also high incidence of gentamicin-resistant lactobacilli was observed by Hummel et al. (2007). With a multimodal distribution of MICs, probably due to the extensive variability of gentamicin resistance mechanisms, some authors indicated that the use of gentamicin in animal farms can induce in bacteria a resistance transmittable via mobile genetic elements (Belletti et al. 2009). Moreover, vancomycin-resistant in species of *Lactobacillus* spp. has been reported to be chromosomally encoded and not inducible or transferable (Klein et al. 2000; Kotsou et al. 2008; Saarela et al. 2002; Tynkkynen et al. 1998). Many species of *Lactobacillus* carry intrinsic resistance towards vancomycin which is due to the presence of D-alanine: D-alanine ligase-related enzymes (Elisha and Courvalin 1995). Mattila-Sandholm et al. (1999) reported that intrinsic vancomycin resistance is not considered as a risk factor in lactobacilli, as many strains have a long history of

safe use as probiotics and there is no indication that they could transfer resistance to other species. In our result all strains were susceptible to cell wall impermeability agents and most of isolates were sensitive to antibiotics that inhibit protein synthesis and more resistant to aminoglycosides such as, streptomycin and neomycin. These results are similar to those of (Charteris et al. 1998; Coppola et al. 2005; Zhou et al. 2005), who reported that lactobacilli are generally susceptible to antibiotics inhibiting the synthesis of proteins, such as chloramphenicol, erythromycin, clindamycin and tetracycline. However, resistant strains to these antibiotic agents have also been described as more resistant to aminoglycosides (neomycin, kanamycin, streptomycin and gentamicin) (Charteris et al. 1998; Danielsen and Wind 2003; Delgado et al. 2005; Flórez et al. 2005). In addition, a recent study reported that the lactobacilli isolated from commercial products in Europe comprised strains resistant to tetracycline, chloramphenicol, and erythromycin and overall, more than 68 % of the isolates exhibited resistance to two or more antibiotics (Temmerman et al. 2003).

Conclusion

The present study demonstrated that there were a large heterogeneity of technological traits and safety differences between the lactobacilli strains isolated from Algerian goat's milk samples collected in different regions of Algeria. A combined approach based on phenotypic and genotypic techniques associate to the evaluation of antibiotic susceptibility and technological traits assessment including acidifying activity and aminopeptidase activity suggest a wide diversity within lactobacilli strains. For acidifying activity results showed that pH of milk unregistered varied between 4.36 and 5.65 after 24 h of incubation in all cultures and between 5.43 and 6.50 and 5.24 to 6.02 after 6 and 12 h of incubation respectively. The values observed for aminopeptidase indicated that all strains expressed activity against the two substrates tested, however, at a somewhat different activity levels. Two *L. plantarum* strains (LAM1 and LAM35) and three *L. pentosus* strains (LAM38, LAM39, LF9 and LF16) showed high ability to release both L-leucine- ρ NA and L-alanine- ρ NA. Moreover, two *L. rhamnosus* strains (LF3 and LF10), had high activity to release Leu- aminopeptidase and Ala-aminopeptidase and all *L. fermentum* strains showed a more homogeneous aminopeptidase activity. The result of antibiotics susceptibility showed that some lactobacilli tested strains had an important potential related to safety properties. The analysis of graphical representation of the PCA based on technological properties of *L. plantarum* and *L. pentosus* classified the strains of each specie into different clusters. Finally the results obtained in our study indicate that the isolates (LAM1, LAM3, LAM21, LAM25, LAM35, LAM38,

LAM39, LF3, LF9, LF10 and LF16), showed potential related to technological traits and safety aspect and could be good candidates to be used as adjunct culture for future application in manufacture of dairy food industry in Algeria such as artisanal cheese or fermented milk.

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