Vol.57, n.3: pp. 410-420, May-June 2014
http://dx.doi.org/10.1590/S1516-89132014005000003
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Lactobacilli Isolated from Algerian Goat's Milk as Adjunct Culture in Dairy Products

Ahmed Marroki[1,2*] **and Leila Bousmaha-Marroki**[1]
[1]*Department of Biology; Faculty of Natural and Life Sciences; University Djillali Liabbès of Sidi Bel Abbes; Algeria.* [2]*Laboratory of Microbial Genetic; Faculty of Natural and Life Sciences; University of Oran; Algeria*

ABSTRACT

In this study, nineteen Lactobacillus *isolated from Algerian goat's milk, 13 belonging to* L. plantarum*, three to* L. pentosus*, two to* L. rhamnosus *and one to* L. fermentum*, were examined* in vitro *in order to be used as adjunct culture in dairy products. The strains were tested for their proteolytic activity, sensory and safety properties. Strains LbMS16 and LbMS21* L. plantarum and *LbMF25* L. rhamnosus *presented the highest proteolytic activity. All the tested lactobacilli were able to grow on MRS agar containing 0.5 and 1% (W/V) of oxgall, whereas none produced biogenic amine* (*BA) from the four tested amino acids and were resistant to pH 2.0 and 3.0, but some strains were able to grow at pH 3.5. None of examined strains were β-haemolytic when grown in hors blood agar. Result of antibiotic resistance showed that all the strains were susceptible to penicillin, erythromycin and resistant to vancomycin. Diacetyl production was observed for two strains of* L. plantarum *and one of* L. rhamnosus*. Most of strains were able to produce pleasant flavours in fermented milk and gave a good acceptance. According to these results, the strains LbMS16, LbMS21and LbMF25 could be good candidates to be used as adjunct culture, playing a probiotic role in dairy products manufacture in Algeria.*

Key words: Lactobacillus, *goat's milk, safety aspect, adjunct culture, sensory properties, antibiotic resistance*

INTRODUCTION

The genus *Lactobacillus* has a long history of safe use in the food industry, especially in the dairy products, and plays an important role in the production of fermented milk products (Maragkoudakis et al. 2006; Bujňáková and Kmeť 2012). They have been an essential part of the natural microflora of many dairy products and play relevant roles in the development of the sensory and organoleptic properties of the final products.

Goats' milk products, especially cheeses and yogurts, are very popular in the Mediterranean region (Tamime and Robinson 1999). These products are manufactured from the raw and pasteurized goat's milk and the quality differences between the products are thought to be due to the presence of indigenous microflora in the milk (McSweeney et al. 1993). Moreover, some artisanal product manufactured in many countries are made under artisanal conditions from the raw milk without using industrial starter cultures, hence, the quality of these products is strictly dependent on the microbial associations responsible for the fermentation, and the biodiversity of lactic acid bacteria involved is considered a fundamental factor for the maintenance of the characteristic features.

There have been some studies focused on selected strains from goat's milk with a technological

*Author for correspondence: a_marroki@yahoo.fr.

potential to be used as adjunct, or starter culture in dairy products (Ayad et al. 2004; Badis et al. 2004a, b). The isolation of strains from goat's milk has the advantage of giving a higher choice of selection and can be considered as proper source for new *Lactobacillus* strains susceptible to be used as adjunct cultures in dairy products with safety and technological potentials. In dairy products, two types of cultures are used, the primary ones, which include all the starter lactic acid bacteria and the secondary presented by the non-starter lactic acid bacteria (NSLAB), known as "adjunct cultures". The addition of selected NSLAB as adjunct cultures could also play a probiotic role (Bertazzoni Minelli et al. 2004; Bude-Ugarte et al. 2006; Maragkoudakis et al. 2006).

The genus *Lactobacillus*, together with *Bifidobacterium* species are most commonly used as probiotic (Saxelin et al. 2005). Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit" (FAO/WHO 2002, 2001). Traditionally, and reinforced by the WHO/FAO guidance document, probiotic candidate strains have been selected on the basis of a few simple properties. The safety of probiotic lactobacilli is important. Current safety criteria for successful probiotics have been also defined in several reviews (Donohue and Salminen 1996; Salminen et al. 1996, 1998; Adams 1999). In recent years, some studies have been focused to characterize and select new strains of *Lactobacillus* that exert a beneficial health effect when ingested by the humans and are applied as adjunct cultures in various types of food products, or in therapeutic preparations (Rodgers 2008). For several aspects, including safety and technological developments, characteristics and sensory properties should be taken into consideration by selection of suitable strains to be used as adjunct culture (Saarela et al. 2000). The strain should be non-pathogenic and should not carry transmissible antibiotic resistance. In addition, other aspects such as strains' acid and bile tolerance, biogenic amine formation and antibacterial activity against pathogenic bacteria should also be considered (Brizuela et al. 2001; Baka et al. 2011). The characterization and some technological properties of 19 *Lactobacillus* strains isolated from Algerian goat's milk were previously reported (Marroki et al. 2011). Few works have been focused to selected *Lactobacillus* strains from Algerian goat's milk with potential characteristics related to safety and probiotic properties. This study had the objective to evaluate *in vitro* some characteristics related to proteolytic activity, safety aspect and sensory properties of 19 lactobacilli isolated from goat's milk in order to select an appropriate adjunct culture, which could play a probiotic role in fermented dairy products in Algeria.

MATERIALS AND METHODS

Lactobacilli Culture Conditions

A total of 19 strains of the *Lactobacillus* genus were investigated in this study belonging to *L. plantarum* (n=13), *L. pentosus* (n=3), *L. rhamnosus* (n=2), *L. fermentum* (n=1). The origin and identification of these isolates at species level were previously described by Marroki et al. (2011). Strains were maintained in MRS medium (Oxoid, UK) containing 20% glycerol at −80°C and were activated on MRS and incubated for 24 to 48 h. The cultures were reactivated by two successive transfers in the same broth before use.

Proteolytic Activity

The strains were tested for their proteolytic activity on skim milk agar according to Essid et al. (2009) as follows. An overnight culture of each strain (MRS broth, 24 h, 30°C, 10^6 CFU/mL) was centrifuged at 13,000 r.p.m for 5 min and pellet was suspended in 20 mM phosphate buffer, pH 7.0. Five microliter of each suspension was spotted on the surface of skim milk agar (10% of skim milk, 0.5% yeast extract, 1.5% agar) incubated at 30°C for 24 h. The proteolytic activity was determined by the measurement of the diameter of clear zones around the spots (mm).

Growth Capacity, pH Tolerance and Acidifying Capacity Test

The growth capacity was evaluated after incubation of the strains (1%) in MRS broth at 30°C. Optical density (OD) at 600 nm was measured until the stationary phase. The average value of each measurement was used for the calculation of the specific growth rate (μ/h) using the equation $\mu = d\ (\ln OD_{600}) / dt$ (Seseña et al. 2005). The pH tolerance was determined according to the method described by Voravuthikunchai et al. (2006). The strains were inoculated in MRS broth adjusted with 4 N HCL to obtain pH 2.0, 3.0, 3.5 and 6.5 (control) and

incubated at 30°C for 24 h. The growth at tested pH was visually assessed. The acidifying ability of the strains (10^6 CFU/mL) was evaluated in MRS broth adjusted at pH 6.5 and incubated at 30°C for 24 h. The pH was measured (glass electrode, Crison, Spain) immediately after inoculation and again after 24 h of incubation and values were expressed as ΔpH (Gati et al. 1999). All the assays were performed in duplicate.

Bile Tolerance

Bile tolerance was determined by the streaking single colony on MRS agar plates containing oxgall bile (0.5 and 1% W/V , Sigma, Germany) as described by Linaje et al. (2004). Control plate contained no oxgall bile. The plates were incubated at 30°C for 24 h and visually examined for the growth.

Biogenic Amine Production

The production of the biogenic amines was assessed by adding 1% (W/V) of each precursor amino acid, tyrosine disodium salt (Sigma,USA), L-histidine monohydrochloride (Sigma), L-lysine (Sigma) and L-ornithine monohydrochloride (Sigma) on MRS agar plate containing 0.06% bromocresol purple. The plates were incubated at 30°C for 48 h and positive reactions were recorded when a purple colour appeared on the plates, or tyrosine precipitates disappeared around the colonies as described by Bover-Cid and Holzapfel (1999).

Hemolytic Activity

Hemolytic activity was determined by streaking single colonies of *Lactobacillus* strains on Colombia agar plates containing fresh hors blood and incubated at 30°C for 24 h as described by Linaje et al. (2004). Blood agar plates were examined for β- haemolysis (clear zones around colonies), α- haemolysis (green-hued zones around colonies), or γ-haemolysis (no zones around colonies) (Maragkoudakis et al. 2006).

Diacetyl Production

Evaluation of diacetyl production in the milk was carried out according to the method described by Franciosi et al. (2009) as follows. *Lactobacillus* strains adjusted at concentration of 10^6 UFC/mL were inoculated in UHT whole milk and after a growth at 30°C for 24 h, 1.0 mL of each cell suspension was added with 0.5 mL of a solution α-naphthol (1% W/V) and KOH (16% W/V) and incubated at 30°C for 10 min. Diacetyl generation was indicated by the formation of a red ring at the top of the tube (King 1948).

Determination of the Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) of eight antimicrobial agents were determined by micro-dilution method 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 representative strains. The following antimicrobials were tested in the concentration ranges (μg/mL) given in parentheses: penicillin G (0.032–64), kanamycin (1–2048), gentamicin (1–2048), streptomycin (2–2048), vancomycin (0.125–256), erythromycin (0.016–32), oxytetracycline (0.063–128) and chloramphenicol (0.125–256). For each isolate, grown in antibiotic-free LSM broth, the inoculum was adjusted to turbidity equivalent to 0.5 McFarland standard (≈1×10^5 CFU/mL). Aliquot of 100 μL of the inoculum was added into each well. As positive control, bacteria were inoculated in LSM without antibiotic and an inoculums-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 at which no growth was observed. Interpretation for susceptibility status was based on EFSA (2008) and values of breakpoint were determined as described by Ammor et al. (2007).

Sensory Analysis

Overnight cultures of strains adjusted at concentration of 10^6 UFC/mL were inoculated (1%, V/V) in 20 mL of UHT skim milk and incubated at 30°C for 7 h (Nieto-Arribas et al. 2009). Then the fermented milk was kept at 4°C for 18 h (Ayad et al. 2004). Sensory evaluations of the fermented milk were carried out by six highly trained judges. Panelists were selected from volunteers, including the students and academic staff of university for the sensory analysis. Sensory analysis was performed in a conditioned room. The samples were coded with four-digit number and served in randomized order. The panelists (n = 6: 2 women, 4 men, aged 20–40 years old) received a 100-h training session on descriptive analysis technique, including basic

tastes and flavor identification using following scale: (0) null; (1) light; (2) medium; (3) strong (Nieto-Arribas et al. 2010). Panelists were instructed to cleanse the palate between each sample with the drinking water provided. Definitions for each of these attributes are shown in Table 1. The attributes selected were as follows: two texture terms (firmness of curd and whey quantity), two odour attributes (yoghurt and butter), five taste attributes (butter, bitter, yoghurt, acid and sweet). All the samples were evaluated in duplicates.

Table 1 - Definitions of sensory attributes.

Sensory attributes	Definitions
Odor attributes/ odour associated with :	
Yoghurt	A flavor similar to that of fresh yoghurt.
Butter	A flavor similar to that of fresh butter.
Texture terms Texture/ appearance :	
Firmness	The firmness is evaluated visually by compress the gel using the little spoonful or middle finger slowly the surface of product and recording how long the surface is retained.
Curd quantity	The curd is partly strained semi-solidse, is white to pale yellow with a good texture and uniforme in consistency. The curd is evaluated visually by mesuring the quantity of curd formed in the bottles.
Whey quantity	Amount of liquid (serum) on the surface of the curd.
Taste attributes/ taste sensation associated with :	
Butter	Fundamental tast sensation of fresh butter.
Bitter	Fundamental taste sensation of which caffeine (Caffeine 0.1% W/V).
Yoghurt	Fundamental tast sensation of commercial yoghurt.
Acid	Fundamental tast sensation of fermented milk, lactic acid sour.
Sweet	Fundamental taste sensation of which sucrose is typical (Sucrose solution 10% W/V).

RESULTS AND DISCUSSION

Proteolytic Activity

The proteolytic activity has been shown desirable for the growth of lactic acid bacteria (LAB) in milk and, in addition, it is involved in the development of some organoleptic characteristics in different fermented milk products (Axelsson 1998). In the present study, proteolytic activity as tested by agar plate method was recognizable by the presence of a clear halo in the plates. The results for 19 *Lactobacillus* tested strains are shown in Table 2. Only 11 strains possessed this feature. Eight strains belonging to *L. plantarum* showed diameter zone ranging between 6 to 19 mm and two *L. rhamnosus* strains LbMF24 and LbMF25 and one *L. fermentum* strain LbMA47, showed 11.5, 23 and 10 mm of diameter, respectively. These results showed that strains LbMS16, LbMS21 and LbMF25 had the highest proteolytic activity (halos ≥ 15 mm). The production of high quality of fermented dairy product such as cheese and fermented milk is dependent on the proteoltytic system of the starter bacteria used. Proteolytic enzymes from LAB play an important role in the degradation of casein and peptides leading the production of free amino acids (Neito-Arribas et al. 2010). These amino acids contribute directly or indirectly in dairy product flavor, since they are precursors of other catabolic reactions, which produce volatile aroma compounds (Fox and Wallace 1997; Williams and Banks 1997). However, highly proteolytic strains are not always the most suitable as starter cultures, since excessive proteolysis can cause uncontrolled production of bitter peptides and other undesirable compounds (Buffa et al. 2005).

Growth Capacity, Low pH Tolerance and Acidifying Capacity Test

Results of growth rate of lactobacilli tested are shown in Table 2. The growth varied between 0.30 to 0.56/h for all the strains. All *L. plantarum* showed the growth rate ranging between 0.31

(LbMS21 and LbMO16) to 0.56/h (LbMS4). The growth rates observed for *L. pentosus* strains were between 0.30 (LbMT10) and 0.32/h (LbMS40). The values were between 0.30 and 0.33/h for *L. rhamnosus* and 0.42/h for *L. fermentum* strains. Similar values were obtained by Seseña et al. (2005) for lactobacilli strains using the same cultures conditions. The key selection criteria of probiotics include many functional aspects, such as tolerance to gastric acidity and bile toxicity (Dunne et al. 2001). Tolerance of low pH *in vitro* was expected to predict the survival of strain in the conditions present in the gastrointestinal tract.

Result of *Lactobacillus* strains tested in MRS broth adjusted at different pH values are shown in Table 2. All the tested strains were not able to grow at pH 2.0 and 3.0 after 24 h of incubation. However, some lactobacilli strains exhibited a resistance at pH 3.5 and all the strains were able to grow at pH 4.0, except strain LbTM9. Generally, tolerance to pH 3.0 is considered as standard for probiotic culture (Gohran 1994). The acidifying capacity observed for *L. plantarum* strains after 24 h of incubation were ranging between 2.08 (LbMO27) and 2.48 (LbMS21) pH. But for *L. pentosus* strains, the ΔpH after 24 h of incubation were between 2.31 (LbMT10) and 2.46 (LbMS40). Concerning *L. rhamnosus* strains, the ΔpH after 24 h was 2.32 and 2.46 for the strains LbMF24 and LbMF25, respectively. The ΔpH for LbMA47 belonging to *L. fermentum* was 2.18 after 24 h of incubation.

Table 2 - Acidifying capacity, pH and Ox gall tolerance, safety aspect, proteolytic activity and diacetyl production of Lactobacilli strains from goat's milk. The result represents the mean ± standard deviation.

Isolats	pH[a]			ΔpH$^{\#}_{24h}$	Growth rate μ(h^{-1})	Haemolysis	Proteolytic activity	Diacetyl[b]
	2.0	3.0	3.5					
L. plantarum								
LbMA9	-	-	-	2.33	0.32 ± 0.01	α	0.00 ± 0.00	+*
LbMF13	-	-	-	2.30	0.33 ± 0.06	γ	0.00 ± 0.00	-**
LbMF33	-	-	-	2.32	0.37± 0.02	γ	0.00 ± 0.00	-
LbMS4	-	-	+	2.34	0.56 ± 0.08	γ	8.50 ± 0.70	+
LbMS9	-	-	-	2.31	0.32 ± 0.01	γ	0.00 ± 0.00	-
LbMS14	-	-	±	2.30	0.41 ± 0.07	γ	8.00 ± 0 .00	-
LbMS16	-	-	-	2.40	0.31 ± 0.01	γ	15.50 ± 0.70	++***
LbMS20	-	-	-	2.36	0.50 ± 0.05	γ	6.00 ± 0.00	-
LbMS21	-	-	+	2.46	0.31 ± 0.11	γ	19.00 ± 1.41	++
LbMS24	-	-	+	2.36	0.33 ± 0.09	γ	6.50 ± 0.70	-
LbMO16	-	-	-	2.25	0.31 ± 0.06	γ	0.00 ± 0.00	-
LbMO27	-	-	-	2.08	0.34 ± 0.12	γ	7.50 ± 0.70	-
LbMO42	-	-	-	2.36	0.36 ± 0.02	γ	10.50 ± 0.70	-
L. pentosus								
LbMS40	-	-	-	2.48	0.32 ± 0.08	γ	0.00 ± 0.00	-
LbMT9	-	-	-	2.34	0.31 ± 0.04	γ	0.00 ± 0.00	-
LbMT10	-	-	+	2.31	0.30 ± 0.01	γ	0.00 ± 0.00	-
L. rhamnosus								
LbMF24	-	-	-	2.32	0.30 ± 0.02	α	11.50 ± 0.70	+
LbMF25	-	-	-	2.46	0.33 ± 0.01	γ	23.00 ±0.41	++
L. fermentum								
LbMA47	-	-	-	2.18	0.42 ± 0.10	γ	10.00 ± 0.00	-

[a] +: Positive growth, - : Negative growth, ±: Weak growth, [b] *+: Low production, **- : no production, ***++ : Fast production
Initial pH : 6.50.

Bile Tolerance

The relevant physiological concentrations of human bile range from 0.3% (Dunne et al. 2001) to 0.5% (Zavaglia et al. 1998). In this study, all the strains were tested for their bile tolerance and were able to grow in 0.5 and 1% oxgall. The resistance of bile by strain 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.

Biogenic Amine (BA) Production

BA production is an undesirable trait in food-grade microorganisms, and it is, therefore, very

important to determine the BA-producing potential of a microorganism before proposing it as a starter culture (Nieto-Arribas et al. 2009). In the present study, the ability to produce BA was screened for the 19 strains of *Lactobacillus* by plate medium method as described by Bover-Cid and Holzapfel (1999). Results obtained exhibited that none *Lactobacillus* strains produced BA from the four tested amino acids. This was a good indication of their acceptability to develop as starter, or adjunct cultures (Olasupo 2001). This result was in agreement with previous results obtained by several authors (Durlu-Ozkaya et al. 2001; Olasupo et al. 2001; Nieto-Arribas et al. 2009). The consumption of food containing high amounts of these amines can have toxic effects (Shalaby 1996). The problems may be more severe in sensitive consumers having a reduce mono-and diamine oxidase activity (Bodmer et al. 1999). Toxicological problems may result from the ingestion of foods containing relatively high levels of BA and may provoke hypertensive crises in the patients treated with monoaminoxidase inhibitors drugs (MAOI) (Arena and Manca de Nadr 2001).

Hemolytic Activity

Hemolytic activity of *Lactobacillus* strains was assessed by detecting the presence of a lytic zone on blood agar plate. As shown in Table 2, none of the strains of *Lactobacillus* examined exhibited β-haemolytic activity when grown on horse blood agar. While most of strains (17 strains) were γ-haemolytic (absence of zones around the colonies), two strains (LbMA9 and LbMF24) exhibited α-haemolysis ability. Haemolytic activity is considered as an important virulence factor and a good indicator in order to select potential probiotics strains.

Diacetyl Production

Diacetyl is key flavor compound generated as the end-product of citrate metabolism by certain LAB. In the present study, 19 *Lactobacillus* strains were investigated to produce diacetyl in UHT skim milk in order to be used as indigenous adjunct cultures, or probiotic strains. As shown in Table 2, different levels (high, medium and low) of diacetyl production were detected. Some strains did not produce diacetyl in skim milk (nine strains of *L. plantarum*, three *L. pentosus* and one *L. fermentum).* Fast production of diacetyl was observed for two *L. plantarum* strains (LbMS16 and LbMS21) and one of *L. rhamnosus* strain (LbMF25); low production of diacetyl was observed for two *L. plantarum* strains (LbMA9 and LbMS4) and one of *L. rhamnosus* (LbMF24). These results were in agreement with those of Skeie et al. (2008a), which suggested that in the presence of a Cit^- O-starter, *L. plantarum* INF15D degraded citrate to aspartate (Asp), which was further converted to acetoin and diacetyl. It has been shown that cheese with added *L. plantarum* INF15D had increased levels of Asp and acetoin (Skeie et al. 2008b), compared with a control cheese without it. Diacetyl is considered as the most important attribute for the consumers, since it greatly influences the quality of dairy products (Heap 1998). Many of the LAB strains produce appreciable buttery and yoghurt flavors in fermented milk. Diacetyl, or 2,3-butanedione is the typical butter flavour/aroma and is an important flavor compound in dairy products such as milk, cheese and yoghurt. It is a natural by-product of fermentation (Harber et al. 2006) and has also attracted interest as one of the parameters on which LAB are characterized (Beshkova et al. 2003).

Determination of the Minimum Inhibitory Concentration (MIC)

The antimicrobial resistance may be considered as one of the criteria to evaluate the safety of strains in food (Borriello et al. 2003). The results of distribution of MICs of eight antimicrobial agents tested against 19 *Lactobacillus* strains using microdilution broth method are presented in Table 3. Evidently all the isolates were resistant to vancomycin (MIC > 256 µg/mL). Resistance to high concentration of vancomycin (MIC ≥ 256 µg/mL) among the lactobacilli has already been demonstrated (Ammor et al. 2007; Danielsen and Wind 2003; Mahur and Singh 2005). 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). Intrinsic vancomycin resistance is not considered 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 (Mattila-Sandholm et al. 1999).

All the lactobacilli isolates were sensitive to penicillin (MIC ≤ 2 µg/mL) and erythromycin (MIC ≤ 1 µg/mL). Generally, lactobacilli seem to be sensitive to cell wall synthesis inhibitors, such

as penicillins (Danielsen and Wind 2003). A wide spread sensitivity toward penicillins has already been observed in lactobacilli used as probiotic, or starter cultures (Danielsen and Wind 2003), and in probiotic strains (Charteris et al. 1998). The present results were in agreement with earlier reports describing that *Lactobacillus* was usually susceptible to ampicillin and erythromycin (Katla et al. 2001; Coppola et al. 2005; Ammor et al. 2007). Seven of the 13 *L. plantarum* were resistant to kanamycin and all *L. rhamnosus, L. fermentum* and *L. pentosus,* except of one strain (LbMS40) were sensitive to it with MIC value < 16 µg/mL. For gentamycin, all *L. rhamnosus, L. fermentum* and *L. pentosus* and seven of the thirteen *L. plantarum* were characterized as resistant. Susceptibility to streptomycin varied among *L. pentosus* (n=3), *L. rhamnosus* (n=2), *L. fermentum* (n=1) and *L. plantarum* (5/13 strains resistant). Nine of the 19 tested strains were sensitive to oxytetracyclin: six *L. plantarm,* two *L. pentosus* and one *L. rhamnosus.* Comparison of the MICs distribution obtained in this study of *Lactobacillus* against tetracycline with recent result showed relatively good. Most of strains were susceptible to chloraphenicol, with MIC value of *L. plantarum* ranging between 2 and 8 µg/mL, and for *L. pentosus, L. rhamnosus* and *L. fermentum* MICs value ranging between 1 and 4 µg/mL. Lactobacilli are generally susceptible to antibiotics inhibiting the synthesis of proteins, such as chloramphenicol, erythromycin, clindamycin and tetracycline, and more resistant to aminoglycosides (neomycin, kanamycin, streptomycin and gentamicin) (Charteris et al. 1998; Coppola et al. 2005; Zhou et al. 2005). Resistance to tetracycline has been observed in *Lactobacillus* species and it has been shown to have a wide range of MICs (Korhonen et al. 2008), also with a multimodal distribution of MICs, probably due to the extensive variability of tetracycline resistance mechanisms conferring diverse levels of susceptibility (Roberts 2005). It is recommended that the antibiotic resistance pattern of potential probiotic strains should be determined to avoid introducing the strains containing transferable antibiotic-resistance genes (Saarela et al. 2000; Mathur and Singh 2005). However, with regard to general concerns on bio-safety of adjunct cultures as probiotics, studies should focus on the location and potential transferability of antibiotic resistance determinants (Mathara et al. 2008).

Table 3 - Distribution of Minimal Inhibitory Concentration (MIC) of *Lactobacillus* strains.

Strains	**Antibiotic with the MIC (µg/mL)**															
	Pen		**Van**		**Kan**		**Gen**		**Strep**		**Ery**		**Oxytet**		**Chlor**	
L. plantarum																
LbMA9	1	S	>256	R	8	S	32	R	16	S	0.5	S	2	S	2	S
LbMF13	1	S	>256	R	64	R	8	S	16	S	0.5	S	4	S	2	S
LbMF33	1	S	>256	R	64	R	16	R	8	S	1	S	2	S	8	R
LbMS4	0.5	S	>256	R	32	R	16	R	8	S	1	S	64	R	4	S
LbMS9	2	S	>256	R	8	S	32	R	8	S	1	S	16	R	2	S
LbMS14	2	S	>256	R	32	R	4	S	16	S	1	S	128	R	2	S
LbMS16	2	S	>256	R	64	R	16	R	64	R	1	S	16	R	2	S
LbMS20	2	S	>256	R	16	R	16	R	64	R	1	S	64	R	2	S
LbMS21	2	S	>256	R	64	R	4	S	128	R	1	S	8	R	4	S
LbMS24	0.5	S	>256	R	2	S	4	S	8	S	0.016	S	16	R	2	S
LbMO16	0.5	S	>256	R	8	S	4	S	16	S	1	S	16	S	2	S
LbMO27	2	S	>256	R	8	S	4	S	64	R	1	S	16	*S*	2	S
LbMO42	2	S	>256	R	8	S	32	R	64	R	0.5	S	16	*S*	8	R
L. pentosus																
LbMS40	1	S	>256	R	16	R	8	R	8	S	0.5	S	128	R	4	R
LbMT9	1	S	>256	R	8	S	32	R	8	S	1	S	0.063	S	1	S
LbMT10	0.5	S	>256	R	8	S	32	R	8	S	1	S	0.063	S	1	S
L.rhamnosus																
LbMF24	1	S	> 256	R	16	S	32	R	16	S	1	S	64	R	2	S
TLF25	1	S	>256	R	16	S	32	R	16	S	1	S	2	S	2	S
L. fermentum																
LbA 47	0.5	S	>256	R	16	S	32	R	16	S	0.5	S	128	R	2	S

The MIC breakpoints were chosen as suggested by EFSA (2008) and by Ammor and al. (2007). S: susceptible, R: resistant, Pen: Penicillin G, Van: Vancomycin, Kan: Kanamycin, Gen: Gentamicin, Strep: Streptomycin, Ery: Erythromycin, Oxytet: Oxytetracycline, Chlor: Chloramphenicol.

Sensory Analysis

The quality of fermented dairy products is largely determined by the sensory perception. Results of mean score sensory analyses of fermented milks with different strains of *Lactobacillus* are shown in Table 4. Generally, most of selected strains presented a good texture (curd consistency and absence of whey). Flavor and consistency are among the most important attributes for the consumers, since they greatly influence the quality of dairy products (Heap 1998). Many of the *Lactobacillus* assayed produced appreciable buttery and yoghurt flavor in fermented milk. The results were in agreement with the result reported by other authors (Garabal et al 2008; Franciosi et al. 2009). The yoghourt and butter taste attributes were detected for most of the strains, but the acid and sweet was detected for many of strains. On basis of the results of sensory analysis obtained in this study, two strains belonging to *L. plantarum* (LbMS16 and LbMS21), one strain of *L. rhamnosus* (LbMF25) and one strains of *L. fermentum* (LbMA47) was selected to be used as adjunct cultures in manufacture of fermented dairy products in Algeria.

Table 4 - Mean score sensory analyses of fermented milks with lactobacilli.

Strains	Texture		Odour attributes		Taste attributes				
	Curd firmness	Whey quantity	Yoghurt	Butter	Yoghurt	Butter	Bitter	Acid	Sweet
L. plantarum									
LbMA9	1	0	2	2	1	2	0	0	1
LbMF13	0	0	1	2	0	2	0	1	1
LbMF33	0	0	0	1	0	1	1	1	0
LbMS4	0	0	1	2	2	2	0	1	1
LbMS9	1	0	0	1	0	2	1	0	0
LbMS14	1	0	1	2	1	2	1	1	1
LbMS16	3	2	2	2	2	2	0	2	2
LbMS20	1	0	0	1	0	2	0	1	0
LbMS21	2	1	2	2	2	2	0	3	1
LbMS24	0	0	0	3	1	2	1	0	0
LbMO16	0	0	1	2	1	2	0	0	0
LbMO27	1	0	0	2	0	2	2	1	1
LbMO24	0	0	0	1	0	1	1	1	0
L. pentosus									
LbS40	1	0	1	2	1	2	1	1	1
LbMT9	0	0	0	1	0	1	0	0	1
LbMT10	0	0	0	1	0	1	1	1	0
L. rhamnosus									
LbMF24	1	0	0	2	0	2	1	0	0
LbMF25	2	1	0	2	1	2	0	1	0
L. fermentum									
LbMA47	2	1	1	2	1	1	1	2	0

CONCLUSION

In conclusion, from the 19 strains of lactobacilli investigated in this study isolated from Algerian goat's milk, the strains LbMS16, LbMS21 (*L. plantarum*) and LbMF25 (*L. rhamnosus*) showing important properties such as proteolytic activity, sensory and safety aspect could be good candidates for application as adjunct culture and probiotic in dairy products in Algeria. Further studies would be required to determine how the selected strains should be evaluated in combination and with the starter strains when used as adjunct culture and probiotic culture and also to investigate their behaviour in the manufacture of artisanal cheese, or fermented milk.

REFERENCES

Adams MR, Marteau P. Safety of industrial lactic acid bacteria. *J Biotechnol*. 1999; 68(2-3):171-178.

Ammor MS, Flóre AB, Mayo B. Antibiotic resistance in non-enterococcal lactic acid bacteria and *Bifidobacterium*. *Food Microbiol*. 2007; 24(6): 559-570.

Arena ME, Manca de Nadra MC Biogenic amine production by *Lactobacillus*. *J Appl Microbiol*. 2001; 90 (6): 158-162.

Axelsson L. Lactic acid bacteria: classification and physiology. In: Salminen, S., and Von Wright, A. (eds.), Lactic Acid Bacteria: Microbiology and Functional Aspects, 2nd ed. Marcel Dekker Inc, New York; 1998. p.1-72.

Ayad EHE, Nashat S, El-Sadek N, Metwaly H, El-Soda M. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. *Food Microbiol*. 2004; 21(6): 715-725.

Badis A, Guetarni D, Moussa-Boudjemaa B, Henni DE, Tornadijo ME, Kihal M. Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiol*. 2004a; 21(3): 343-349.

Badis A, Guetarni D, Moussa-Boudjemaa B, Henni DE, Tornadijo ME, Kihal M. Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiol*. 2004b; 21(5): 579-588.

Baka AM, Papavergou EJ, Pragalaki T, Bloukas JG, Kotzekidou P. Effect of selected autochthonous starter cultures on processing and quality characteristics of Greek fermented sausages. *LWT - Food Sci Technol*. 2011; 44 (1): 54-61.

Bertazzoni M E, Benini A, Marzotto M, Sbarbati A, Ruzzenente O, Ferrario R, Hendriks H, Dellaglio F. Assessment of novel Probiotic *Lactobacillus casei* strains for the production of functional dairy foods. *Int Dairy J*. 2004; (14): 723-736.

Beshkova DM, Simova ED, Frengova GI, Simov Z I, Dimitrov Zh P. Production of volatile aroma compounds by kefir starter cultures. *Int Dairy J*. 2003; 13(7): 529-535.

Bodmer S, Imark C, Kneubühl M. Biogenic amines in foods: Histamine and food processing. *Inflamm Res.* 1999; 48(6): 296-300.

Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, et al. Safety of probiotics that contain lactobacilli or bifidobacteria. *Clin Infect Dis*. 2003; 36 (6): 775-780.

Bover-Cid S, Holzapfel WH. Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int J Food Microbiol*. 1999; 53 (1): 33-41.

Brizuela MA, Serrano P, Pérez Y. Studies on Probiotics Properties of Two *Lactobacillus* Strains. *Braz Arch Biol Technol*. 2001; 44(1): 95-99.

Bude-Ugarte M, Guglielmotti D, Giraffa G, Reinheimer JA, Hynes E. Nonstarter lactobacilli from Argentinean cheeses. *J Food Prot*. 2006; (69): 2983-2991.

Buffa M, Morais J, Jiménez-Belenguer A, Hernández-Giménez, E, Guamis B. Technological characterization of lactic acid bacteria isolated from raw ewes' milk for cheese making. *Milchwissenschaft*. 2005; 61(4): 404-407.

Bujňáková D, Kmeť V Functional properties of *Lactobacillus* strains isolated from dairy products. *Folia Microbiol*. 2012; 57: 263-267.

Charteris WP, Kelly PM, Morelli L, Collins JK. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *J Food Prot.* 1998; 61: 1636-1643.

Coppola R, Succi M, Tremonte P, Reale A, Salzano G, Sorrentino E. Antibiotic suscebtibility of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *Lait*. 2005; 85 (3): 193-204.

Danielsen M, Wind A. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *Int J Food Microbiol*. 2003; 82 (1): 1-11.

Donohue DC, Salminen S. Safety of probiotic bacteria. *Asia Pac J Clin Nutr*. 1996; 5(1): 25-28.

Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, et al. *In vitro* selection criteria for probiotic bacteria of human origin: Correlation with *in vivo* findings. *Am J Clin Nutr*. 2001; 73(2 Suppl):386S-392S.

Durlu-Ozkaya F, Xanthopoulos V, Tunaï N, Litopoulou-Tzanetaki E. Technologically important properties of lactic acid bacteria isolated from Beyaz cheese made from raw ewes' milk. *J Appl Microbiol*. 2001; 91(5): 861-870.

Elisha B. G, Courvalin P. Analysis of genes encoding D-alanine:D-alanine ligase-related enzymes in *Leuconostoc mesenteroides* and *Lactobacillus* spp. *Gene*.1995; 152: 79-83

Essid I, Medini M, Hassouna M. Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. *Meat Sci*. 2009; 81: 203-208.

European Food Safety Authority (EFSA). Scientific opinion of the panel on biological hazards on a request from the European food safety authority on food borne antimicrobial resistance as a biological hazard. *EFSA J* 2008; 765: 1-87

FAO/WHO. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Cordoba, Argentina: Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. 2001; 1-34.

FAO/WHO. Guidelines for the evaluation of probiotics in food. London, Ontario:Food and Agriculture Organization of the United Nations and World Health OrganizationWorking Group Report 2002; 1-11.

Fox PF, Wallace JM. Formation of flavour compounds in cheese. *Adv Appl Microbiol*. 1997; 45: 17-85.

Franciosi E, Settanni L, Cavazza A, Poznanski E. Biodiversity and technological potential of wild lactic acid bacteria from raw cows' milk. *Int Dairy J*. 2009; 19(1): 3-11.

Garabal JI, Rodriguez-Alonso P, Centeno JA. Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). *LWT -Food Sci Technol*. 2008; 41(8): 1452-1458.

Gatti M, Contarini G, Goutarini G, Neviani E. Effectiveness of chemometric techniques in discrimination of *Lactobacillus helveticus* biotypes from natural dairy starter cultures on the basis of phenotypic characteristics. *Appl Environ Microbiol*. 1999; 65(4): 1450-1454.

Gohran M. *Lactobacillus* with the ability of colonization and establishing in the intestinal tracts, assignee. *Japan Patent*. 1994; No. 6-501624.

Harber P, Kaochoy Saechao K, Boomus C. Diacetyl-Induced Lung Disease. *Toxicol Rev*. 2006; 25 (4): 261-272.

Heap HA. Optimising starter culture performance in NZ cheese plants. *Aust J Dairy Technol*. 1998; 53:74-78.

Katla AK, Kruse H, Johnsen G, Herikstad H. Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *Int J Food Microbiol*. 2001; 67 (1-2): 147-152.

King N. Modification of Voges–Proskauer test for rapid colorimetric determination of acetyl methyl carbimol plus diacetyl in butter. *Dairy Ind*. 1948; 13: 860-866.

Klare I, Konstabel C, Muller-Bertling S, Reissbrodt R, Huys G, Vancanneyt M, et al. Evaluation of New Broth Media for Microdilution Antibiotic Susceptibility Testing of Lactobacilli, Pediococci, Lactococci, and Bifidobacteri. *Appl Environ Microbiol*. 2005; 71(12):8982-8986.

Korhonen, JM, Danielsen M, Mayo B, Egervärn M, Axelsson L, Huys G, et al. Antimicrobial susceptibility and proposed microbiological cut-off values of lactobacilli by phenotypic determination. *Int J Probiotics Prebiotics.* 2008; 3(4): 257-268.

Linaje R, Coloma MD, Pérez-Martinez G, Zúñiga M. Characterization of faecal enterococci from rabbits for the selection of probiotic strains. *J Appl Microbiol*. 2004; 96: 761-771.

Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E. Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int Dairy J*. 2006; 16 (3): 189-199.

Marroki A, Zúñiga M, Kihal M, Pérez-Martínez G. Characterization of *Lactobacillus* from Algerian goat's milk based on phenotypic, 16S rDNA sequencing and their technological properties. *Braz J Microbiol*. 2011; 42(1):158-171.

Mathara JM, Schillinger U, Guigas C, Franz C, Kutima P M, Samuel K, et al. Functional characteristics of *Lactobacillus* spp. from traditional Maasai fermented milk products in Kenya. *Int J Food Microbiol.* 2008; 126: 57-64.

Mathur S, Singh R. Antibiotic resistance in food lactic acid bacteria-a review. *Int J Food Microbiol.* 2005; 105(3): 281-295.

Mattila-Sandholm T, Matto J, Saarela M. Lactic acid bacteria with health claims-interactions and interference with gastrointestinal flora. *Int Dairy J*. 1999 ; 9: 25-35.

McSweeney PLH, Fox PF, Lucey JA, Jordan KN, Cogan TM. Contribution of the indigenous microflora to the maturation of cheddar cheese. *Int Dairy J*. 1993; 3: 613-634.

Nieto-Arribas P, Poveda JM, Seseña S, Palop LI, Cabezas L. Technological characterization of *Lactobacillus* isolates from traditional manchego cheese for potential use as adjunct starter cultures. *Food Control*. 2009; 20: 1092-1098.

Nieto-Arribas P, Seseña S, Poveda JM, Palop L, Cabezas L. Genotypic and technological characterization of *Leuconostoc* isolates to be used as adjunct starters in Manchego cheese manufacture. *Food Microbiol*. 2010; 27 (1): 85-93.

Olasupo NA, Shillinger U, Holzapfel WH. Studies on some technological properties of predominant lactic acid bacteria isolated from Nigerian fermented food. *Food Biotechnol*. 2001; 15 (3): 157-167.

Roberts MC. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett*. 2005; 245(2): 195-203.

Rodgers S. Novel applications of live bacteria in food services: probiotics and protective cultures. *Trends Food Sci. Technol*. 2008; 19(4): 188-197.

Saarela M, Mogensen G, Fondèn R, Matto J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. *J Biotechnol*. 2000; 84 (3): 197-215.

Salminen S, von Wright A, Laine M, Vuopio-Varkila J, Korhonen T, Mattila-Sandholm T. Development of selection criteria for probiotic strains to assess their potential in functional foods: a Nordic and European approach. *Biosci Microflora*. 1996; 15: 61-67.

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, et al. Demonstration of safety of probiotics - a review. *Int J Food Microbiol*. 1998; 44(1-2): 93-106.

Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. *Curr Opin Biotechnol*. 2005; 16 (2): 204-211.

Seseña S, Sánchez I, Palop L. Characterization of *Lactobacillus* strains and monitoring by RAPD-PCR in controlled fermentations of "Almagro" eggplants. *Int J Food Microbiol*. 2005; 104(3): 325-335.

Shalaby AR. Significance of biogenic amines to food safety and human health. *Food Res Int*. 1996; 29 (7): 675-690.

Skeie S, Kieronczyk A, Næss RM, Østlie H. *Lactobacillus* adjuncts in cheese: Their influence of the degradation of citrate and serine during ripening of a washed curd cheese. *Int Dairy J*. 2008a; 18(2): 158-168.

Skeie S, Kieronczyk1 A, Eidet S, Reitan M, Olsen K, Østlie H. Interaction between starter bacteria and adjunct *Lactobacillus plantarum* INF15D on the degradation of citrate, asparagine and aspartate in a washed-curd cheese. *Int Dairy J*. 2008b; 18 (2): 169-177.

Tamime AY, Robinson RK. Yogurt Science and Technology, 2nd ed. Woodhead Publishing Ltd., Cambridge. 1999.

Voravuthikunchai SP, Bilasoi S, Supamala O. Antagonistic activity against pathogenic bacteria by human vaginal lactobacilli. *Anaerobe*. 2006; 12 (5-6): 221-226.

Williams AG, Banks JM. Proteolytic and other hydrolytic enzyme activities in non starter lactic acid bacteria (NSLAB) isolated from Cheddar cheese manufactured in the United Kingdom. *Int Dairy J*. 1997; 7(12): 763-774.

Zavaglia AG, Kociubinsky G, Pérez P, de Antoni G. Isolation and characterization of *Bifidobacterium* strains for probiotic formulation. *J Food Prot*. 1998; 61:865-873.

Zhou JS, Pillidge CJ, Gopal PK, Gill HS. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *Int J Food Microbiol*. 2005; 98(2): 211-217.

Received: March 20, 2013;
Accepted: September 20, 2013.