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# Screening of technological and probiotic properties of lactic acid bacteria isolated from Algerian traditional fermented milk products

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**Abstract**

A total of 208 strains of lactic acid bacteria (LAB) were isolated from Algerian traditional fermented milk (Jben, Klila, Raib and Lben), in order to evaluate their technological and probiotic properties. These strains were tested for their antimicrobial activity against *Listeria monocytogenes* using agar well diffusion technique. Only 11 strains were retained, they were also characterised in respect to their technological properties. The retained strains were identified by analyses of the 16S rDNA gene as; *Lb. curvatus* (1 strain), *Ln. mesenteroides* subsp *mesenteroides* (1 strain), *Lb. plantarum* (3 strains), *Lb. brevis* (2 strain), *Lactobacillus acidophilus* (2 strains), and *Lactococcus lactis* subsp *lactis* (2 strains). All the antimicrobial compounds produced by the selected lactic acid bacteria were fully or partially inactivated by the proteolytic enzymes. The compounds were heat stable up to 100°C for 20 min, and were active from pH 3.0 to 10.0. The enzymatic potential of the strains evaluated with API ZYM system show that all strains exhibited high leucine, cystine aminopeptidase and β-Galactosidase activities. *Lactococcus* strains were those that showed the greatest degree of proteolytic activity, however very low lipolytic and amylolytic capacity was detected for same strains. Two out of the 11 selected strains showed moderate survival rates under simulated gastric, however all tested strains showed a high stability under intestinal conditions. According to the results, the selected LAB strains could be used as potential probiotics to a possible inhibition of food-borne pathogenic bacteria and also would be of considerable interest to use as starter culture.



## Introduction

Fermented milk is a dairy product provides the human diet with nutritious compounds of varied flavours, aromas, and textures. These which product may have been made from milk with or without any modification of their composition, via the action of appropriate microorganisms and which result in a lowering of the pH with or without coagulation (Borresen *et al*., 2012). Several types of fermented milk products have been reported to exist throughout the world (Stanley, 1998; Tamime, 2002). The most popular of them in North African are Jben, Lben, Klila and Raib, (Benkerroum and Tamime, 2004; Benkerroum, 2013).

Jben a soft variety cheese is made in the mountainous area of eastern Algeria (souk ahras, Guelma, Tebessa, Khanchla and Batna) of cows' milk, with sometime additions of goats' and sheep's milk from January to March, although some manufacturers who provide a wider market use rennet to coagulate the milk just a few hours after milking. Traditional protocol includes rennet coagulation of raw whole cow's milk; to which a salt was added in the proportion of 10-20 Nacl per litre of milk at room temperature for 2 to 15 d (Benkerroum and Tamime, 2004). Klila a hard variety cheese is made from raw milk by heating whey of curd at 65°C for 30 minutes without using a starter culture of lactic acid bacteria. Then the obtained curd is sieving through a muslin doth or straw basket to discard whey (Benkerroum, 2013).

Raib and its by-products (Lben and zebda) are traditional fermented dairy products, still widely produced and consumed in Maghreb countries (Morocco, Tunisia, and Algeria) (Benkerroum and Tamime, 2004; Koussou *et al.*, 2007). This traditional fermented dairy product is obtained after spontaneous curdling of raw milk within 24 to 36 h at ambient temperature; however Lben (buttermilk) is obtained by churning spontaneously soured milk to remove butter.

Fermented milk production is based on the metabolic activity of LAB to ferment sugars, especially glucose and galactose, so to produce lactic acid and aroma substances that give typical flavours and tastes to fermented products (Jay, 2000; Marshall, 2005). LAB also release antimicrobial metabolites so

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called bacteriocins, which are considered safe and natural preservatives, with great potential to be used on their own, or synergistically with other methods in food preservation. Over the past two decades, a large number of LAB, isolated from various fermented food systems in different parts of the world have been studied for their probiotic potential and ability to produce industrially important substances (Baruzzi *et al*., 2011; Senaka *et al*., 2012) .

The aim of the present study was to characterize autochthonous lactic acid bacteria isolated from fermented milk products and to study their technological and probiotic properties in order to select those used as lactic acid starter in the manufacturing of fermented dairy products and which are suitable to local conditions.

## Materials and Methods

### *Isolation of LAB and culture conditions*

A total of 208 LAB strains were obtained from Algerian traditional fermented milks samples (Raib, Lben, Jben and Klila) collected from rural areas in eastern Algeria (table 1). Phenotypic characterization of LAB was done according to the procedures described by Hammes and Vogel (1995), Schillinger and Lücke (1987), and Stiles and Holzapfel (1997). The carbohydrate fermentation profiles of the selected isolates were investigated using API 50 CH strips and API CHL medium according to manufacturer's instructions (API system, Bio-Merieux, France).

### *Molecular characterization*

Those LAB isolates sharing some of the screened properties were further identified by molecular biology. Total genomic DNA was extracted from overnight culture of bacteria isolates using Bacterial Genomic DNA extraction kit (SpinKlean Genomic DNA Extraction Kit, Canada) (VanHoorde *et al*., 2008). Polymerase chain reaction (PCR) amplification of 16S rDNA was carried out using Taq PCR Master Mix Kit (Biomatik, Canada). An approximately 1500-bp fragment of the 16S rDNA was amplified by using the following universal primers 1492R (Bacteria/Archaea-specific) 5'-GGTTACCTTGTTACGACTT-3' and 27F (Bacteria-specific) 5'-AGAGTTTGATCCTGGCTCAG-3'.

### *Screening for antibacterial activity*

Isolated colonies of the assumed LAB were screened for antimicrobial-producing activity essentially using the spot method as described by Spelhaug and Harlander (1989). The spectrum of activity against different bacteria (Table 2) was determined by the well-diffusion assay (schillinger and Lúcke, 1989) and disk diffusion assay (Tagg and McGiven, 1971).

### *Characterization of antimicrobial substance*

Cell-free supernatants (CFS) from the lactic acid cultures were collected by centrifugation (7500 g, 10 min, 4°C) of overnight MRS broth cultures. The supernatant fluids were adjusted to pH 6.5 and exposed to heat treatments of 65°C for 40 min, 95°C for 20 min, 100°C for 20 min, and 121°C for 20 min, and then were tested for remaining antimicrobial activity. In order to determine the effect of pH on Semi-purified preparations of the bacteriocin were adjusted to various pH values in the range of 2 to 12. The pH-adjusted bacteriocin samples were incubated at 37°C for 20 min and then neutralized to pH 6 and tested for bacteriocin activity. The following enzymes were tested for their hydrolytic activity on the antimicrobial compounds contained in the supernatants: proteinase K (2.6 U $mg^{-1}$), pronase E (22 U $mg^{-1}$), α-Chymotrypsin (16U $mg^{-1}$), catalase (adjusted to a final activity of 2600 U $mg^{-1}$), lipase (50 U $mg^{-1}$), and α-amylase (15 U $mg^{-1}$).

### *Technological and probiotics properties*

#### *Assessment of proteolytic activity*

Proteolytic activity was tested using Plat Count Agar PCA with 2% (w/v) skim milk. The presence of clear zones around the colonies was recorder as positive activity. All strain with positive reaction in MRS with 1% skimed milk was considered as strains with slight activity. Extracellular quantitative proteolytic activity was determined by the o-phthaldialdehyde (OPA) method (Church *et al.*, 1983). The results were calculated from a calibration curve obtained from the dilution of glycine in distilled water and were expressed in mM glycine $L^{-1}$ of milk.

#### *Assessment of amylolytic activity*

Surface-dried plates of starch agar (Gordon *et al*., 1973) were streaked with 24 h-old cultures, incubated at 30°C for 48 h. After incubation, the plates were flooded with iodine solution for 15-30 min and examined the clear zone underneath (after the growth was scrapped off) for amylolytic activity.

#### *Assessment of Lipolytic activity*

Tested strains were grown overnight at 37°C in MRS broth. A loopful fresh culture was placed on Tributyrin Agar (Leuschner *et al*., 1997). Plates were incubated at 37°C for 4 days and observed daily for halo formation around the colonies. The radius of the halo formation (in mm) at the end of

incubation was measured

*Enzymatic profile by API-zym system*

Enzymatic activities of selected LAB strains were assayed using the API- ZYM (bioMérieux, France) galleries as described by the manufacturer. The enzymatic activity was graded from 1 to 5 according to the colour reaction chart. The approximate number of free nmol hydrolyzed substrate may be obtained from the colour intensity, 0: no activity; 1: liberation of 5 nmol; 2:10 nmol; 3: 20 nmol; 4:30 nmol and 5: ≥40 nmol (Papamaloni *et al*., 2003).

*Survival under simulated gastro-intestinal tract condition*

The survival under simulated gastro-intestinal tract condition of LAB was performed according to the method of Charteris *et al.* (1998). Simulated gastric and pancreatic juices were prepared by suspending pepsin (3 mg $ml^{-1}$; Sigma) and pancreatin USP (1 mg $ml^{-1}$; Sigma) in sterile sodium chloride solution (0.5%, w/v), which was then adjusted with hydrochloric acid (3.0 M) and NaOH (1 M) to pH 2.5 and 8.0, respectively. Total viable counts (CFU $ml^{-1}$) were evaluated after incubation for 180 min in cultures tested for gastric transit tolerance, and for 240 min in cultures tested for small intestinal transit tolerance.

## Results and Discussion

In the present 208 LAB were isolated from sixteen samples of fermented skimmed milk (Raib and Lben) and twenty-two samples of Traditional cheeses (Jben and Klila) collected from local markets and individual households of rural areas in eastern Algeria. Direct antagonism tests carried out on solid medium revealed that 52 of isolates produced antimicrobial substances that were active against the indicator strain *L. monocytogenese* ATCC7644. from which, 11 bacteriocinogenic strains were identified and selected for further study.

Generally, the bacteriocins from the selected LAB were shown to be ineffective against Gram negative bacteria. The partially purified bacteriocin preparations from the strains (JBB76 and JBB65) showed broad antimicrobial activity including against Gram-negative *Pseudomonas* and *E. coli* strains (Table 2). Messi *et al.* (2001) reported the inhibitory action of bacteriocin of *L. plantarum* against Gram negative strains. The bacteriocins produced from *L. plantarum* have been found to be inhibitory towards closely related LAB, particularly the mesophilic and thermophilic lactobacilli (Aymeriche *et al*., 2000). Of all the

Table 1. Grouping of LAB strains isolated from Algerian fermented milk products

| Species | Fermented milk product | | | | Grouped strain | Representative strains |
|---|---|---|---|---|---|---|
| | Jben (12) | Klila (10) | Raib (10) | Lben (6) | | |
| *Lactobacillus plantarum* | 15 | 2 | 8 | 7 | 32 | JBB71, JBB75, JBB76 |
| *Lactobacillus paracasei* | 9 | 0 | 12 | 3 | 24 | |
| *Lactobacillus rhamnosus* | 0 | 0 | 6 | 6 | 12 | |
| *Lactobacillus brevis* | 8 | 5 | 3 | 9 | 25 | JBB02, JBB07 |
| *Lactobacillus curvatus* | 8 | 2 | 10 | 2 | 22 | JBB65 |
| *Lactobacillus acidophilus* | 4 | 2 | 4 | 10 | 20 | JBB102, JBB75 |
| *Enterococcus faecium* | 8 | 0 | 3 | 1 | 12 | |
| *Lactococcus lactis* subsp. *lactis* | 22 | 5 | 12 | 3 | 42 | JBL10, JBL15 |
| *Leuconostoc mesenteroides* subsp. *cremoris* | 2 | 1 | 4 | 2 | 05 | JBLC125 |
| *Leuconostoc mesenteroides* subsp. *dextranicum* | 0 | 0 | 3 | 0 | 08 | |
| *Pediococcus acidilactici* | 2 | 1 | 3 | 0 | 06 | |

Table 2. Inhibitory spectrum of the pH neutralized cell-free supernatants of the LAB strains isolated from fermented milk products, as determined with the well-diffusion assay

| Isolated strains | | Indicator strains | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Gram positive | | | | Gram negative | | |
| | | *Bacillus cereus* ATCC 14578 | *Bacillus subtilis* ATCC8 | *Staphylococcus aureus* ATCC 25293 | *Listeria monocytogenes* ATCC 7644 | *Ent. faecalis* ATCC 19433 | *Escherichia coli* ATCC 25422 | *Pseudomonas aeruginosa* ATCC 27853 |
| *Lactococcus lactis subsp lactis* | JBL10 | +++ | +++ | +++ | +++ | ++ | - | - |
| | JBL15 | +++ | +++ | - | +++ | - | - | - |
| *Lactobacillus brevis* | JBB02 | ++ | ++ | + | + | - | - | - |
| | JBB07 | ++ | ++ | +++ | +++ | ++ | - | - |
| *Lactobacillus curvatus* | JBB65 | +++ | +++ | +++ | +++ | ++ | ++ | ++ |
| *Lactobacillus plantarum* | JBB71 | + | + | - | ++ | ++ | - | - |
| | JBB75 | +++ | +++ | +++ | +++ | ++ | - | - |
| | JBB76 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| *Lactobacillus acidophillus* | JBB102 | ++ | + | +++ | +++ | ++ | - | - |
| | JBB110 | ++ | + | +++ | +++ | +++ | - | - |
| *Ln. mesenteroides* subsp *mesenteroides* | JBLC125 | - | + | + | ++ | - | - | - |

- = no inhibition zone, + = inhibition zone up to 5 mm, ++ = inhibition zone up to 10 mm; +++ = inhibition zone over 12 mm.

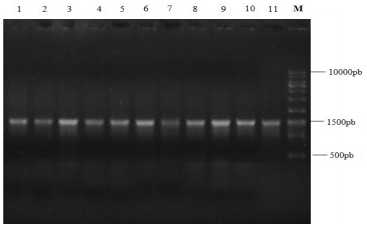


Figure 1. Electrophoretical pattern of 16S rDNA amplicons (1500 bp) obtained on genomic DNA from selected isolates: Lane M, 1kb ladder (10000-500 bp); 1: *Lactococcus lactis* subsp *lactis* (JBL10). 2: *Lactococcus lactis* subsp *lactis* (JBL15). 3: *Lactobacillus brevis* (JBB02). 4: *Lactobacillus brevis* (JBB07). 5: *Lactobacillus curvatus* (JBB65). 6: *Lactobacillus plantarum* (JBB71). 7: *Lactobacillus plantarum* (JBB75). 8: *Lactobacillus plantarum* (JBB76). 9: *Lactobacillus acidophillus* (JBB102). 10: *Lactobacillus acidophillus* (JBB110). 11: *Ln. mesenteroides* subsp *mesenteroides* (JBLC125).

indicator strains tested, *L. monocytogenes* and *S. aureus*, were the most sensitive, being nhibited by all 11 strains. However the cultures that produced "high" inhibition zones against *L. monocytogenes* were: JBL10, JBL15, JBB07, JBB65, JBB75, JBB76, JBB102 and JBB110. Therefore, the high sensitivity of the *Listeria* strain to the bacteriocins produced by our isolates is not surprising, since a wide sensitivity of *L. monocytogenes* strains to bacteriocins has been reported (Ennahar and Deschamps, 2000; Achemchem *et al.*, 2006). Our results has also revealed that the inhibitory compounds produced by the 11 isolates demonstrated a high résistance to heat treatments ranging in temperature from 30 to 121°C (Table 3). In the other hand the bacteriocins were shown to be stable over a broad pH range with all peptides maintaining some antimicrobial activity within the pH range of pH

Table 3. Effect of heat treatment, pH and proteolytic enzymes on the antimicrobial compounds produced in the supernatant by selected lactic acid bacteria isolated from Algerian fermented milk products ab

| Treatments | *Lactococcus lactis* subsp *lactis* | | *Lactobacillus brevis* | | *Lactobacillus curvatus* | *Lactobacillus plantarum* | | | *Lactobacillus acidophillus* | | *Ln. mesenteroides* subsp *mesenteroides* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | JBL10 | JBL15 | JBB02 | JBB07 | JBB65 | JBB71 | JBB75 | JBB76 | JBB102 | JBB110 | JBLC125 |
| enzymes | | | | | | | | | | | |
| Pronase E | - | - | - | - | - | - | - | - | - | - | - |
| Proteinase K | - | - | - | - | - | - | - | - | - | - | - |
| α-Chymotrypsin | - | - | - | - | - | - | - | - | - | - | - |
| Lipase | + | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Catalase | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| pH | | | | | | | | | | | |
| 2 | + | + | + | + | - | - | + | - | + | - | - |
| 3 | ++ | ++ | +++ | +++ | +++ | ++ | ++ | +++ | +++ | ++ | ++ |
| 5 | +++ | +++ | +++ | +++ | ++ | ++ | +++ | +++ | +++ | +++ | +++ |
| 7 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| 9 | ++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ |
| 11 | + | - | - | - | +++ | - | - | +++ | - | - | - |
| 12 | - | - | - | - | ++ | - | - | + | - | - | - |
| Heat Treatment | | | | | | | | | | | |
| 65° C/40 min | ++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ |
| 95° C/20 min | ++ | ++ | +++ | +++ | +++ | +++ | ++ | +++ | ++ | ++ | ++ |
| 100° C/20 min | + | + | - | - | +++ | +++ | + | +++ | + | + | + |
| 121° C/20 min | - | - | - | - | +++ | - | - | - | - | - | - |

[a]: All assays were conducted with *Listeria monocytogenes* ATCC 7644 as indicator strain.
[b]: - = no inhibition zone, + = inhibition zone up to 5 mm, ++ = inhibition zone up to 10 mm; +++ = inhibition zone over 12 mm.

Table 4. Enzymatic activitya detected using API-ZYM system, of selected LAB isolated from Algerian fermented milk products

| Isolated strains | | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lactococcus lactis subsp lactis* | JBL10 | 0 | 1 | 0 | 3 | 1 | 4 | 5 | 1 | 2 | 1 | 1 | 2 | 4 | 5 | 0 |
| | JBL15 | 0 | 1 | 0 | 3 | 1 | 4 | 3 | 1 | 2 | 3 | 2 | 3 | 0 | 3 | 0 |
| *Lactobacillus brevis* | JBB02 | 0 | 3 | 0 | 5 | 1 | 5 | 2 | 1 | 4 | 3 | 3 | 4 | 4 | 4 | 0 |
| | JBB41 | 0 | 3 | 0 | 4 | 2 | 4 | 2 | 2 | 5 | 5 | 3 | 3 | 0 | 3 | 0 |
| *Lactobacillus curvatus* | JBB65 | 0 | 2 | 0 | 3 | 4 | 5 | 2 | 2 | 4 | 5 | 2 | 3 | 1 | 3 | 0 |
| *Lactobacillus plantarum* | JBB71 | 0 | 2 | 0 | 5 | 4 | 4 | 2 | 1 | 4 | 5 | 2 | 3 | 0 | 3 | 0 |
| | JBB75 | 0 | 1 | 0 | 5 | 4 | 5 | 2 | 1 | 4 | 4 | 2 | 4 | 0 | 3 | 0 |
| | JBB76 | 0 | 1 | 0 | 5 | 4 | 5 | 5 | 1 | 4 | 3 | 2 | 4 | 0 | 3 | 0 |
| *Lactobacillus asidophillus* | JBB102 | 0 | 2 | 1 | 4 | 1 | 4 | 4 | 2 | 3 | 4 | 2 | 4 | 1 | 3 | 0 |
| | JBB110 | 0 | 1 | 0 | 3 | 1 | 4 | 4 | 2 | 4 | 4 | 2 | 3 | 1 | 3 | 0 |
| *Ln. mesenteroides* subsp *mesenteroides* | JBLC125 | 0 | 2 | 0 | 4 | 1 | 4 | 3 | 1 | 5 | 1 | 1 | 3 | 4 | 4 | 0 |

01: Alcaline phosphatase ; 02: Esterase ; 03: Esterase and lipase ; 04: Leucine arylamidase ; 05: Valine arylamidase ; 06: Cystine arylamidase ; 07: Acid phosphatase ; 08: Naphtol phosphohydrolase ; 09: α-Galactosidase ;10: β-Galactosidase; 11: β-Glucuronidase; 12: α- Glucosidase; 13: β-Glucosidase; 14: N-acetyl-β-glucosaminidase; 15: α-Mannosidase

Table 5. Enzymatic activity of selected LAB isolated from Algerian fermented milk products

| Isolated strains | | Protease activity[a] | Lipolytic activity[b] | α-amylase[b] |
|---|---|---|---|---|
| *Lactococcus lactis* subsp *lactis* | JBL10 | 3.13±0.02 | -/+ | -/+ |
| | JBL15 | 3.95±0.12 | - | -/+ |
| *Lactobacillus curvatus* | JBB65 | 2.60±010 | - | -/+ |
| *Lactobacillus brevis* | JBB02 | 2.10±0.00 | - | - |
| | JBB07 | 2.88±0.04 | + | - |
| *Lactobacillus plantarum* | JBB71 | 1.98±0.05 | + | - |
| | JBB75 | 2.01±0.02 | - | -/+ |
| | JBB76 | 2.52±0.01 | - | -/+ |
| *Lactobacillus acidophillus* | JBB102 | 2.14±0.04 | - | - |
| | JBB110 | 2.21±0.02 | - | -/+ |
| *Ln. mesenteroides* subsp *mesenteroides* | JBLC125 | 1.12±0.00 | - | - |

[a]: Proteolytic activity measured using the o-phthaldialdehyde (OPA) spectrophotometric assay and expressed as mM Gly $L^{-1}$ of milk, after a 24 h period of incubation of the strains in the milk
[b]: Strains showing positive hydrolysis test (>2.0 mm) were assayed.

3 to 10.

According to Tagg *et al.* (1976) bacteriocins differ greatly with respect to sensitivity to pH. Many of them are considerably more tolerant of acid than alkaline pH values. In the present study bacteriocin produced by the strain *Lactobacillus curvatus* (JBB65 ) exhibited the same profile and was active at pH values between 4 - 9. Maximum inhibitory activity was demonstrated at pH 4 and 5. Similar properties have been reported for other bacteriocins including lactacin, lactacin 27, acidolin, pediocin A, and pediocin PA-1 (Hastings *et al*., 1996). These bacteriocins were also stable over a wide range of pH. Piard and Desmazeaud (1992) reported that temperature stability is very convenient if the bacteriocin is to be used in food preservative, because many processing procedures involve a heating step, and cold is one of the most popular preservation procedures. Furthermore, activity at neutral pH constitutes an advantage over other bacteriocins used as food preservatives and particularly over nisine, whose maximal solubility and stability are at pH 2, with these parameters decreasing significantly as the pH increases.

Regarding proteolytic activity, the tow strains of the genus *Lactococcus* presented the greater proteolytic activity. These results agree with those obtained by Pérez *et al.* (2003) and Herreros *et al.* (2003) in studies about the technological characterization of lactic acid bacteria isolated from craft cheeses. Tributyrin, and soluble starch were used for detecting, lipid and starch digestive capabilities, respectively. Neither starch nor lipid digestions were detected. The absence of amylolytic activity indicating that selected LAB they have no role in saccharification and liquefaction of starchy substrates. It has been reported that Information on the contribution of LAB to the lipolysis in fermented milk products is rather scarce and are generally considered to be weakly lipolytic, as compared with other groups of microorganisms (Fox *et al*., 2004). On the basis of the results of lipolytic activity and in agreement with our results, Papamanoli *et al.* (2003) reported that *Lactobacillus* species are weakly lipolytic.

Concerning the activities of the enzymes correlated with carbohydrate catabolism, as shown in Table 5, α-galactosidase exhibited by 10/11 of tested strains, whereas *Lactobacillus* strains exhibited a high β-galactosidase activity, while relatively low β-galactosidase activity was observed among lactococci. β-glucosidase and N-acetyl-β-glucosaminidase activities were exhibited by strains (JBL10, JBB02, JBLC125), whereas very weak β-glucuronidase activity was displayed by only *Lactobacillus brevis* (JBB02, JBB41).

Presence of strong β-galactosidase suggests that fermented products derived from the collected samples may be suitable for lactose-intolerant patients. The lower activity of esterase-lipase among the tested strains indicates that these isolates are not important to reduce bitterness, develop typical flavor, body, and taste during fermentation process. All selected strain had high leucin and cystine arylamidase. Further, *L. curvatus* and *L. plantarum* strains possess valine

Table 6. Effect of simulated gastric and intestinal juices on viability of the lactic acid bacteria isolated from Algerian fermented milk (log CFU $ml^{-1}$)

| Isolated strains | | Viable count in simulated gastric juice[a] | | Viable count in simulated intestinal juice[b] | |
|---|---|---|---|---|---|
| | | control | pH 2.5 (180 min) | control | pH 8.0 (240 min) |
| *Lactococcus lactis* subsp *lactis* | JBL10 | 9.12±0.02 | 7.14±0.03 | 9.07±0.02 | 8.87±0.01 |
| | JBL15 | 9.08±0.01 | 6.23±0.01 | 9.10±0.03 | 8.90±0.03 |
| *Lactobacillus brevis* | JBB02 | 8.85±0.06 | 5.06±0.04 | 8.91±0.05 | 8.65±0.04 |
| | JBB07 | 8.99±0.04 | 4.98±0.01 | 8.94±0.03 | 6.44±0.01 |
| *Lactobacillus curvatus* | JBB65 | 8.95±0.06 | 4.97±0.08 | 9.10±0.11 | 8.59±0.15 |
| *Lactobacillus plantarum* | JBB71 | 9.00±0.02 | 6.46±0.04 | 8.98±0.04 | 7.08±0.05 |
| | JBB75 | 8.91±0.02 | 6.94±0.03 | 8.88±0.03 | 8.28±0.04 |
| | JBB76 | 8.78±0.01 | 6.90±0.02 | 8.70±0.07 | 7.40±0.02 |
| *Lactobacillus acidophillus* | JBB102 | 8.97±0.01 | 5.17±0.05 | 8.91±0.01 | 8.65±0.05 |
| | JBB110 | 8.99±0.01 | 5.03±0.02 | 8.91±0.02 | 8.52±0.04 |
| *Ln. mesenteroides* subsp *mesenteroides* | JBLC125 | 8.65±0.02 | 4.99±0.01 | 8.71±0.04 | 8.66±0.01 |

[a]: Pepsin (3 mg $ml^{-1}$) and sodium chloride (5 mg $ml^{-1}$).
[b]: Pancreatin (1 mg $ml^{-1}$) and sodium chloride (5 mg $ml^{-1}$).

arylamidase activities. According to Ammor *et al.* (2005), lactic acid bacteria with these enzymes contribute to the catabolism of proteins and peptides generating free amino acids, precursors of flavour compounds in the final product.

With respect to the resistance to simulated gastric juice conditions (pH 2.5), the results of these study illustrated in table 6 shows a significant decrease in the population of *Lactobacillus curvatus* (JBB65), *Lactobacillus brevis* (JBB07) and *Ln. mesenteroides* subsp *mesenteroides* (JBLC125). However all *L. plantarum* strains and those of *Lactococcus lactis* subsp *lactis*, survived better after incubation for 180 min. These results agreed with those obtained by Madureira *et al.* (2005), who reported that the resistance of probiotic strains to passage through the gastrointestinal tract is strain-dependent and several parameters may determine the extent to which probiotic strains survive; passage through the upper gastrointestinal tract, the degree of stomach acidity and the period of exposure. Concerning the survival in the simulated pancreatic juice (pH 8.0), all selected strains survived well after incubation for 240 min. Most studies have shown that the majority of the strains survived well under such conditions, suggesting a potential recuperation of the initial levels during the passage of the small intestine However, the susceptibility or resistance of probiotic cultures to bile is species as well as strain specific (Millette *et al*., 2008).

## Conclusions

Over the past two decades, there is an increasing research on the traditional dairy products obtained from raw milk produced. Milk fermentation, like many traditional fermenting processes, is spontaneous and uncontrolled and could be a valuable source of autochthonous Lactic Acid Bacteria (LAB). In the present study several LAB isolated from Algerian fermented milk products were characterized and shown to exhibited favourable functional properties with industrial potentials, including enzymatic profiles, acidifying and coagulating ability, and antimicrobial activity. Based on their technological properties, they were considered as potential candidate lactic acid bacteria for use as starter culture in dairy milk fermented production.

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