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Identification and Characterization of Functional and Technological *Lactobacillus plantarum* Strains Isolated from Raw Goat and Camel Milk Collected in Algeria

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The role of lactic acid bacteria (LAB) in food preservation, prevention of poisoning, increased nutritional value and improved organoleptic quality of food, is indirectly related to food hungry from different continents and so the ensuring a sustainable development. This work identifies five strains *Lb. plantarum*, with an atypical growth at 45 °C. The results of technological test are satisfactory for industrial use. The strains are heat resistant, produce flavors and have a proteolytic activity. However, they do not produce dextran on MSE media. Strains of *Lb. plantarum* have a kinetics growth and acidification in MRS broth and skim milk at 30 °C, almost identical. But the strain *Lb. johnsonii* has a different kinetic growth and acidification. The best growth temperature for *Lb. plantarum* LbG22 strain was 37 °C. The strains *Lb. plantarum* LbC5 and LbC6 have the best antibacterial activity, they inhibited all tested strains. The *Lb. plantarum* LbG22 strain has the greatest inhibitory activity against the strains of *Staphylococcus aureus*, compared to other lactic acid strains studied. In mixed culture, growth of *S. aureus* ATCC 43300 was inhibited after 24h of culture with *Lb. plantarum* LbG22. Susceptibility to antibiotics was also evaluated with 33 antibiotics.

Key words: *Lactobacillus plantarum*, Growth kinetics, Antagonism, Antibiotics, Probiotics, Milk, Poultry animals.

In Algeria, the gene pool of domestic and wild fauna is rich and diverse. The breed goats, 70% in localized areas steppe is valued at more than 2 million head. Similarly, the camel (dromedary) has a flock of 100,000 head localized exclusively in the Saharan zone (Ghazi, 2006). The scientific exploitation of this wealth is the basis for sustainable development and assurance of future generations. Goat's milk plays a vital role in the lives of rural communities, whether in its raw or processed (Raïb, L'ben "local traditional fermented milk" and J'ben "traditional local cottage cheese"). In these products, spontaneous fermentation is obtained by natural lactic flora (Moulay *et al*., 2006; Badis *et al*., 2004).

Lactobacilli, and specifically *Lactobacillus plantarum*, are an important group of micro-organisms in the microbial flora of raw goat and camel milk. Also they often occur spontaneously in large numbers in most fermented foods. Despite this, *Lactobacillus plantarum* is not regarded as lactic acid bacteria starter. However,

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in dairy lactic acid strains are selected on the basis of their technological properties (production of lactic acid, aroma production, proteolytic activity and growth kinetics), their functional characteristics (antibacterial activity, resistance gastrointestinal transit and resistance to antibiotics) and other factors to take into account (Tamime, 2002; Mayra-Makinen and Bigret, 2004; Molin, 2008).

In this context, the identification and characterization of functional and technological lactic strains (*Lactobacillus plantarum*) isolated from raw goat and camel milk, have been achieved in this study.

MATERIAL AND METHODS

Bacterial Strains and Growth Conditions:

From the collection of the Laboratory of Applied Microbiology, University of Oran, six strains pre-identified as the genus *Lactobacillus* were selected, three (LbG22, LbG68, LbG58) were isolated from raw goat's milk, and three others (LbC5, LbC6 and $LbCA_2$) from raw camel's milk.

Nine pathogenic strains were used in this study: three strains of *Staphylococcus aureus* (ATCC 25923, ATCC 29213 and ATCC 43300), *Listeria ivanovii* ATCC 19119, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus* and *Salmonella enterica*. Pathogenic strains were generously offered by the University Hospital of Oran, except that strains of *Listeria ivanovii* ATCC 19119 from the Department of Analytical Chemistry, Nutrition and Food Science, University of Santiago de Compostela (Spain). Lactic strains were grown on MRS medium, pathogenic bacteria in nutrient broth and agar.

Identification of lactic strains

Isolates Gram-positive, rod-shaped and catalase-negative thus obtained were characterized according to the methods and criteria of Carr *et al* (2002), Axelsson (2004) and Hammes and Hertel (2006). Growth at different temperatures (15°C and 45°C) was followed in MRS broth after incubation for 24 to 48 hours. Gas production from glucose and gluconate was determined in MRS broth containing inverted Durham, and hydrolysis of arginine was tested on M16BCP medium (Thomas, 1973). For the identification of *Lactobacillus* species, the API 50 CHL carbohydrate fermentation strips (bioMérieux, Inc., Marcy l'Etoile, France) were used.

Technological characterization

Citrate utilization, in the presence of carbohydrates, was studied on the media of Kempler and Mc Kay (1980). Heat resistance was tested at 60.5°C for 30 min (Samelis et al., 1994). Production of dextran was recorded on MSE Medium (Mayeux *et al*., 1962). Production of acetoin from glucose was determined by using the Voges–Proskauer test (Zourari *et al*., 1992). The ability to proteolysis of milk casein was also performed (Badis *et al.,* 2004).

Growth kinetics

The observation of the kinetics of growth and acidification in MRS medium (de Man *et al*., 1960) at 30 °C, was conducted following the observation points 0, 2, 4, 6, 8, 18, 24 and 48h, bacterial growth is followed by counting on solid medium and the variation of pH is followed by a pH meter., growth characteristics (growth rate, generation time) are calculated according to Hassan *et al.* (1989). The study of the kinetics of acidification and lactic acid production on skim milk at 30 °C was performed in skim milk is reconstituted to 10% enriched with 3 g/l extract yeast and sterilized at 110 °C for 10 min. The kinetics of acidification is conducted in the following interval times 0, 3, 6, 9, 24, 48, 72 h. The production of lactic acid is expressed in mM by the method of Kumar *et al*. (1991). The study of the kinetics of growth at different temperatures was applied on the strain LbG22, the growth kinetics was performed on MRS medium. At each time intervals, bacterial growth was followed by measuring the optical density at 600 nm and acidification by pH meter.

Screening for antibacterial substances production by *Lactobacillus* strains

To study the bacterial antagonism of *Lactobacillus* strains, we used the method described by Fleming *et al.* (1975), with some modifications; the MRS medium is inoculated with lactic strains with a Multipoint. After 24 hours of incubation, a layer of agar (0.7%) [MRS for lactic acid bacteria and Muller Hinton for pathogenic strains] inoculated with the strain to be tested is added to the surface and re-incubated for 24 -48 hours. Strains showing a clear zone of the lateral

extension greater than 0.5 mm are considered as producers of antibacterial substances (Fleming *et al.*, 1975). This test was repeated at least three times for each indicator strain studied and the Student t test was used to evaluate the results statistically. To eliminate the effect of lactic acid (an important factor in the inhibition by lactic acid bacteria), we use the previous method on MRS agar buffered to pH 7 with phosphate buffer (0.1 M). To determine if the substance is proteinaceous, the supernatant culture of 18-24 hours, obtained after centrifugation (8000g for 10 min) was treated with two types of proteolytic enzymes [pepsin (Prolabo), chymotrypsin-α (Merck)]. Then, the method of Tagg and McGiven (1971) was applied. This test was performed in the interaction between *Lactobacillus plantarum* LbG22 strain and *Staphylococcus aureus* ATCC 43300. The study of growth kinetics of *Lactobacillus plantarum* LbG22 and *Staphylococcus aureus* ATCC 43300 alone or in mixed culture was performed in skim milk at 30°C, as has been described by Mami *et al*. (2008). The estimation of bacterial concentration is by counting on solid medium. The enumeration of staphylococci is on Chapman medium and lactobacilli on MRS medium containing bromocresol green (Guiraud and Rosecea, 2004).

Antibiotic susceptibility testing

For each strain tested, typical colony morphology was selected and subcultured on liquid MRS medium and incubated for 18h. The cell density of cultures was around 10^8 CFU / ml. The strains were spread on MRS medium using swabs, and Thirty three antibiotics were tested. Penicillin (6 µg / 10 IU), oxacillin (1 µg), ampicillin (10 µg), amoxycillin + clavulanic acid (20 µg + 10 µg), Piperacillin (75 µg), Ticarcillin (75 µg), Imipenem (10 µg), Cephalothin (30 µg), cefazolin (30 µg), cefoxitin (30 µg), cefotaxim (30 µg), ceftazidim (30 µg), cefsulodin (30 µg), amikacin (30 µg), netilmicin (30 µg), tobramycin (10 µg), tetracyclin (30 µg), erythromycin (15 µg), spiramycin (100 µg), lincomycin (15 µg), clindamycin (2 µg), pristinamycin (15 µg), vancomycin (30 µg), bacitracin (0,02 t 0,04 IU), colistin (50 µg), trimethoprim-sulfamethoxazol (co-trimoxazol) (1.25 µg + 23.75 µg), nitrofurantoin (300µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), pefloxacin (5 µg), fusidic acid (10 µg), rifampin (30 µg). Agar discs with antibiotics were then incubated for 48 hours. The diameters of inhibition zones were measured using a ruler. The results were expressed as sensitive (S), intermediate (I) and resistant (R) according to recommended standards (Committee of antibiogram of the French Society for Microbiology, 2007). Two reference strains of known antibiotic resistance, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, were included in the tests (Zhou *et al.*, 2005, Taheri *et al.*, 2009).

RESULTS AND DISCUSSION

Species identification

After rounds of purification on solid and liquid medium (MRS pH 5,4), the colonies of the strains were small, whitish, smooth, convex surface. Gram staining showed that all strains are rods Gram-positive bacteria. Under the microscope the bacilli were isolated or grouped in pairs or chain. The catalase test, production of gas (CO_2) from glucose and hydrolyzed arginine on the M16-BCP medium were negative for all strains. While in the production of gas from gluconate have two aspects: strains LbG22, LbG58, LbG68, LbC5 and LbC6 were heterofermentative and strain $LbCA_2$ homofermentative. The six strains grow well at 15 and 45°C after 24h of incubation, and ferment ribose in the API 50 CHL gallery. From the above results, we conclude that little: The six strains belong to the genus *Lactobacillus*. Strain LbG22, LbG58, LbG68, LbC5 and LbC6 belong to Group II of lactobacilli, facultatively heterofermentative. The strain $LbCA_2$ belongs to Group I, Obligatory homofermentative.

The results of the fermentation of carbohydrates on the API gallery 50 CHL, were used to identify species, Table II. Based on these results, and following recommendations of Carr *et al.* (2002), Axelsson (2004) and Hammes and Hertel (2006), we conclude that: strain LbG22, LbG58, LbG68, LbC5 and LbC6 belong to the species *Lactobacillus plantarum*, these results are confirmed by the profile of *Lactobacillus plantarum* ATCC 14917 (obtained from the database BioMérieux), which has the percentage of similarity of 100% for strains LbG22 and LbG58, 97,95% for strains LbG68 and LbC6 and

91.83% pure strain LbC5. The strain $LbCA_2$ belongs to the species *Lactobacillus johnsonii.*

Technological analysis

The six strains resisted heating for 30 min at 60.5 °C water bath. They produce acetoin on Clark and Lubs medium, does not produce dextran on MSE medium, use of citrate in the presence of glucose "KMK medium", and have the ability to hydrolyze milk casein.

Growth kinetics

The growth kinetics and acidification in MRS broth

The growth rate of strains LbG22, LbG58, LbG68 and LbC6 was almost the same and equal to 0.49 ± 0.03 h^{-1} strain LbC5 shows a growth rate of 0.33 h^{-1}. The $LbCA_2$ strain (*Lb. johnsonii*) has different growth kinetics to those of strains *Lb. plantarum*, with a growth rate during the exponential phase of 0.90 h^{-1}. For the kinetics of acidification, strains of the species *Lb. plantarum* (LbG22, LbG58, LbG68, LbC5 and LbC6) have almost the same rate of acidification around 0.156 ± 0.007 pH units per hour. While $LbCA_2$ strain (*Lb. johnsonii*) was a rate of acidification of 0.043 pH units per hour. These results are consistent with identification results, in fact, strains of *Lb. plantarum* has growth kinetics and acidification significantly different to that of strain *Lb. johnsonii*.

Kinetics of acidification and production of lactic acid in skim milk

It appears that the five *Lb. plantarum* strains (LbG22, LbG58, LbG68, LbC5 and LbC6) have almost the same rate of acidification is estimated to 0.03 ± 0.002 pH units per hour and produce lactic acid by a speed of 0,77 ± 0,06 mM/h. The $LbCA_2$ strain (*Lb. johnsonii*), acidifies the medium with a velocity of -0.019 pH units per hour, and produces lactic acid by a velocity of 0.43 mM/h.

Growth kinetics at different temperatures

The maximum growth rate of *Lb. plantarum* LbG22 was recorded at 37 °C, it is estimated at 0.66 h^{-1}, with a rate of acidification of 0.23 pH unit/h. In the incubation at 15 °C, the growth rate was 0.02 h^{-1}, with a variation of pH: 0.015 pH unit/h at 30°C. The growth rate was 0.37 h^{-1}, the variation of pH is estimated at 0.163 pH unit/h. While at 45°C, the growth rate was 0.13 h^{-1}, the rate of change of pH is 0.082 pH unit/h.

Bacterial antagonism.

Bacterial interactions

Results of interactions between *Lactobacillus* strains with them and with pathogenic strains are presented in Table III.

In this work, we studied the ability of *Lb. plantarum* and *Lb. johnsonii* strains to inhibit three reference of *Staphylococcus aureus* strains (ATCC

Table 1: Phenotype characteristics of *Lactobacillus* strains isolated from Algerian raw goat and camel's milk to identify the genus level

Characters	Strains					
	LbG 22	LbG 58	LbG 68	LbC 5	LbC 6	LbC A_2
morphology	Rods	rods	rods	rods	rods	Rods
Gram stain reaction	+	+	+	+	+	+
Catalase	-	-	-	-	-	-
Ribose fermentation	+	+	+	+	+	+
CO_2 from glucose	-	-	-	-	-	-
CO_2 from gluconate	+	+	+	+	+	-
Arginine hydrolysed	-	-	-	-	-	-
Growth at 15 / 45°C	+/+	+/+	+/+	+/+	+/+	+/+
Conclusion Genus Group	*Lactobacillus* Group II, Facultatively heteroferme-ntative	*Lactobacillus* Group II, Facultatively heteroferme-ntative	*Lactobacillus* Group II, Facultatively heteroferme-ntative	*Lactobacillus* Group II, Facultatively heteroferme-ntative	*Lactobacillus* Group II, Facultatively heteroferme-ntative	*Lactobacillus* Group I, Obligately Homofer mentative

Table 2. Differential characteristics of related species of the genus *Lactobacillus* based on API 50 CHL analyses

	Strains					
Carbohydrates	LbG 68	LbG 22	LbG 58	LbC A_2	LbC 5	LbC 6
Witness	-	-	-	-	-	-
Glycérol	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-
D- Arabinose	-	-	-	-	-	-
L- Arabinose	+	+	+	+	+	+
D- Ribose	+	+	+	+	+	+
D- Xylose	-	-	-	+	+	-
L- Xylose	-	-	-	-	-	-
D- Adonitol	-	-	-	-	-	-
Méthyl-βD-Xylopyranoside	-	-	-	-	-	-
D- Galactose	+	+	+	+	+	+
D- Glucose	+	+	+	+	+	+
D- Fructose	+	+	+	+	+	+
D- Mannose	+	+	+	+	+	+
L- Sorbose	-	-	-	-	-	-
L- Rhamnose	-	-	-	+	-	-
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
D- Mannitol	+	+	+	+	+	+
D- Sorbitol	+	+	+	+	+	+
Méthyl-αD-Mannopyranoside	-	+	+	-	-	-
Méthyl-αD-Glucopyranoside	-	-	-	-	-	-
N-AcétylGlucosamine	+	+	+	+	+	+
Amydaline	+	+	+	+	+	+
Arbutine	+	+	+	+	+	+
Esculin iron citrate	+	+	+	+	+	+
Salicine	+	+	+	+	+	+
D- Celiobiose	+	+	+	+	+	+
D- Maltose	+	+	+	+	+	+
D- Lactose (bovine)	+	+	+	+	+	+
D- Melibiose	+	+	+	+	+	+
D-Saccharose	+	+	+	+	+	+
D- Trehalose	+	+	+	+	+	+
Inuline	-	-	-	-	-	-
D- Mélézitose	+	+	+	+	+	+
D- Raffinose	+	+	+	-	+	+
Amidon	-	-	-	-	-	-
Glycogène	-	-	-	-	+	-
Xylitol	-	-	-	-	-	-
Gentiobiose	+	+	+	+	+	+
D-Turanose	+	+	+	+	+	+
D- Lyxose	-	-	-	-	-	-
D- Tagatose	-	-	-	+	+	-
D- Fucose	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-
D- Arabitol	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-
Potassium Gluconate	+	+	+	-	+	+
Potassium 2-cétogluconate	-	-	-	-	-	-
Potassium 5-cétogluconate	-	-	-	-		-
Identified as	*Lb. plantarum*	*Lb. plantarum*	*Lb. plantarum*	*Lb. plantarum*	*Lb. Johnsonii*	*Lb. plantarum*

Table 3. Antimicrobial activity of isolated *Lactobacillus* strains against pathogenic species

Indicator strains	LbG22		LbG58		LbG68		LbC5		LbC6		$LbCA_2$	
	A	B	A	B	A	B	A	B	A	B	A	B
Gram +												
*S. aureus*ATCC 25923	++	+	++	+	++	+	++	+	++	+	++	+
S. aureus ATCC 29213	++	++	++	+	++	+	++	+	++	+	++	-
S. aureus ATCC 43300	++	++	++	+	++	-	++	+	++	+	++	-
Listeria ivanovii ATCC 19119	++	+	++	+	++	+	++	+	++	+	++	-
Gram -												
E. coli ATCC 25922	++	+	++	+	++	+	++	+	++	+	+	-
Klebsiella pneumoniae	++	-	++	-	++	+	+	+	+	+	-	-
Acinetobacter calcoaceticus	+	+	+	+	++	+	++	++	++	+	-	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	-	+
Salmonella enterica	++	-	++	-	+	-	++	-	++	-	-	-
Lactobacillus												
Lb. plantarum LbG 22	-	-	-	-	+	-	++	++	++	++	-	-
Lb. plantarum LbG 58	-	-	-	+	+	-	+	+	++	++	-	-
Lb. plantarum LbC 5	+	+	+	+	+	+	++	+	++	+	-	-
Lb. johnsonii LbC A_2	-	-	-	-	+	+	++	++	++	++	-	-

A : on MRS medium pH 6.2, B : on buffered MRS medium, < 0,5 mm : (-), ≥ 0,5 mm : (+), ≥ 5 mm : (++).

Table 4. Determination of antibiotic susceptibility of lactobacilli strains

Antibiotics	Strains tested					
	LbG 22	LbG 58	LbG 68	LbC 5	LbC 6	LbC A_2
PENICILLINS						
Penicillin	I	I	I	I	I	S
Oxacillin	R	R	R	R	R	R
Ampicillin	S	S	S	S	S	S
Amoxycillin + Clavulanic Acid	I	I	I	I	I	I
Piperacillin	S	S	S	S	S	S
Ticarcillin	S	S	S	S	S	S
CARBAPENEMS						
Imipenem	S	S	S	S	S	S
CEPHALOSPORINS						
Cephalothin	I	I	R	R	R	S
Cefazolin	S	S	I	I	R	I
Cefoxitin	R	R	I	R	R	R
Cefotaxim	I	S	I	R	I	S
Ceftazidim	R	R	R	R	R	R
Cefsulodin	R	R	R	R	R	R
AMINOSIDS						
Amikacin	R	R	R	R	R	R
Netilmicin	R	I	R	I	I	S
Tobramycin	R	R	R	R	R	R
TETRACYCLINS						
Tetracyclin	R	I	S	S	S	I
MACROLIDS						
Erythromycin	S	S	S	S	S	S
Spiramycin	R	R	R	R	R	R
LINCOSAMIDS						
Lincomycin	R	R	S	S	S	S
Clindamycin	R	R	S	S	S	S
STREPTOGRAMINS						
Pristinamycin	S	S	S	S	S	S
GLYCOPEPTIDS						
Vancomycin	R	R	R	R	R	R
POLYPEPTIDS						
Bacitracin	R	R	R	R	R	R
Colistin	R	R	R	R	R	R
SULFAMIDS-TRIMETHOPRIM						
Trimethoprim-Sulfamethoxazol	S	S	S	S	S	R
NITROFURANS						
Nitrofurantoin	S	S	S	S	S	S
QUINOLONS						
Nalidixic Acid	R	R	R	R	R	R
FLUOROQUINOLONS						
Ciprofloxacin	R	R	R	R	R	R
Ofloxacin	R	R	R	R	R	R
Pefloxacin	R	R	R	R	R	R
DIVERSE						
Fusidic Acid	R	R	R	R	R	I
Rifampin	S	S	S	S	S	S

25923, ATCC 29213, ATCC 43300). On normal MRS medium (pH 6.2), staphylococci were inhibited by *Lactobacillus* strains studied. By cons, on buffered (pH 7), only strains *Lb. plantarum* inhibited staphylococci. The strain *Lb. plantarum* LbG22, is the strain that has the highest inhibitory activity against staphylococci (figure 1). *S. aureus* ATCC 25923 strains was the most resistant strains compared to *S. aureus* studied. Inhibition of strains of *S. aureus* strains by *Lb. plantarum* has been previously described. For example, Mami *et al.* (2008), indicated that strains of *Lb. plantarum* isolated from raw goat's milk inhibit strains of *S. aureus*. *Lb. plantarum* strains inhibit *Listeria ivanovii* ATCC 19119 on normal and buffered medium. Inhibition of strains of the genus *Listeria* by some strains of *Lb. plantarum* has been previously described (Ouwehand and Vesterlund, 2004). *Lb. plantarum* strains inhibited *E. coli* ATCC 25922 in normal and buffered medium, although inhibition in the first case is largely greater (Fig. 2).

Inhibition of *E. coli* strains of *Lb. plantarum* has already been described by several studies (Todorov *et al.* 2004; Karthikeyan and Santosh, 2009). The other Gram negative strains studied, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Salmonella enterica*, were inhibited at pH 6,2, by *Lb. plantarum* strains. By cons, in buffered medium, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus* were inhibited mainly by strains Lb. plantarum LbG68, LbC5 and LbC6. However, *Salmonella enterica* was not inhibited by any lactic strains on buffered medium. Karthikeyan and Santosh (2009) have described *Lactobacillus plantarum* strains that inhibit *Salmonella* Typhimurium, *Salmonella* Paratyphi, *Klebsiella sp* and *Pseudomonas aeruginosa*. The interaction between lactic acid bacteria strains showed that the bacteria that have become increasingly competitive inhibition are LbC5 and LbC6 strains, which are also the most sensitive to inhibition (Fig. 3).

Nature of inhibitor agent

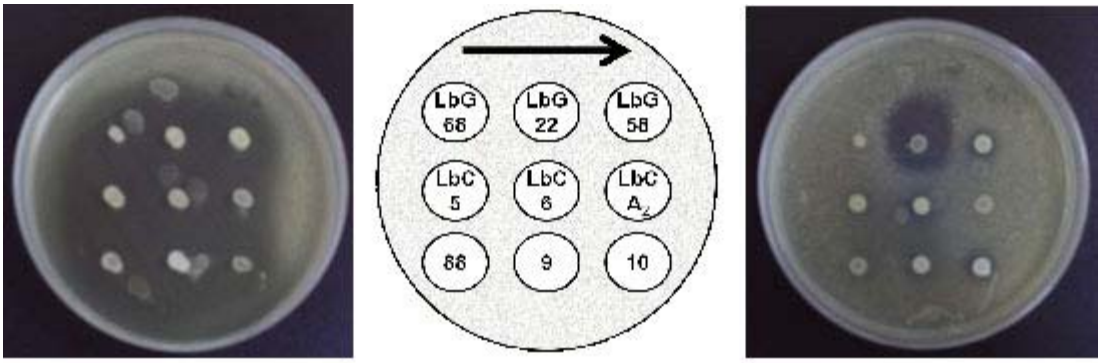
In MRS medium pH 6.2, the supernatant (a culture of *Lb. plantarum* LbG22 strain of 24h) inhibited *S. aureus* ATCC 43300 with an activity of 9.90 ± 3.63 mm. On buffered, inhibition activity was 8.37 ± 4.50 mm. While no inhibition zone was observed after treatment with proteolytic enzyme pepsin and á-chymotrypsin. After applying the statistical test of *Student*, apparently, there is no significant difference between the activity in MRS broth pH 6.2 and buffered MRS pH 7. From these results, we can say that the *Lactobacillus* strains possess antibacterial activity, which is due to lactic acid, and a proteinaceous agent may be bacteriocin.

Growth kinetics in mixed culture

The study of the growth kinetics of *Lactobacillus plantarum* LbG22 and *Staphylococcus aureus* ATCC 43300 alone or in mixed culture was made in skim milk. The results of this test are presented in Figures 4, 5 and 6. The linear regression lines (of the exponential phase of growth) are plotted for the time interval of 0-9 hours. In the case of the culture of *Lb. plantarum* LbG22 alone, regression analysis provides a value of 0.243 $\log_{10}$ CFU per unit of hours (a growth rate of 0.80 h^{-1}, or a doubling time of 75 minutes). After 72 h, the bacterial concentration was estimated at 9.00 $\log_{10}$ CFU. ml^{-1}. The rate of acidification of the medium is estimated by calculating the slope of the regression of the curve of pH variation, it is - 0.027 pH units per hour, so that production of lactic acid, estimated 0.7 mM/h. With culture *S. aureus* ATCC 43300 alone, the growth rate was 0.99 h-1. After 72 h, the bacterial concentration was estimated by 8.73 $\log_{10}$ CFU. ml^{-1}. The rate of acidification is - 0.02 pH units per hour, so that the production of lactic acid is estimated at 0.51 mM/h. In the mixed culture, *Lb. plantarum* LbG22 increases with a growth rate of 0.82 h^{-1}. After 72 h, the bacterial concentration was estimated of 8.43 $\log_{10}$ CFU. ml^{-1}. For the strain *S. aureus* ATCC 43300, the growth rate was 0.70 h^{-1}. After 72 h, the bacterial concentration was estimated at less than 6 $\log_{10}$ CFU. ml^{-1}. The rate of acidification is 0.024 pH units per hour, so that the production of lactic acid, is estimated at 0.65 mM/h. From the results we can say that the strain *Lb. plantarum* LbG22 strain inhibited *S. aureus* ATCC 43300 from 24 hours of mixed culture.

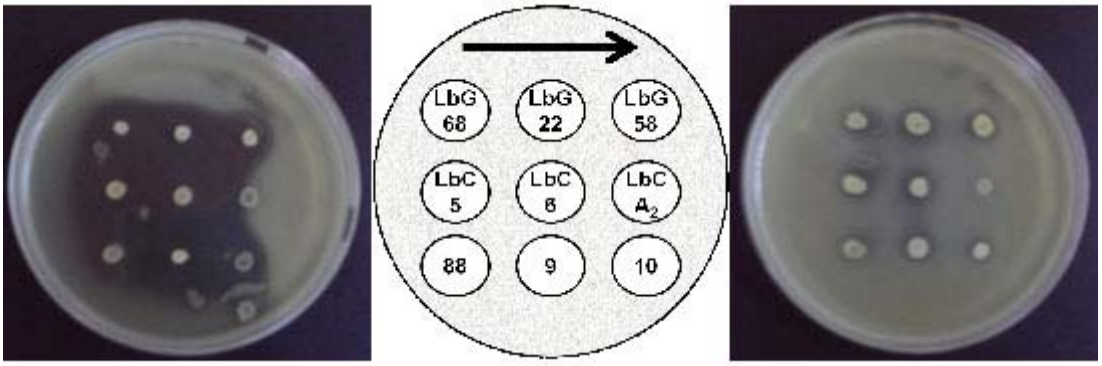
Antibiotic susceptibility of Lactobacilli

The results of this test are presented in table IV. According to test results, lactic strains studied were sensitive to ampicillin, ticarcillin, imipenem, erythromycin, pristinamycin, rifampin, nitrofurantoin, trimethoprim-sulfamethoxazol (except LbC A_2). By cons they were resistant to:



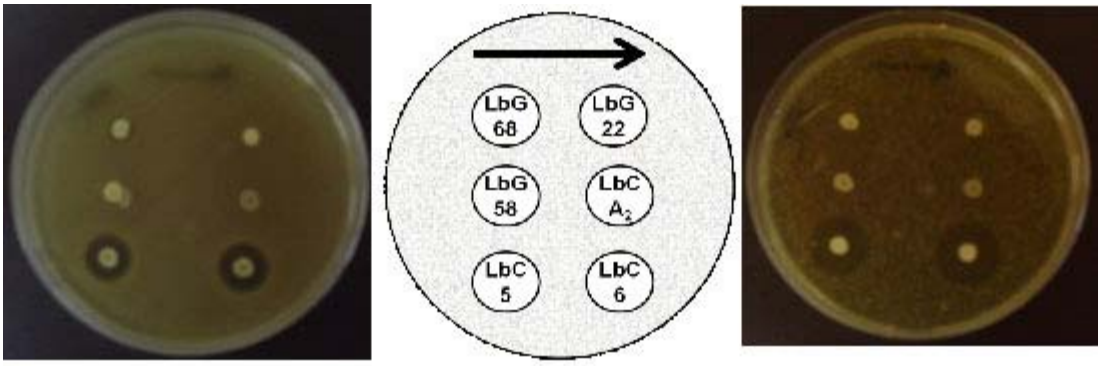
(a) (b)

Fig. 1. Test of inhibition of *Staphylococcus aureus* ATCC 43300 by *Lactobacillus* strains, (a) in MRS pH 6.2, (b) in buffered MRS pH 7



(a) (b)

Fig. 2. Test of inhibition of E. coli ATCC 25922 by *Lactobacillus* strains, (a) in MRS pH 6.2, (b) in buffered MRS pH 7.



(a) (b)

Fig. 3. Test of inhibition of Lactobacillus plantarum LbG22 by *Lactobacillus* strains, (a) in MRS pH 6.2, (b) in buffered MRS pH 7

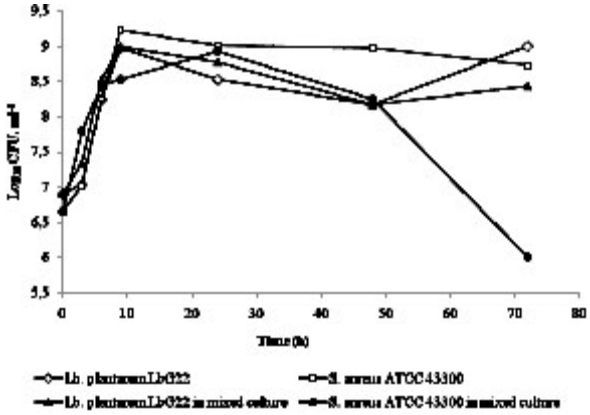


Fig. 4. Growth kinetics in skim milk at 37 °C, in culture alone and in mixed culture of *Lb. plantarum* LbG22 and *S. aureus* ATCC 43300 strains

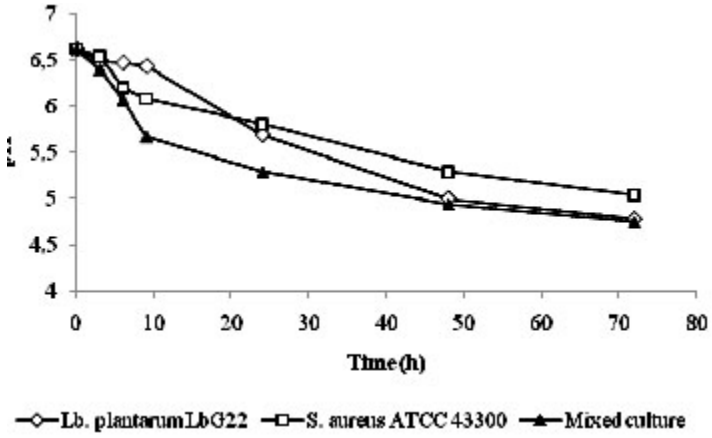


Fig. 5. Kinetics of acidification of skim milk at 37 °C, in culture alone and in mixed culture, *Lb. plantarum* LbG22 and *S. aureus* ATCC 43300 strains

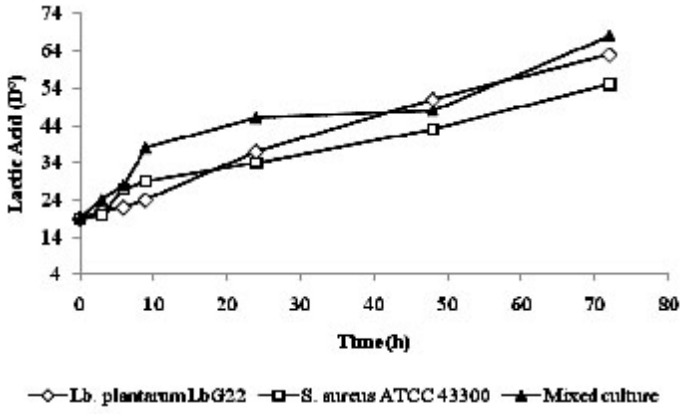


Fig. 6. Kinetics of lactic acid production in skim milk at 37 °C, in culture alone and in mixed culture, *Lb. plantarum* LbG22 and *S. aureus* ATCC 43300 strains

oxacillin, cefoxitin, ceftazidime, cefsulodin, amikacin, tobramycin, spiramycin, vancomycin, bacitracin, colistin, nalidixic acid, ciprofloxacin, ofloxacin, pefloxacin, fusidic acid. For other antibiotics the results were variable among strains. However little we notice the presence of three groups according to the results of this test: [LbG 22, LbG 58], [LbG 68, LbC 5 and LbC 6] and [LbC A_2].

DISCUSSION

In this work, the identification and characterization of functional and technological *Lactobacillus plantarum* strains isolated from raw milk of goat and camel have been performed.

The identification of strains was performed following the recommendations of Carr *et al* (2002), Axelsson (2004) and Hammes and Hertel (2006). The results of the identification of the genus, shows that the six strains studied belong to the genus *Lactobacillus*. The LbC A2 strain belongs to the species *Lb. johnsonii*, and the five other strains (LbG22, LbG58, LbG68, LbC5 and LbC6) belong to the species *Lb. plantarum*. This identification is reinforced by the comparison with the profile of fermentation of the strain *Lb. plantarum* ATCC 14917, where the percentages of similarity were impourtants. Have high percentages of similarity is very encouraging, because the *Lb. plantarum* species is much diversified phenotypically (it is a strain which is ubiquitous in various environments) (Axelsson, 2004).

However, these strains grow at 45 °C. The classic literature indicates that the *Lb. plantarum* species did not grow at this temperature (Carr *et al*. 2002; Axelsson, 2004; Hammes and Hertel, 2006). But Carr *et al.* (2002) do not consider this feature as a key in his dichotomous identification pattern, it only requires: growth at 15°C, ADH (-), Ribose (+), Raffinose (+) and mannitol (+).

In 2004, An *et al*. identified three *Lb. plantarum* strains which grow at 45°C. They use the molecular identification by sequencing the 16S rDNA. So there really *Lb. plantarum* grown at 45°C. The *Lb. plantarum* species, although it is very diversified phenotypically, genetically it is very stable, and in several studies (Axelsson, 2004, An *et al*., 2004) and every time when we compare the traditional identification and molecular, we find that the traditional identification is reliable for the study of this species.

In our laboratory (LMA-University of Oran, Algeria), researchers have isolated strains which are resistant to atypical temperatures, as is the case Drici *et al.* (2009), which was able to isolate strains *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* from raw camel's milk, which are thermotolerant to 50°C, and confirming identification has been verified by molecular methods. Isolation of strains belonging to the species *Lactobacillus plantarum* from Algerian raw milk of goat and camel has already been described in several studies (Guessas and Kihal, 2004; Badis *et al*., 2004; Badis *et al*., 2005; Laouabdia Sellami *et al.*, 2007; Mami *et al.*, 2008).

The technological results are satisfactory for industrial utilisation. The strains are heat resistant; they produce flavours and have a proteolytic activity. However, the six strains studied did not produce dextran on MSE medium. However, further studies are essential for a real application in the food industry. The thermotolerant should be further studied and the proteolytic activity quantified. For the industrial starters, it should be less proteolytic but more aminopeptidase, in the aim to reduced risk of getting bitter peptides (from casein). Releasing more amino acids, they also produce more aroma precursors (Mayra-Makinen and Bigret, 2004).

The kinetics of growth and acidification in MRS broth and skim milk at different temperatures has been addressed in this work. From the results, the *Lactobacillus plantarum* strains have identical growth kinetics and acidification in MRS broth and skim milk at 30 °C. However *Lactobacillus johnsonii* has growth kinetics and acidification different. These results are consistent with the results of identification.

The best growth temperature for *Lb. plantarum* LbG22 strain was at 37°C, although some authors generally recommend incubation at 30°C, for the culture of lactic strains from milk and dairy products. However, the best grow at 37°C, was observed in *Lactobacillus* strains of intestinal origin and yogurt (Corry *et al*., 2003). This suggests that the strain *Lb. plantarum* LbG22 may be of intestinal origin.

In this work we studied the interaction of the strains *Lactobacillus plantarum* and *Lactobacillus johnsonii* with pathogenic strains and with *Lactobacillus* strains. The strains *Lb. plantarum* LbC5 and LbC6 have the best antibacterial activity, they inhibited all studied strains. The *Lb. plantarum* LbG22 strain has the greatest inhibitory activity against *S. aureus* strains, among other lactic strains studied. In mixed culture, growth of *S. aureus* ATCC 43300 was inhibited after 24h of culture *with Lb. plantarum* LbG22.

The antibacterial activity of lactic strains may be due to the production of several antibacterial agents. The lactic acid and acidification inhibits several types of bacteria. Also, these strains also produce diacetyl, which also has a power of inhibition. Even The H_2O_2 can be the source of this inhibition (Ouwehand and Vesterlund, 2004).

However, in a buffered medium at pH 7, and the interaction with bacteria that have catalase, *S. aureus*, for example, the antibacterial activity of the studied strains persist. After treatment with proteolytic enzymes, α-chymotypsine and pepsin, the inhibition disappears. That suggests that strains *Lb. plantarum* studied produce proteinaceous agents that cause inhibition of other bacteria. These compounds can be protein bacteriocins. The production of bacteriocins by *Lb. plantarum* strains is widely accepted. (Olasupo, 1996; Ouwehand and Vesterlund, 2004; Todorov *et al.*, 2004; Karthikeyan and Santosh, 2009).

The technique used to study the sensitivity of *Lactobacillus* strains to antibiotics, was that of "Disks agar diffusion method". Several researchers have already used this method in the study of lactobacilli (Zhou *et al.*, 2005; Ortu *et al.*, 2007; Taheri *et al.*, 2009).

The results obtained in this work are comparable to those found by other researchers. Mikelsaar *et al.* (2004) have reported that *Lb. plantarum* strains, showed a resistance of 100% to cefoxitin and vancomycin, and 71% to ciprofloxacin. For tetracycline, 43% of strains were sensitive, 29% intermediate and 29% resistant. Vescovo *et al.* (1982) have found that strains of *Lb. acidophilus* and *Lb. reuteri*, are resistant to most antibiotics tested (21 antibiotics), and even Strompfová and Lauková (2004) reported lactobacilli resistant to vancomycin. Many researchers have speculated that commensal bacteria such as lactic acid bacteria may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens bacteria. The main threat to these bacteria is that they can transfer resistance genes to pathogenic bacteria. Genes conferring resistance to tetracycline, erythromycin and vancomycin have been detected and characterized in *Lactococcus lactis*, Enterococci and, more recently, in *Lactobacillus* species isolated from meat and fermented milk products (Mathur and Singh, 2005). West and Warner (1985) have shown that some of *Lb. plantarum* strain can become resistant to streptomycin and rifampin, after the transfer of a plasmid from *Streptococcus*.

CONCLUSION

In this work, we were able to identify strains of Lb. plantarum, which growth at 45 °C. The technological results are satisfactory for industrial use. Strains of *Lb. plantarum* studied, has a good antibacterial activity, it is mostly gram-positive strains that are most sensitive. The resistance of strains to certain antibiotics, demand further studies to determine the source of this gene resistance. Similarly, other molecular biology studies and technological skills are necessary before the introduction of these strains as starters in dairy industry.

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