SCICELL

Nova Biotechnol Chim (2021) 20(2): e958
DOI: 10.36547/nbc.958

Nova Biotechnologica *et* Chimica

Typical Moroccan goat lactic acid bacteria and their assay as starters

Linda Zaaraoui[1,2,✉], Abdellah Bouksaim[1], Maha Elhamdani[1,2], Aouatif Benali[1], Mohammed Oukassou[3], Khadija Ounine[2] and Mohammed Bouksaim[1]

[1]*Laboratory of Food Technology, INRA, RCAR-Rabat, P.O.Box 6570, Rabat Institutes, Rabat 10101, Morocco*

[2]*IbnTofail University, Faculty of Sciences, P.O.Box 242, Kénitra, Morocco*

[3]*Therapeutic Physiology Laboratory, Institute of Agronomy and Veterinary Hassan II, P.O.Box 6202 Madinat Al Irfane, Rabat, Morocco*

✉ *Corresponding author: zaraoui.linda@gmail.com*

Article info

Article history:
Received: 1st September 2020
Accepted: 5th May 2020

Keywords:
adjunct culture
adjunct starter cultures
goat's milk
Lactobacillus
Moroccan dairy products

Abstract

The knowledge of lactic acid bacteria of raw milk and the main factors affecting their variability are particularly important issues for the control of cheese processing and the bioconservation of farm raw milk food products. The present research study concerned the isolation and identification of twenty strains of the *Lactobacillus* genus from goat milk originating from the Oulmes region, using the API 50 CH system. All isolates found represented five species: *Lactobacillus plantarum* (43.75 %), *Lactobacillus brevis* (37.75 %), *Lactobacillus pentosus* (6.25 %), *Lactobacillus salivarus* (6.25 %), and *Lactobacillus acidophilus* (6.25 %). According to biochemical activities, the majority of the strains displayed weak acidification and autolysis activities in milk. In contrast, they showed high extracellular proteolytic activity. All isolates produced exopolysaccharides and most of them could metabolize citrate. The absence of hemolytic activity may suggest the use of these isolates as adjunct starters in the food fermentation process.

Introduction

Lactic acid bacteria are food-grade microorganisms that play a vital role in the fermentation of animal and plant raw materials. Their ability to ferment carbohydrates and to a lesser degree, degrade proteins and lipids, leads to the synthesis of a wide range of compounds, such as organic acids, peptides, antimicrobial and aromatic compounds, and exopolysaccharides. These compounds may contribute to the organoleptic, technological, and nutritional characteristics of fermented foods (Mozzi *et al.* 2010; Ray *et al.* 2014). The discovery of the action of lactic bacteria on milk was probably accidental, but their use was perpetuated in the form of natural leavens (Chammas *et al.* 2006; Zamfir *et al.* 2006). Over the past fifteen years, considerable interest has developed around the use of lactic cultures with beneficial effects on health or "probiotics" (*Bifidobacterium, Lactobacillus*). The probiotics are living microorganisms which, when administered in adequate quantities, are beneficial to the health of the host (FAO / WHO 2001). This definition could not specify the nature of the benefit, and as a result, many health benefits were attributed to lactic acid bacteria with probiotic potential. They include anti-cancer, anti-diabetic, anti-obesity, anti-diarrheal,

immune, anti-allergic, anti-oxidant, antimicrobial, and microbial-balanced activities of the colon (Teitelbaum and Walker 2002; Park *et al*. 2016). The objective of the present study is to identify strains of lactobacilli isolated from goat milk of the Oulmes region and to test some technological aptitudes of these strains.

Experimental

Sample collection

The samples of goat milk originated from the AIT Ichou region situated in a rural mountainous region called Oulmes city belongs to the province of Khemisset, Rabat-Salé-region Kenitra in Morocco. The raw goat milk samples were immediately cooled and delivered to the microbiology laboratory in an isotherm container, being analyzed in arrival.

Isolation and purification of lactic acid bacteria

To carry out this operation, ten milliliters of each raw milk samples were aseptically added into 90 ml of sterile 0.9 % NaCl solution and mixed thoroughly. Serial dilutions (10 – 1 to 10 – 8) were performed and 1 mL aliquots of appropriate dilution were directly inoculated in triplicate on the following media for lactic acid bacteria such as the Man Rogosa and Sharpe (MRS) (Fluka, Sigma-Aldrich) and M17 (Oxoid) agar media. After incubation in Petri plates for 24 h at 15 ºC, 32 ºC, 38 ºC, and 45 ºC, representative strains of lactic acid bacteria were obtained from M17 and MRS plates of highest sample dilutions. Colonies were either randomly picked up or when the plate contained less than 10 colonies (Leisner *et al.* 1997). The purity of the isolates was checked by streaking again to fresh agar plates, followed by macroscopic and microscopic examinations.
A total of one hundred purified isolates were collected whose twenty strains occurring in pairs or chains of size baton gram-positive and catalase-negative were selected for further studies. For the conservation, the strains of lactic acid bacteria were stored at -80 °C in MRS broth supplemented with 15 % (v/v) glycerol until use. And their regeneration was monitored by an overnight incubation under 37 °C in MRS broth.

API Systems

The bacteria gram-positive, catalase-negative was the subject of biochemical identification using the bioMérieux API system using API 50 CH gallery with API 50 CHL medium (bioMerieux, Marcy star, France) (Ghanbari *et al.* 2009).

Technological characteristics

To perform these bacteria technological characteristics, the following parameters are tested under the optimum conditions.

Acidifying activity

To evaluate the acidifying power, the concerning 20 strains were initially grown in MRS broth at 37 °C for 24 h. And there are inoculated at a level of 1 % in reconstituted sterile skim milk solution (10 % w/v) (Fluka, Sigma-Aldrich). Then, the pH was measured (pH 211 precision pH meter, HANNA Instruments Inc., Italy) after 2, 4, 6, and 24 h incubation at 37 °C.
The acidification rate was calculated as (Eq. 1):

$$\Delta pH = pHf\ (finalvalue) - pH0\ (initialvalue) \quad (1)$$

The experiments were carried out in duplicate.

Proteolytic activity

To determine the proteolytic activity, the isolates were subcultured twice in reconstituted skim milk (10 % w/v), containing yeast extract (0.3 % w/v), for 24 h at 37 °C and using 1 % (v/v) inoculum. Final growth was performed in skim milk (10 % w/v) for 24 h at 37 °C (1 % v/v inoculum). The proteolytic activity was determined by the quantity of free amino acids released according to the method of Church *et al*. (1983). Results were expressed as glycine equivalents (mM) according to a standard curve, prepared using pure glycine in the range of 0 – 10 mM.

Autolytic activity

For determining the important autolytic activity factor, the overnight cultures were centrifuged (5000 × g for 15 min at 4 °C). The cell pellet was washed twice using potassium phosphate buffer (10 mM, pH 7.0) and then suspended in potassium phosphate buffer (10 mM, pH 5.5). The obtained cell suspension was subjected to one cycle of freezing (-20 °C for 22 h) and thawing, then incubated at 45 °C for 2 h. The autolytic activity was determined as the percentage decrease in the absorbance at 650 nm at different time intervals as described by Boutrou *et al*. (1998), which was defined as follows (Eq. 2):

$$\text{Autolytic activity \%} = (A0 - At) \times 100/A0 \qquad (2)$$

where A_0 = initial absorbance and A_t = absorbance measured after t hours of incubation.

Citrate metabolism

It is noticed in the technological microorganism's field that bacteria citrate utilization in the presence of carbohydrates was studied on the special agar medium as described by Kempler and McKay (KMK agar) (Kempler and McKay 1980). The blue bacteria colonies and/or large blue center colonies were considered citrate positive.

Exopolysaccharides (EPS) production

It is known that the production of the exopolysaccharide was evaluated as reported by Mora *et al.* (2002). For this special and important production, overnight cultures were streaked on the surface of plates containing ruthenium red milk (10 % skim milk powder, 1 % sucrose, 0.5 % yeast extract, 0.08 g 1-1 ruthenium red, 1.5 % agar). After incubation at 37 °C for 24 h, non-ropy isolates gave red colonies due to the staining of the bacterial cell wall, while ropy isolates appeared as white colonies.

Hemolytic activity

In this step of the present research work, the hemolytic activity was performed as described by Maragkoudakis *et al.* (2009). The strains were examined for signs of β-haemolysis (clear zones around colonies), α-haemolysis (green zones around colonies) or γ-haemolysis (no clear zones around colonies), and the results are illustrated and interpreted as shown in the part of the results and the discussion.

Antibacterial activity determination

This parameter can play an important role in food preservation. So, to evaluate the antibacterial activity of identified strains, the test was monitored against some known pathogenic bacteria by the good diffusion assay using cell culture or cell supernatant (Du Toit *et al.* 1998; Jamaly *et al.* 2011). Fresh overnight MRS cultures were centrifuged at 8,000 × g for 10 min, and the cell-free supernatants were used directly or after being filtred aseptically (0.22 µm pore size; Serva, Heidelberg, Germany), neutralized with 1 mol.L^{-1} NaOH (pH 6.5 – 7) and treated with catalase (0.5 mg.mL^{-1}) (Sigma-Aldrich). The lactobacillus cells were diluted with MRS and used for their antimicrobial activity. It is noticed that the indicator pathogenic strains included *Listeria innocua* (LMHAE-LI 107), *Staphylococcus aureus* (LMHAE-SA 105), *Pseudomonas aeruginosa* (ATCC 29753), *Klebsiella pneumonia* (CIP 53153), *Micrococcus luteus* (ATCC15957), and *Escherichia coli* (ATCC54127), were tested. They were grown overnight in LB broth (Sigma-Aldrich) at pH 7.0 and diluted with sterile phosphate-buffered saline (PBS) (pH 7.2). After dilution, the pathogenic strains were mixed with 5 mL of LB soft agar (0.7 %, w/v) to reach a final concentration of 104 CFU.mL^{-1}, this medium was poured into Petri plates prepared in advance with 10 ml of basal agar containing 2 % (w/v) agar. Wells of 5 mm diameter were performed using the top of a Pasteur pipette and were filled with approximately 50 µl of 108 CFU.$mL^{-1}$of identified strains in 0.1 % saline peptone, of cell-free treated supernatant and cell-free untreated supernatant. The plates were then stored at 4 °C for 4 h to allow the radial diffusion of any antimicrobial compound. Following incubation at 37 °C for 24 h, the plates were monitored for the appearance of clear zones of inhibition. Each test was performed in triplicate.

Results and Discussion

API 50 CHL systems identification

For bacteria selection and identification, the biochemical identification by API 50 CH system is carried out after incubation of the galleries at 37 °C for 24 h. The results are read directly on the seeded gallery. The profiles obtained were analyzed by APILAB software, in collaboration with the microbiology laboratory of the National Center for Scientific and Technical Research (CNRST). It should be specified here that in the case of inaccurate identifications or unidentified profiles in the API database; the isolate is thus considered as unidentified.

Table 1. Results of bacteria identification by API 50 CH.

Strains	Identification API 50 CHL	[% I.D]
1	*LB. Sp*	-
2	*LB. brevis*	99.7
3	*LB. pentosus*	87.6
4	*LB. plantarum*	98.8
5	*LB. plantarum*	86.9
6	*LB. Sp*	-
7	*LB. plantarum*	99.4
8	*LB. salivarus*	94.9
9	*LB. plantarum*	99.9
10	*LB. brevis*	99.9
11	*LB. plantarum*	99.8
12	*LB. Sp*	-
13	*LB. Sp*	-
14	*LB. brevis*	99.5
15	*LB. acidophilus*	98.7
16	*LB. brevis*	99.8
17	*LB. plantarum*	94.4
18	*LB. plantarum*	99.6
19	*LB. brevis*	99.7
20	*LB. brevis*	99.1

Twenty isolates under study and identified by API 50 CH showed a variety of *lactobacillus* species. Five different species were identified between the 20 isolates (Table 1). However, four bacterial species have not been identified; these are isolates 1; 6; 12, and 13. In light of the present results, a dominance of LB has observed *LB. plantarum* with a percentage of 43.75 %, followed by *LB. brevis* with a percentage of 37.5 %. The remainder were distributed among *LB. acidophilus*; *LB. pentosus*, and *LB. salivarus* with a percentage of 6.25 % each.

First of all the presence of *LB. plantarum* as the majority species in goat milk samples from Oulmes may be explained by a natural selection due to the region biotic environment, giving that these species are considered as the habitual host of plants (Zadi Karam *et al.* 2006). Also, the two species *LB. plantarum* and *LB. brevis* are widely studied, have proved to possess probiotic potential (Jamaly *et al.* 2011; Guidone *et al.* 2014; Jia *et al.* 2017) and are worth exploiting to improve fermented milk products, such as yogurt, cheese, etc. Also, the marketed systems are with limited databases, the manufacturer of the gallery can only do their updates. A bacterium absent from the repertoire of identification galleries will not be recognized.

In general, the error percentage in the galleries is a function of several parameters that may be due to the experimenter, the API system itself, or a mutation of the microorganism in identification. Also, it has been reported that API identification is only about 65 % reliable (Soto *et al.* 1994; Ouadghiri *et al.* 2005), hence the importance of using genotypic techniques. However, the API system remains a useful means for an initial microorganism's identification. However, it's coupling with other tests such as immunological and molecular could be, a fortiori, interesting to deepen the scientific and technological knowledge that concern the lactic bacteria in Morocco. These bacteria can also play an important role in the transformation and valorization of agricultural products of economic and social interest (olives, milk, etc.).

Acidifying power

The results of acidifying activity in milk at 37 °C are shown in Fig. 1. The initial pH of the milk was 6.59. All isolates showed low acidifying activity after 2 h (ΔpH2), 4 h (ΔpH4), and 6 h (ΔpH6) incubation, with values ranging from 0.02 to 0.37, from 0.07 to 0.53, and from 0.07 to 0.63 pH units, respectively. As regards their ability to reduce the pH of skim milk in 24 h (ΔpH24), the values of the acidification activity of bacteria isolates studied ranged from 1.75 ± 0.03 to 0.35 ± 0.01 pH units.

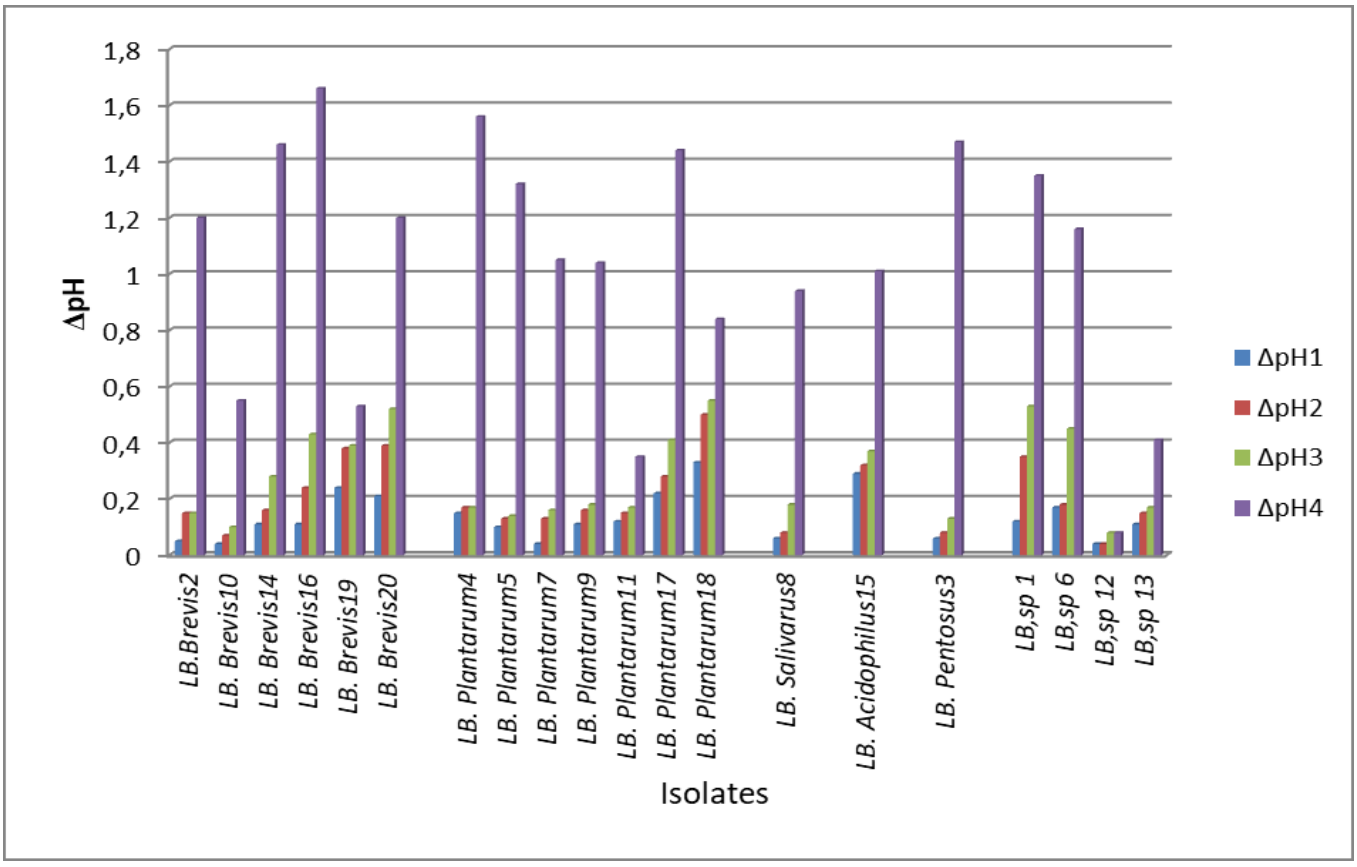

Fig. 1. The pH decreasing in reconstituted skim milk after 2 h (ΔpH2), 4 h (ΔpH4), 6 h (ΔpH6), and 24 h (ΔpH24) of incubation at 37 °C, respectively. Values are mean ± standard deviation (n = 3).

It was noted that *LB. brevis16* showed the highest acidification activity (1.75 ± 0.01 pH units), while *LB. plantarum11* showed the lowest one (0.35 ± 0.01 units pH). After 24 h none of the lactobacillus strains can be characterized, as fast as they did not reach a pH of the milk below 5.3 after 6 h in optimal growth temperature (Beresford *et al.* 2001). These results are not consistent with those reported by Buket *et al.* (2012) who observed that *L. plantarum* had a fast acidifying capacity.

So, it is known that a rapid decrease in pH during the initial step of cheese preparation is essential for coagulation and for the prevention or reduction of the growth of adventitious microbiota they could not be used as starter organisms. However, they may be useful as adjunct cultures depending on their other important properties associated with industrial needs.

Proteolytic activity

In this phase of the research, all species showed proteolytic activity evaluated at values greater than 1 mM glycine. Indees, isolates *LB. brevis2*, *LB. plantarum9*, *LB. plantarum7*, and *LB. pentosus3* have shown its highest activity with values (3.8 mM Gly, 4.7 mM Gly, 9.4, and 7.75 mM Gly, respectively). In previous studies (El-Ghaish *et al.* 2010; Kholif *et al.* 2011; Moslehishad *et al.* 2013) also reported that *Lb. plantarum* had the highest proteolytic activity among the tested strains. Thus, the values obtained for the lactobacillus strains tested are superior when compared to those reported by Herreros *et al.* (2003) and Ballesteros *et al.* (2006). It should be noted that species exhibiting high acidifying activity do not necessarily have the highest proteolytic activity, the same observation was previously reported by Requena *et al.* (1991), Fortina *et al.* (1998); Durlu-Ozkaya *et al.* (2001). This proteolytic activity is one of the most important desirable criteria for complementary cultures as it may be responsible for aroma production, flavor enhancement, cell growth, and inhibitory activity enhancement of the

fermented final product (Yvon *et al.* 2006; Donkor *et al*. 2007).

Autolytic activity

Cell autolysis allows to release of intracellular enzymes, including peptidases that can contributes to maturation and contribute to the development of cheese flavors (Fitzsimons *et al*. 2001; Collins *et al.* 2003; Lortal *et al.* 2005). Nevertheless, the degree of autolysis is straining dependent (Wilkinson *et al.* 1994; El-Soda *et al.* 2000).
All of the strains tested in the present study showed variable autolytic activities (Table 2). In general, they exhibited a low autolysis rate as described by Ayad *et al*. (2004): 0.78 and 17.04 %, respectively. This result is not in agreement with those published by Boutrou *et al*. (1998); Wainrichter *et al.* (2001); Salima *et al*. (2009); Dako *et al. (*1995) and El-Soda *et al*. (1995), who observed higher autolysis (superior of 50 %) for *Lb. plantarum* and *Lb. brevis*. Nevertheless, these values are compatible with the potential role of the strains as adjunct cultures (Franciosi *et al*. 2009; Jamaly *et al*. 2010).

Citrate metabolism

Based on the results obtained in this study, the Lactobacillus strains studied under the present research conditions showed a difference in their ability to use citrate (Table 2). Of the 16 *Lactobacillus* strains studied just one strain *LB. plantarum18* was found to be negative citrate. Several studies have shown that some species of non-starter lactic acid bacteria (NSLB) isolated from milk and cheese, like *Lb. plantarum*, possess the ability to use citrate as the only carbon source and thereby metabolize it by producing different aromatic compounds such as acetate, lactate, acetone (Palles *et al.* 1998; Adesulu-Dahunsi *et al.* 2017).

Table 2. Summary of the results of the biochemical tests of *Lactobacillus* isolates.

Strains	Metabolism of citrate	EPS production	Autolysis[a] [%]	Proteolysis[a] [mM Gly]
LB. brevis2	+	+	2.90 ± 0.5	3.79 ± 0.27
LB. brevis10	+	+	1.76 ± 0.6	1.10 ± 0.09
LB. brevis14	+	+	2.96 ± 0.82	1.48 ± 0.25
LB. brevis16	+	+	2.71 ± 0.2	1.25 ± 0.04
LB. brevis19	+	+	1.32 ± 0.55	1.82 ± 0.16
LB. brevis20	+	+	2.06 ± 0.7	1.22 ± 0.15
LB. plantarum4	+	+	3.40 ± 0.5	1.17±0.14
LB. plantarum5	+	+	17.04 ± 0.63	1.36±0.14
LB. plantarum7	+	+	3.23 ± 0.68	9.39±0.94
LB. plantarum9	+	+	4.19 ± 0.89	4.63±0.11
LB. plantarum11	+	+	2.33 ± 0.59	1.17±0.34
LB. plantarum17	+	+	0.77 ± 0.15	1.30 ± 0.12
LB. plantarum18	-	+	3.01± 0.12	1.91 ± 0.03
LB. salivarus8	+	+	3.58 ± 0.57	2.14 ± 0.05
LB. acidophilus15	+	+	5.89 ± 0.89	1.40 ± 0.09
LB. pentosus3	+	+	4.07 ± 0.1	7.75 ± 0.36

[a] Presented values are means of duplicate determinations ± SD.

Exopolysaccharides (EPS) production and hemolytic activity

The EPS produced by lactic acid bacteria are used as thickeners or viscosifiers, stabilizing or emulsifying agents, and as gelling and water-binding agents or texturizers. It appears that all *Lactobacillus* were able to produce EPS as shown in Table 2. These cultures will be used as adjuncts cultures for their ability to improve the texture of "Rayeb" milk (Marshall and Rawson 1999). Stabilize the yogurt gel and decrease its tendency to

syneresis (Parente *et al.* 2017). And to product drinking yogurt, cheese, fermented cream, and milk based desserts, (Cerning 1995; Crescenzi 1995). They may also be involved in prebiotic, probiotic, and biological activities, as well as having potential application in the food industry (Harutoshi 2013; Shiby *et al*. 2013; Silva *et al*. 2019).

None of the strains tested produced hemolysis when tested on sheep blood. The absence of such activity should be a criterion for selecting strains to be used as a starter or adjunct cultures in dairy products (Giraffa 1995).

Antibacterial activity determination

Lactobacillus species had strong antibacterial against many bacterial pathogens (Jamaly *et al.* 2011; Eid *et al*. 2016) as well as perform essential roles in the preservation of food dairy product for human consumption (Mufandaedza *et al.* 2006; Batdorj *et al.* 2007). According to the results observed in the present work (Table 3), four isolates: *LB. plantarum5*, *LB. plantarum9*, *LB. brevis14*, and *LB. plantarum17* have no activity against the six pathogens; and none of *Lactobacillus* strains study showed bacteriocin activity spectrum against the indicator organisms since no inhibition was observed when treated supernatants (pH 6.5) was tested. It was the same results found by (Jamaly *et al.* 2011). While for the untreated supernatant it was observed that only the supernatant of the isolates *LB. pentosus LB. salivarus8*, *LB. acidophilus15*, *LB. brevis19*, and *LB. brevis20* had antibacterial activity against *Klebsiella pneumonia* CIP 53153.

However, we notice that the majority of the cells of

Table3. The antimicrobial activity determination of *Lactobacillus* against pathogenic bacteria.

Strains	***Listeria innocua* LMHAE-LI 107**			***Staphylococcus aureus* LMHAE-SA 105**			***Pseudomonas aeruginosa ATCC 29753***			***Klebsiella pneumonia* CIP 53153**			***Escherichia coli ATCC54127***			***Micrococcus luteus* ATCC15957**		
	S	**ST**	**C**	**S**	**ST**	**C**	**S**	**ST**	**C**	**S**	**ST**	**C**	**S**	**ST**	**C**	**S**	**ST**	**C**
LB. brevis 2	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. pentosus3	-	-	-	-	-	-	-	-	-	++	-	++	-	-	-	-	-	-
LB. plantarum4	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. plantarum5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LB. plantarum7	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. salivarus8	-	-	-	-	-	-	-	-	-	+	-	++	-	-	-	-	-	-
LB. plantarum9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LB. brevis10	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. plantarum11	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
LB. brevis14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LB. acidophilus15	-	-	-	-	-	-	-	-	+	++	-	++	-	-	-	-	-	+
LB. brevis16	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. plantarum17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LB. plantarum18	-	-	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-
LB. brevis19	-	-	+	-	-	-	-	-	-	++	-	++	-	-	-	-	-	++
LB. brevis20	-	-	-	-	-	-	-	-	-	++	-	++	-	-	-	-	-	+

Note: - No inhibition; + inhibition zone between 2 and 6 mm; ++ Inhibition zone larger than 6 mm. **C**: Cells of strains in fresh MRS broth; **S**: Cell-Free supernatant; **TS**: Cell-Free supernatant adjusted to pH 6.5 – 7 and treated with catalase. Results are averages of three experiments.

lactobacillus had an effective anti-pathogenic biological activity. The *lactobacillus LB. brevis19* presents an antibacterial activity against *Listeria innocua* LMHAE-LI 107. While *LB. acidophilus15* presents an antibacterial activity against *Pseudomonas aeruginosa* ATCC 29753. When *LB. acidophilus15*, *LB. brevis*19, and *LB. brevis20* showed activity on *Micrococcus luteus* ATCC15957. For all the *lactobacillus* isolates, except of *LB. plantarum5*, *LB. plantarum9*, *LB. plantarum17*, and *LB. brevis14*, they showed activity on *Klebsiella pneumonia* CIP 53153. Finally, none of the bacteria studied has activity on *Staphylococcus aureus* LMHAE-SA 105 and *Escherichia coli* ATCC54127.

Conclusion

Presented research concerned to the lactic acid bacteria isolated from local raw goat milk from the Oulmes region. It showed a special and important diversity of bacteria that may be due to the biotope of this area. So, *Lactobacillus plantarum* (43.75 %); *Lactobacillus brevis* (37.75 %); *Lactobacillus pentosus* (6.25 %); *Lactobacillus salivarus* (6.25 %); *Lactobacillus acidophilus* (6.25 %) were identified. This bacteria isolated from milk belongs to species commercially used for milk fermentation. However, according to biochemical activities, it is not suitable as starter cultures due to their low acidifying ability. Due to this reason it is necessary to make soon a comparative study between these isolates and commercially available starter cultures. Nevertheless, they could be used as good adjunct cultures because of their important technological potential, such as high extracellular proteolytic activity. All isolates also revealed ability to produce exopolysaccharides, while most of them could metabolize citrate and no hemolytic activity was observed.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Adesulu-Dahunsi AT, Sanni AI, Jeyaram K, Banwo K (2017) Genetic diversity of Lactobacillus plantarum strains from some indigenous fermented foods in Nigeria. LWT-Food Sci. Technol. 82: 199-206.

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

Ballesteros C, Poveda JM, González-Viñas, MA, Cabezas L (2006) Microbiological, biochemical and sensory characteristics of artisanal and industrial Manchegocheeses. Food Control. 17: 225-249.

Batdorj B, Trinetta V, Dalgalarrondo M, Prevost H, Dousset X, Ivanova I, Haertle T, Chobert JM (2007) Isolation, taxonomic identification, and hydrogen peroxide productionby *Lactobacillus delbrueckiisubsplactisT31*, isolated from Mongolian yogurt: inhibitory activity on food-borne pathogens fool-borne pathogens. J. Appl. Microbiol. 103: 584-593.

Beresford TP, Fitzsimons NA, Brennan NL, Cogan TM (2001) Recent advances in cheese microbiology. Int. Dairy J. 11: 259-27.

Boutrou R, Sepulchre A, Pitel G, Durier C, Vassal L, Gripon JC, Monnet V (1998) *Lactococcallysis* and curd proteolysis: Two predictable events important for the development of cheese flavor. Int. Dairy. J. 8: 609-616.

Buket K, Ozlem E, Yekta G, Ismail A, Sevil P, Asya C (2012) Genotypic identification and technological characterization of lactic acid bacteria isolated from traditional Turkish Kargi tulum cheese. Afr. J. Biotechnol. 11: 7218-7226.

Cerning J (1995) Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. Lait. 75: 463-472.

Chammas GI, Saliba R, Corrieu G, Beal C (2006) Characterization of lactic acid bacteria isolated from fermented milk “laban”. Int. J. Food Microbiol. 110: 52-61.

Church FC, Swaisgood HE, Porter DH, Catignani GL (1983) Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. J. Dairy. Sci. 66: 1219-1227.

Collins YF, Mcsweeney PLH, Wilkinson MG (2003) Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. Int. Dairy. J. 13: 841-866.

Crescenzi V (1995) Microbial polysaccharides of applied interest: Ongoing research activities in Europe. Biotechnol. Prog. 11: 251-259.

Dako E, El-Soda M, Vuillemard JC, Simard RE (1995) Autolytic properties and aminopeptidases activities of lactic acid bacteria. Food. Res. Int. 28: 503-509.

Donkor ON, Henriksson A, Vasiljevic T, Shah NP (2007) Proteolytic activity of dairy lactic acid bacteria and probiotics as determinant of growth and in vitro angiotensin-converting enzyme inhibitory activity in fermented milk. Lait. 87: 21-38.

Du Toit M, Franz CM, Dicks LM, Schillinger U, Haberer P, Warlies B, Ahrens F, Holzapfel WH (1998) Characterization and selection of probiotic *lactobacilli* for a preliminary minipig feeding trial and their effect on serum cholesterol levels, feces pH, and feces moisture content. Int. J. Food. Microbiol. 40: 93-104.

Durlu-Ozkaya F, Xanthopoulos V, Tunail N, Litopouloutsaneki E (2001) Technologically important properties of lactic acid bacteria isolates from Beyaz cheese made from raw ewes milk. J. Appl. Microbiol. 91: 861-870.

Eid R, El Jakee J, Rashidy A, Asfour H, Omara S, Kandil MM, Mahmood Z, Hahne J, Seida AA (2016) Potential antimicrobial activities of probiotic *Lactobacillus* strains isolated from raw milk. Journal Probiotics & Health. 4: 2.

El-Ghaish S, Dalgalarrondo M, Choiset Y, Sitohy M, Ivanova I, Haertle T, Chobert J-M (2010) Characterization of a new isolate of *Lactobacillus* fermentum IFO 3956 form Egyptian Ras cheese with proteolytic activity. Eur. Food Res. Technol. 230: 635-643.

El-Soda M, Madkor SA, Tong PS (2000) Evaluation of commercial adjuncts for use in cheese ripening: 4.

Comparison between attenuated and not attenuated lactobacilli. Milchwissenschaft. 55: 260-263.

El-Soda M, Farkye N, Vuillemard J, Simard R, Olson N, El Kholy W, Dako E, Medrano E, Gaber M, Lim L (1995) Autolysis of lactic acid bacteria.Impact on flavor development in cheese. *In* Charalambous G (Eds). Food flavour: Generation analysis and process influence, Amsterdam, The Netherlands. pp: 2205-2223.

FAO/WHO Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, October 2001.

Fitzsimons NA, Cogan TM, Condon S, Beresford T (2001) Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. J. Appl. Microbiol. 90: 600-606.

Fortina MG, Nicastro G, Garminati D, Neviani E, Manachini PL (1998) *Lactobacillus* heterogeneity in natural cheese starters. The diversity in phenotypic characteristics. J. Appl. Microbiol. 84: 72-80.

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

Ghanbari M, Rezaei M, Jami M, Nazari RM (2009) Isolation and characterization of *Lactobacillus* species from intestinal contents of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*). Iran. J. Vet. Res. 10: 152-157.

Giraffa G (1995) *Enterococcal* bacteriocins: their potential as anti Listeria factors in dairy technology. Food. Microbiol. 12: 291 -299.

Guidone A, Zotta T, Ross RP (2014) Functional properties of Lactobacillus plantarum strains: A multivariate screening study. LWT-Food Sci. Technol. 56: 69-76.

Harutoshi T (2013) Exopolysaccharides of lactic acid bacteria for food and colon health applications. *In* Kongo M (Eds.) Lactic acid bacteria-R & D for food, health and livestock purposes, InTech, Tokyo.

Herreros MA, Fresno JM, González Prieto MJ, Tornadijo ME (2003) Technological characterization of lactic acid bacteria isolated from Armada cheese (a Spanish goat's milk cheese). Int. Dairy. J. 13: 469-479.

Jamaly N, Benjouad A, Bouksaim M (2011) Probiotic potential of *Lactobacillus* strains isolated from known popular traditional Moroccan dairy products. Br. Microbiol. Res. J. 1: 79-94.

Jamaly N, Benjouad A, Comunian R, Daga E, Bouksaim M (2010) Characterization of *Enterococci* isolated from Moroccan dairy products. Afr. J. Microbiol. Res. 4: 1768-1774.

Jia FF, Zhang LJ, Pang XH, Gu XX, Abdelazez A, Liang Y, Sun SR, Meng XC (2017) Génomique (Séquence complète du génome de *Lactobacillus plantarum* KLDS1.0391 producteur de bactériocine, une souche probiotique avec résistance du tractus gastro-intestinal et adhérence aux cellules épithéliales intestinales. 109: 432-437.

Kempler GM, McKay LL (1980) Improved medium for detection of citrate-fermenting *Streptococcus lactis subsp. diacetylactis*. Appl. Env. Microbiol. 39: 926-927.

Kholif AM, Mahran GA, El-Nawawy MA, Ismail AA, Salem MME, Zaky WM (2011) Evaluation de l'activité protéolytique de certains *lactobacilles* laitiers. Journal mondial des sciences laitières et alimentaires. 6: 21-26.

Leisner JJ, Rusul G, Wee BW, Boo HC, Muhamad K (1997) Microbiology of chili bo, a popular Malaysian food ingredient. J. Food Prot. 60: 1235-1240.

Lortal S, Chapot-Chartier MP (2005) Role mechanisms and control of lactic acid bacteria lysis in cheese. Int. Dairy. J. 15: 857-871.

Maragkoudakis PA, Konstantinos CM, Psyrras D, Cremonese S, Fischer J, Cantor MD, Tsakalidou E (2009) Functional properties of novel protective lactic acid bacteria and application in raw chickenmeat against *Listeria monocytogenes* and *Salmonella enteriditis*. Int. J. Food. Microbiol. 130: 219-226.

Marshall V, Rawson HL (1999) Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. Int. J. Food Sci. Technol. 34:137-143.

Mora D, Fortina MG, Parini C, Ricci G, Gatti M, Giraffa G, Manachini PL (2002) Genetic diversity and technological properties of *Streptococcus thermophilus* strains isolated from dairy products. J. Appl. Microbiol. 93: 278-287.

Moslehishad M, Mirdamadi S, Ehsani MR, Ezzatpanah H, Moosavimovahed AA (2013) The proteolytic activity of selected lactic acid bacteria in fermenting cow's and camel's milk and the resultant sensory characteristics of the products. Int. J. Dairy Technol. 66: 279-285.

Mozzi F, Raya RR, Vignolo GM (2010) New approaches for the study of lactic acid bacteria biodiversity: A focus on meat ecosystem. *In* Mozzi F, Raya RR, Vignolo GM (Eds.), Biotechnology of lactic acid bacteria: Novel applications, Blackwell Publishing. pp. 251-272.

Mufandaedza J, Viljoen BC, Feresu SB, Gadaga TH (2006) Antimicrobial properties of lactic acid bacteria and *yeast* LAB cultures isolated from traditional fermented milk against pathogenic *Escherichia coli* and *Salmonelle enteritidis* strains. Int. J. Food Microbiol. 108:146-152.

Ouadghiri M, Amar M, Vancanneyt M, Swings J (2005) Biodiversity of lactic acid bacteria in Moroccan soft white cheese (Jben). FEMS Microbiol. Lett. 251: 267-271.

Palles T, Beresford T, Condon S, Cogan TM (1998) Citrate metabolism in *Lactobacillus casei* and *Lactobacillus plantarum*. J. Appl. Microbiol. 85: 147-154.

Parente E, Cogan TM, Powell IB (2017) Starter cultures: General aspects. *In* McSweeney PLH, Fox PF, Cotter PD, Everett DW (Eds.), Cheese (4th Edn.), Chemistry, Physics and Microbiology, Academic Press, pp. 201-226.

Park S, Ji Y, Park H (2016) Évaluation des propriétés fonctionnelles des *lactobacilles* isolés à partir de kimchi blanc coréen. *Contrôle des aliments*. 69 : 5-12.

Ray RC, Joshi VK (2014) Fermented Foods : scénario passé, présent et futur. *In* Ray RC, Montet D, (Eds.), Microorganismes et fermentation des aliments traditionnels, CRC Press, Boca Raton, Floride, USA, pp. 1-36.

Requena T, Pelaez C, Desmazeaud MJ (1991) Characterization of *lactococci* and *lactobacilli* isolated from semi-hard goat's cheese. J. Dairy. Res. 58: 137-145.

Salima R, Khadidja B, Halima ZK, Nour-Eddine K (2009) Proteolysis and autolysis properties of two *Lactobacilli* isolated from camel milk of South-Western Algeria. Eur. J. Sci. Res. 34: 218-227.

Shiby VK, Mishra HN (2013) Fermented milks and milk products as functional foods – a review. Crit. Rev. Food Sci. Nutr. 53: 482-496.

Silva LA, Lopes Neto JHP, Cardarelli HR (2019) Exopolysaccharides produced by *Lactobacillus plantarum*: technological properties, biological activity, and potential application in the food industry. Ann. Microbiol. 69: 321-328.

Soto A, Zapardiel J, Soriano F (1994) Evaluation of API Coryne system for identifying coryneform bacteria. J. Clin. Pathol. 47:756-459.

Teitelbaum J, Walker W (2002) Impact nutritionnel des pré- et probiotiques en tant qu'organismes gastro-intestinaux protecteurs . Annu. Rev. Nutr. 22: 107-138.

Wainrichter B, Luginbuhl W, Rohm H, Jimeno J. (2001) Differentiation of facultatively hetero fermentative *lactobacilli* from plants, milk and hard type cheeses by SDS-PAGE, RAPD, FTIR, energy source utilization and autolysis type. LWT-Food Sci. Technol. 34: 556- 566.

Wilkinson MG, Guinee, TP, O'Callaghan DM, Fox, PF (1994) Autolysis and proteolysis in different strains of starter bacteria during cheese ripening. J. Dairy Res. 61: 249-262.

Yvon M (2006) Key enzymes for flavour formation by lactic acid bacteria. Aust. J. Dairy. Technol. 61: 16-24.

Zamfir M, Vancanneyt M, Makras L, Vaningelgem F, Lefebvre K, Pot B, Swings J (2006) Biodiversity of lactic acid bacteria in Romanian dairy products. Syst. Appl. Microbiol. 29: 487-495.

Zadi Karam H, Karam NE. (2006). Bactéries lactiques du lait de chamelle d'Algérie: mise en évidence de souches de *Lactococcus* résistantes au sel. Tropicultura, 24: 153-156.