

Full Length Research Paper

Identification and major technological characteristics of *Lactococcus* and *Lactobacillus* strains isolated from "hamoum", an Algerian fermented wheat

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Twenty-eight strains of lactic bacteria were isolated from fermented wheat "hamoum" and phenotypically attributed to the following species: seven strains of *Lactococcus lactis* subsp. *lactis*, six strains of *Lactobacillus brevis* and 15 strains of *Lactobacillus plantarum*. The acidifying behavior of the strains is considerably variably demanding on considered strain. The amounts of lactic acid produced reached 9.7 g for lactococci. Strains (27) showed proteolytic activity in the presence of 1% skimmed milk. The lipolysis activity of *L. lactis* strains was greater than that expressed by lactobacilli. The search for aromatic activity showed that four out of ten citratase producing strains can produce acetoin. The results indicate that *L. plantarum* is the most dominant strain in the "hamoum" with the most important technological characteristics.

Key words: "Hamoum", *Lactococcus lactis* subsp. *lactis*, *Lactobacillus brevis*, *Lactobacillus plantarum*, identification, proteolysis, lipolysis, exopolysaccharides (EPS), aromatic activity.

INTRODUCTION

Cereals are by far the most important food resource in the world for both human and animal. Wheat (*Triticum* species), by its important nutritional power, remains one of the main human food resource (Cassman, 1999).

In Algeria, wheat was historically conserved in underground silos called "Matmor" or "Matmora". Due to the accidental infiltration of precipitation water into the "matmor", the humidified or flooded wheat grains undergo a spontaneous fermentation at the periphery and depth of the silo, which depends also on the nature of the soil. Humidity, uncontrolled temperature and the absence of air in the matmor cause microbial fermentation

phenomena that can last several years (\leq nine years). Fermented wheat taste is then discovered and entered into the eating habits for the manufacture of fermented wheat, bread or couscous, "lemzeiet", "elmechroub" or "hamoum". This fermented wheat has a variety of flavors, textures and aromas that are highly coveted by consumers in specific regions (Bekhouche et al., 2013).

Balance of total microbial population present in wheat grains can be affected by many factors (Wang et al., 2015). Elements of this imbalance include climatic conditions, mainly temperature and humidity, and biotic conditions associated with insect and mold attack and

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pesticide application. Among the microorganisms associated to wheat grains, lactic acid bacteria play a very important role in preserving the balance of the microbial flora and stabilizing the final fermentation products (Corsetti et al., 2017).

Lactic acid bacteria are a heterogeneous group of microorganisms producing lactic acid as the main product of metabolism. They colonize many food products such as dairy products, meat and vegetables. They are involved in a large number of spontaneous fermentations of food products and intervene in the dairy industry and fermentation of many other food products. They contribute to both texture and flavor of food and the production of aromatic compounds. They constitute a group of bacteria united by a multitude of morphological, metabolic and physiological characteristics. In general, they are described as Gram-positive bacteria, immobile, rods and cocci, non-sporulating, free of cytochromes and catalase, anaerobic micro-aerophiles, strictly fermentative, with complex nutritional requirements (amino acids, peptides, vitamins, salts, fatty acids, fermentable carbohydrates) and produce lactic acid as the main end product during carbohydrates fermentation (Axelsson, 2004).

Currently, lactic acid bacteria encompass 13 different bacterial genera: *Lactobacillus*, *Bifidobacterium*, *Leuconostoc*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Carnobacterium*, *Oenococcus*, *Weissella*, *Aerococcus*, *Tetragenococcus* and *Vagococcus*. Classification can be done according to phylogenetic criteria by the use of molecular methods. However, the classical phenotypic/biochemical characterization remains practical in the preliminary identification of microorganisms. Some phenotypic characteristics are used to identify species within genus such as ability to ferment carbohydrates, different bile concentrations toleration, extracellular polysaccharides production, growth factors requirement, acetoin production, and some enzymes synthesis. The G+C composition of the DNA, the fatty acid composition, the electrophoretic mobility of lactate dehydrogenase are criteria that can also be studied for the identification of lactic species (Vandamme, 1996; Stiles and Holzappel, 1997; Ho et al., 2007).

Lactobacillus is one of the most important genus involved in food microbiology, due to its role in food production and preservation. Lactobacilli contribute to the flavor of fermented foods by diacetyl production. The genus *Lactobacillus* was proposed by Beijerinck in 1901. They are long and fine (sometimes curved) rods often grouped in chains, immobile, non-sporulated, with negative catalase, and developed at 30 to 40°C. Lactobacilli have very complex nutritional requirements lactic acid bacteria. It is therefore quite heterogeneous, contains species with a wide phenotypic/biochemical variety, and physiological properties. Heterogeneity is reflected by the type of molecular percentage G+C of the DNA in the species of this genus (Schleifer and Ludwig,

1995; Axelsson, 2004; Hammes and Hertel, 2006).

The lactococci have been used primarily as starter cultures for various dairy products (yogurt, Cheddar, and hard cheeses). For most parts, they have been limited to N and D Streptococci and *Leuconostoc cremoris* and *Leuconostoc dextranicum*. The lactococci are Gram-positive cocci, nonmotile, grow at 10 and 40°C, but not 45°C, grow in 4% NaCl (except for *L. cremoris*); some species grow in 0.1% methylene blue milk medium. The lactococci ferment glucose by the hexose diphosphate pathway with the formation of L(+) lactic acid. In general, *Lactococcus* species produce smooth colonies with an entire edge on agar media (Carr et al., 2002).

Lactococcus lactis is predominantly found on plant material and in the dairy environment. It is extensively used in dairy fermentations, which is mainly due to its role in the development of texture and flavor through, for example proteolysis and the production of volatile flavor compounds. It also contributes to food preservation through the production of organic acids and bacteriocins such as nisin. Four *L. lactis* subspecies have been defined: subsp. *lactis*, subsp. *cremoris*, subsp. *hordniae*, and subsp. *tractae* (Backus et al., 2017).

To our knowledge, very few studies have been carried out on the fermented wheat "hamoum", but without a special interest regarding the technological interest in lactobacilli and lactococci. The metabolic activities of bacterial species sought by food industry, like production of lactic acid, aroma or thickening saccharides. Then, the objectives of this study were to isolate these lactic acid bacteria from "hamoum" in order to identify and highlight their technological characteristics.

MATERIALS AND METHODS

Isolation and storage of lactic acid bacteria

To carry out this study, 28 strains of lactic acid bacteria were isolated from fermented wheat "hamoum": three samples of hamoum of three different matmors (underground silos) were taken in sterile bottles; an aliquot of 5 g was homogenized with Stomacher, then was added to 10 ml of sterile skimmed milk and placed to coagulate at 30°C for 24 h in order to promote the development of the endogenous lactic flora. After coagulation of milk, the first dilutions were prepared by mixing 1 ml of each milk sample with 9 ml of physiological water (0.90% w/v NaCl solution). Decimal dilutions were then made in the same solution. Purification of bacterial strains was performed by the method of the streaks on solid MRS medium (De Man et al., 1960). Incubation was carried out at 30°C for 48 h. Obtained colonies were examined macroscopically and bacteria were characterized microscopically after Gram staining. The search for catalase activity was performed for all strains. Gram positive and catalase negative bacteria were then stored at -20°C in MRS medium supplemented (v/v) with 40% glycerol.

Strains identification

Physiological and biochemical study of strains

Bacterial growth was followed by spectrophotometric measures

(A_{600nm}) at different temperatures: 10, 15, 37, and 45°C, different pH: 4, 4.5 and 8 and different concentrations of NaCl: 2, 4 and 6.5% in liquid MRS medium. Each strain was seeded into two tubes containing the Falkow medium (Falkow et al., 1958): a control tube (without arginine) and a test tube (with arginine). The presence of arginine dihydrolase results in turning the pH indicator towards violet, whereas glucose fermentation in the control tube leads to the turning of the colored indicator towards the yellow.

Fermentation type was searched out for all the strains. The strains were seeded in tubes containing 10 ml of MRS medium and a Durham tube, and then incubated at 30°C for 48 h. The accumulation of gas in the Durham tube shows that the path of degradation of the sugar is heterofermentary, otherwise it is homofermentary.

Identification with API50 CHL galleries

Fermentation profiles of the strains were established using the API50 CHL biochemical galleries according to manufacturer instructions. The identification of strains was performed using Apiweb™ software of Biomerieux.

Study of technological characteristics of strains

Measurement of acidity produced by bacteria: The acidity produced by the bacteria in MRS medium was estimated by pH-meter using Dornic soda (N/9) (Karam and Karam, 1994) after an incubation period of 24 h at 30°C. Results were expressed in Dornic degrees according to the formula: Acidity (°D) = $n \times 10$ (n = average volume of soda to titrate 10 ml of milk; 1 °D = 0.1 g/L of produced lactic acid) (Accolas et al., 1971).

Bacterial proteolysis activity: Cells capacity for proteolysis was sought in MRS medium Na/Na₂-phosphate buffered to pH 7 supplemented with 2% of reconstituted sterile skimmed milk at 10%, according to the method described by Van Den Berg et al. (1993) and adapted by Roudj et al. (2009).

Lipolytic activity: Lipolytic activity was sought on solid MRS medium Na/Na₂-phosphate buffered to pH 7 and supplemented with 1% of milk fat as sole lipid source.

Aromatic activity: (1) Search for acetoin (Hydroxy-3-butanone-2 or acetylmethylcarbinol): Bacteria were inoculated into Clark and Lubs medium. After incubation at 30°C for 48 h, the production of acetoin was demonstrated by means of Voges-Proskauer colored reaction (Eddy, 1961). (2) Search for citratase: The production of citratase was demonstrated by bulk culture in semi-solid agar with citrated milk; prepared by adding 0.5 ml of 10% sodium citrate solution to 10 ml of milk with 1% (0.1 ml) of the preculture and then adding 4 ml of molten agar at 48°C (Harrigan, 1998).

Exopolysaccharides (EPS) production: The production of EPS was sought on Mayeux medium (*Leuconostoc* specific medium) and on hypersaccharosed solid MRS medium containing 50 g of sucrose per liter (Messens et al., 2002).

RESULTS AND DISCUSSION

Strains identification

After purification series on MRS medium (pH 5.4), bacterial colonies presented the following characteristics:

small, whitish, smooth, curved and with regular outline. Results of morphologic tests have also shown that all strains were Gram positive and catalase negative. Microscopic observation has revealed two cell forms: 7 strains have presented the shape of small cocci rallied in chains, recalling the form of *Lactococcus* strains, and 21 strains whose cells have presented the shape of isolated rods or short chains belong to *Lactobacillus* genus.

Among the lactobacilli, 14 isolates were homofermentatives and did not possess arginine dihydrolase. All these strains did not grow at 10°C but grew at 15 and 37°C. Out of them, only nine were able to grow at 45°C. These isolates belong to Group II of lactobacilli (Streptobacteria) according to Axelsson (2004) and Hammes and Hertel (2006) recommendations. The six other strains of bacilli belong to Group III of the lactobacilli (Betabacteria) because they are heterofermentatives with positive ADH, and grew at 15°C but not at 45°C. Eight strains of *Lactococcus* were homofermentary, possessed ADH and grew at 15, 37 and 45°C. Table 1 shows the physiological and biochemical characteristics of the strains.

Results of fermentation of the carbohydrates on the API 50CHL gallery allowed the identification of the strains. The results (Table 1) show that the 7 strains of *Lactococcus* belong to *L. lactis* subsp. *lactis*, the 6 heterofermentative strains of *Lactobacillus* are part of *Lactobacillus brevis*. The 15 homofermentative lactobacilli belong to *Lactobacillus plantarum*; which was confirmed by the ATCC 14917 *L. plantarum* carbohydrate fermentation profile obtained from Biomerieux database.

Technological characteristics of strains

Acidity produced by bacteria

The results (Figure 1) lead us to note that the acidifying behavior of these bacteria is variable from one strain to another in the same species. In this study, comparison between means of acidity production in all readings has not revealed any significant difference in all strains, which reflects stability of this characteristic (Table 2).

The strains of *L. plantarum* has produced acidity varying from 10 to 90°D, which is greater than that produced by strains of *L. brevis*. These results are in agreement with those of Zhang and Vadlani (2014). *L. plantarum* is known to be homofermentative to hexoses, producing 2 moles of lactic acid per hexoses mole (Passos et al., 1994). However, the higher acidifying behavior was that of the strains of *L. lactis*. They produced lactic acid amounts of up to 9.7 g/L, which is in agreement with the work of Åkerberg et al. (1998). In general, lactobacilli ferment lactose by producing lesser amounts than *Lactococcus* and this was also suggested by Herreros et al. (2003). In fact, comparison between means of acidity production of the different strains has revealed a significant difference ($P < 0.05$) (Table 3).

Table 1. Phenotypic characteristics of strains isolated from "hamoum".

Species	Code of the strain	Arginine dihydrolase	Gas production	Growth at different									
				Temperature (°C)				% NaCl			pH		
				10	15	37	45	2	4	6.5	4	4.5	8
<i>Lactobacillus brevis</i>	HMTK10	+	+	-	+	+	-	-	-	-	+	+	-
	HMTK24	+	+	-	+	+	-	+	-	-	+	+	-
	HMTK29	+	+	-	+	+	-	+	+	-	-	-	-
	HMTK52	+	+	-	+	+	-	+	-	-	-	-	-
	HMTK56	+	+	-	+	+	-	-	-	-	-	-	-
	HMTK57	+	+	-	+	-	-	-	-	-	-	-	-
<i>Lactobacillus plantarum</i>	HMTK2	-	-	-	+	+	+	+	+	+	+	+	+
	HMTK6	-	-	-	+	+	+	+	+	-	-	-	-
	HMTK8	-	-	-	+	+	+	+	+	-	-	+	-
	HMTK9	-	-	-	+	+	+	+	+	+	-	-	+
	HMTK21	-	-	-	+	+	-	+	-	-	-	+	-
	HMTK23	-	-	-	+	+	+	+	+	-	-	-	+
	HMTK25	-	-	-	+	+	-	+	-	-	-	-	-
	HMTK26	-	-	-	+	+	-	+	-	-	-	-	-
	HMTK28	-	-	-	+	+	-	+	-	-	-	-	-
	HMTK50	-	-	-	+	+	-	+	-	-	+	+	-
	HMTK51	-	-	-	+	+	-	+	+	-	+	+	-
	HMTK53	-	-	-	+	+	+	+	-	-	-	-	-
	HMTK58	-	-	-	+	+	+	+	+	-	-	+	-
	HMTK59	-	-	-	+	+	+	+	+	+	-	-	+
<i>Lactococcus lactis ssp lactis</i>	HMTK1	+	-	-	+	+	+	+	+	-	-	-	+
	HMTK3	+	-	-	+	+	+	+	+	+	-	-	-
	HMTK4	+	-	-	+	+	+	+	-	-	+	+	-
	HMTK7	+	-	-	+	+	+	+	-	-	-	-	+
	HMTK20	+	-	-	+	+	+	+	+	+	-	+	+
	HMTK22	+	-	-	+	+	+	+	+	-	-	-	+
	HMTK54	+	-	-	+	+	+	+	+	-	-	-	+
	HMTK55	+	-	-	+	+	+	+	+	+	-	-	+

+, Positive reaction; -, negative reaction.

According to these results, it may be suggested that strains with good acidifying activity can be proposed for application in the dairy industry, in which they lead to pH decrease, which plays an important and essential part in the coagulation of milk by destabilizing the casein micelles on one hand and giving the product its distinct and characteristic taste, thus contributing to flavor and aroma production. They may also act as inhibitors of undesirable micro-organisms.

Proteolysis activity

All the tested strains except the HMTK24 strain showed a growth with proteolysis activity confirmed by the appearance of a clear halo around the colonies seeded in

a touch on the surface of the MRS medium supplemented with 1% of skimmed milk reconstituted at 10% (Figure 2).

According to Vuilleumard (1986), the strain is considered as a proteolytic one if it presents a lysis zone with a diameter of 5 to 15 mm. In comparison with this data, our strains are revealed to be proteolytic, with proteolysis zone diameters between 6 and 14 mm. *L. lactis* subsp. *lactis* strains are more proteolytic than *Lactobacillus* strains, of which 50% have a lysis zone greater than or equal to 10 mm. The statistical study showed significant differences ($p < 0.05$) between the results obtained for the three species (Table 4). These results are consistent with those of Hassaïne et al. (2007). *L. lactis* possesses a complex proteolysis system comprised of multiple intracellular peptidases and a single protease anchored

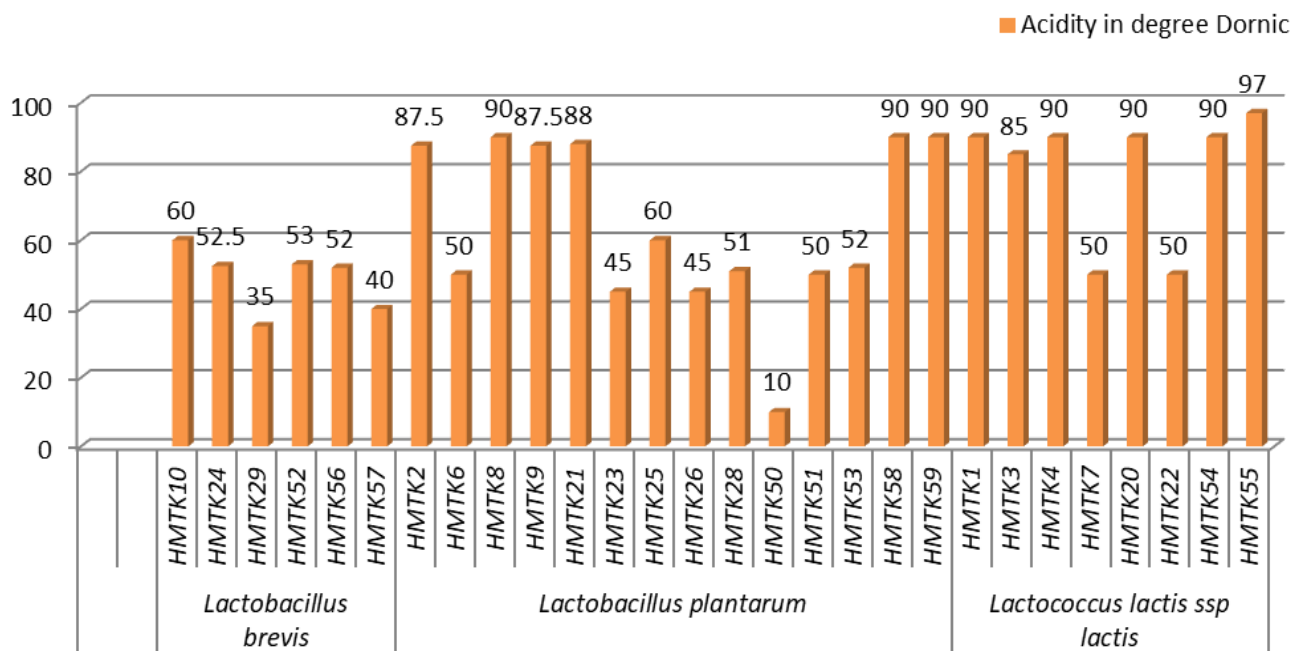


Figure 1. Acidity produced by the strains.

Table 2. Mean \pm standard deviation of acidity produced by strains during experiment assays.

Parameter	<i>Lactobacillus brevis</i>	<i>Lactobacillus plantarum</i>	<i>Lactococcus lactis</i>
1 st assay	46.50 \pm 8.41	64.00 \pm 24.91	80.25 \pm 18.95
2 nd assay	49.25 \pm 8.75	64.04 \pm 24.80	79.75 \pm 18.85
3 rd assay	48.25 \pm 10.01	64.68 \pm 24.77	80.88 \pm 18.87

to the cell surface, PrtP, whose gene is plasmidic, a serine protease that allows growth in milk by hydrolyzing caseins (Kunji et al., 1996). Some strains of *L. lactis* possess a surface protease specific to the maturation of the precursor of nisin. A functional protease of the HtrA family was demonstrated in *L. lactis* (Poquet et al., 2001).

The strains of *Lactobacillus* exhibit proteolytic activity with lysis zones with a diameter ranging from 6 to 12 mm, which is in line with the results of Roudj et al. (2009). *L. brevis* have shown a moderate level of proteolysis compared to *L. plantarum* with lysis diameters not exceeding 8 mm, these results being in agreement with the work of Belkheir et al. (2017). Strains exhibiting high proteolytic activity could be used with other ferments as complement or secondary culture. These strains can contribute to the development of the flavors during the maturation stage of cheese or in the manufacture of the fermented beverages.

L. lactis and *Lactobacillus* are largely deficient in the capacity of amino acid biosynthesis, which is compensated for by the ability to synthesize a large number of peptidases, amino acid permeases and

multiple oligopeptide transport systems (Opp) (Klaenhammer et al., 2005). A large number of *Lactococcus* and *Lactobacillus* peptidases have been purified and biochemically characterized; in most cases, the corresponding gene has been cloned and sequenced (Kirsi et al., 2006). The first step in the use of casein by lactic acid bacteria is performed by CEP. Five different types of these enzymes (PrtP endoprotease, 2 general PepN and PepC aminopeptidases, PepO1 endopeptidase and Opp oligopeptide transport system) were cloned and characterized, including PrtP from *L. lactis*, whose gene (*prtP*) can be found either on plasmidic or chromosomal DNA, while the CEPs of lactobacilli are coded by genes on chromosomal DNA (Holck and Naes, 1992; Guédon et al., 2001; Kelleher et al., 2017).

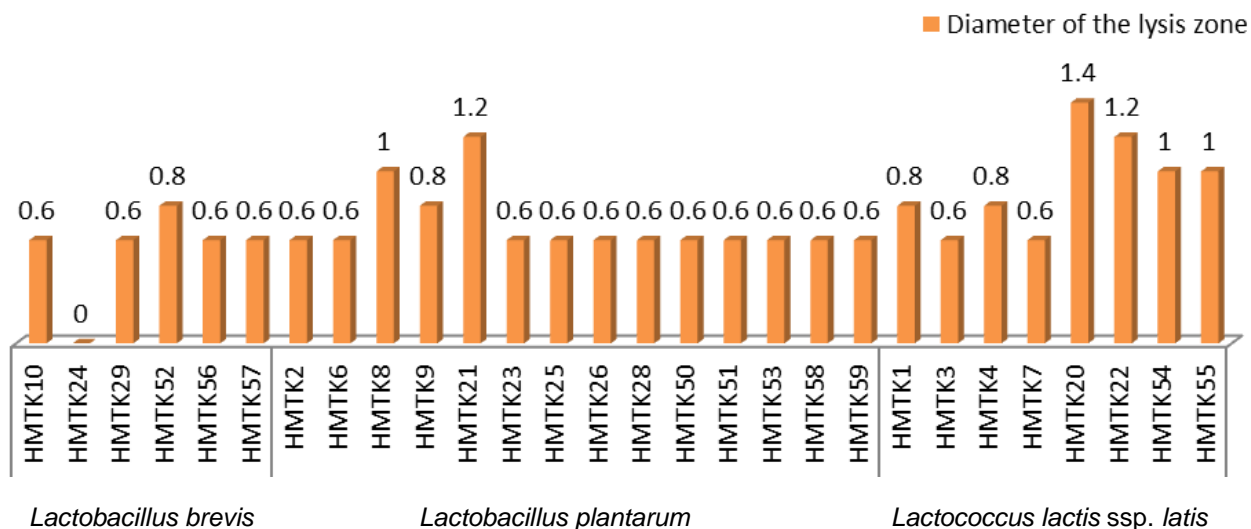
Lipolytic activity

The lipolytic activity of the strains of the same species is highly variable. An example of the result is as shown in

Table 3. Acidity production expressed by different strains.

Strain	Mean \pm standard deviation
<i>Lactobacillus brevis</i>	48.75 \pm 9.33 ^(a)
<i>Lactobacillus plantarum</i>	64.00 \pm 24.91 ^(a)
<i>Lactococcus lactis</i> subsp <i>lactis</i>	80.25 \pm 18.95 ^(a)

Values are mean \pm standard deviation. ^(a)No significant difference was obtained by Duncan's test between three assay.

**Figure 2.** Proteolytic activity of the bacterial strains.**Table 4.** Proteolysis expressed by different strains.

Strain	Mean \pm standard deviation
<i>Lactobacillus brevis</i>	0.533 \pm 0.273 ^(a)
<i>Lactobacillus plantarum</i>	0.6857 \pm 0.1875 ^(a)
<i>Lactococcus lactis</i> subsp <i>lactis</i>	0.9250 \pm 0.2816 ^(a)

Values are mean \pm standard deviation. ^(a)No significant difference was obtained by Duncan's test between three assay.

Figure 3. The ratio of the clear halo to the colony diameter (h/c) expressing the lipolytic activity was calculated for each strain (Figure 4). The statistical study of the means obtained for the lipolytic activity shows significant differences ($p < 0.05$) by Duncan's post hoc test (Table 5). The strains of *Lactococcus lactis* ssp *lactis* express a higher activity with ratios ranging from 2 to 5 by comparing it with that expressed by *L. plantarum*. The two species preferentially hydrolyze short chain fatty acids knowing that the milk fat is rich of these fatty acids. *L. lactis* exhibits a stronger esterase activity than that of *L. plantarum* according to Macedo et al. (2003) and Karam

et al. (2012), which confirms the results of the present study. Several studies have characterized the esterases of *L. plantarum* (Gobbetti et al., 1997; Brod et al., 2009; Esteban-Torres et al., 2013, 2014, 2015; Kim et al., 2017). The lipolytic activity of *L. brevis* remains the lowest with ratios (h/c) not exceeding 3.

The study of Herreros et al. (2004) suggests that *L. plantarum* strains hydrolyze C8 and C14 fatty acids while those of *L. brevis* have shown greater esterase activity on C4 and C8 fatty acids. The study of the genome of *L. plantarum* WCFS1 reveals the presence of a rich repertoire of esterases and lipases suggesting their important role in cellular metabolism, among them LpEst

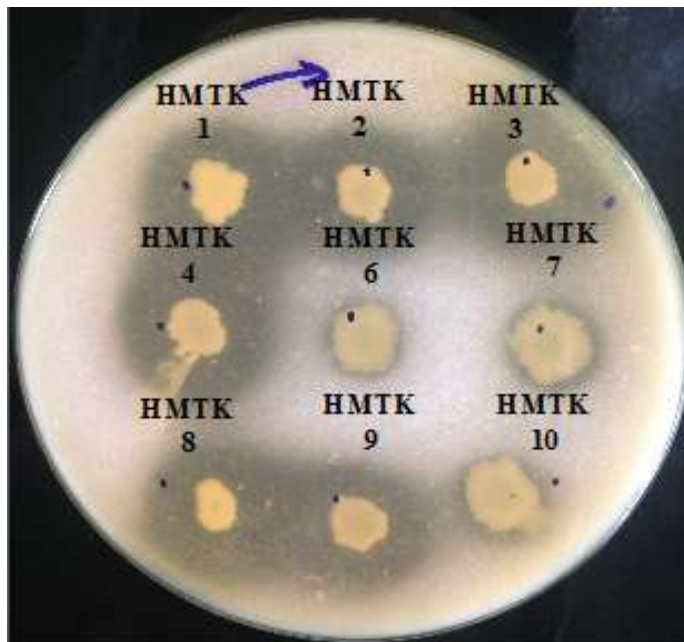


Figure 3. Lipolytic activity on MRS medium deprived of tween 80 and supplemented with 1% milk fat.

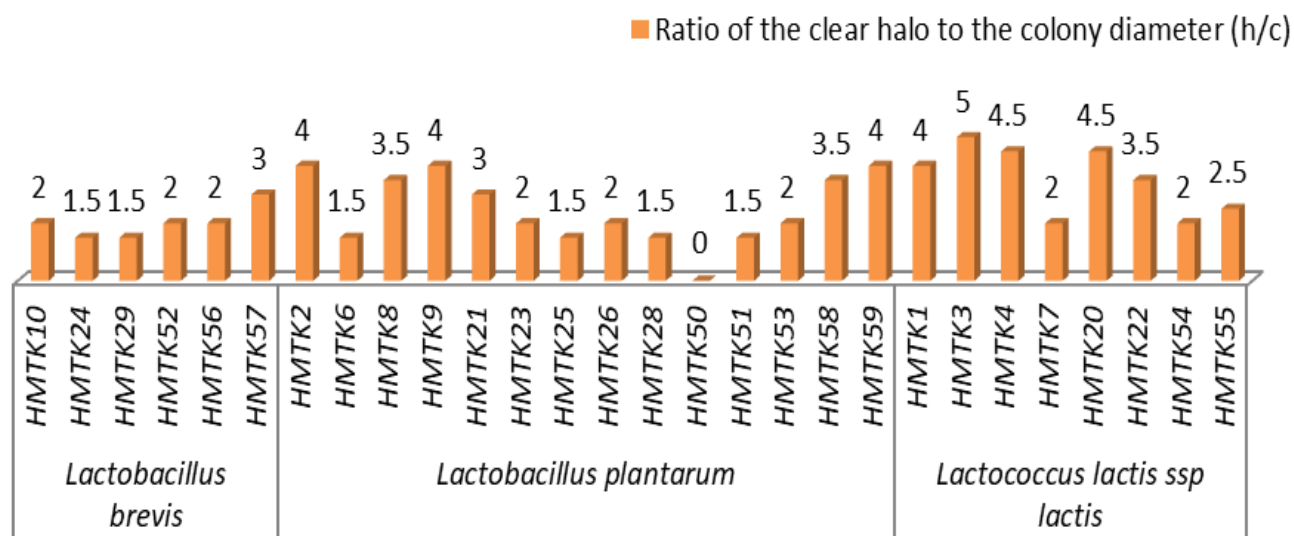


Figure 4. Lipolytic activity expressed by the strains on the milk fat.

carboxylesterase (Alvarez et al., 2014).

Lipases find promising applications in various fields: hydrolysis and synthesis of long-chain acylglycerols, manufacture of detergents, manufacture of food ingredients, application in the paper industry and biocatalysis of stereoselective transformations. They are widely used in the treatment of products of organic chemistry, the manufacture of cosmetic and pharmaceutical products as well as increased stability or

enantioselectivity (Kapoor and Gupta, 2012).

Production of exopolysaccharides

The production of EPS by lactic bacteria is a favorable trait to many industrial food processes. The main advantage of the use of exopolysaccharide-producing lactic bacteria in lactic ferments during the production of

Table 5. Lipolysis expressed by different strains.

Strain	Mean \pm standard deviation
<i>Lactobacillus brevis</i>	2.000 \pm 0.548 ^(a)
<i>Lactobacillus plantarum</i>	2.429 \pm 1.238 ^(a)
<i>Lactococcus lactis</i> subsp <i>lactis</i>	3.500 \pm 1.195 ^(a)

Values are mean \pm standard deviation. ^(a)No significant difference was obtained by Duncan's test between three assay.

**Figure 5.** Production of exopolysaccharides on hypersaccharosed MRS medium.

fermented milks is the improvement of the texture and the reduction of the syneresis (expulsion of liquid from a gel). According to this test, negative results were obtained on the Mayeux medium for all the lactic strains, which were unable to develop by forming colonies with a more or less glutinous aspect, testifying to the production of a thickening agent, exopolysaccharides. Nevertheless, four strains, HMTK2, HMTK4, HMTK10 and HMTK24, produce EPS on hypersaccharosed MRS medium (Figure 5).

Several works focus on the EPS production by *Lactobacillus* (Ismail and Nampoothiri, 2010; Dilna et al., 2015; Fontana et al., 2015; Salazar et al., 2015; Oleksy and Klewicka, 2016). To date, about 30 species of *Lactobacillus* producing EPS have been identified, the most well-known being *Lactobacillus casei*, *Lactobacillus acidophilus*, *L. brevis*, *Lactobacillus curvatus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *L. plantarum* and *Lactobacillus johnsonii*. The genetic determinants of EPS are carried either by a plasmidic or a chromosomal DNA. The genes encoding for proteins responsible for EPS synthesis by mesophilic lactic bacteria are generally located on a plasmid. In *Lactococcus*, the production of EPS is less stable, the main reasons being the plasmidic

location of the production genes and the presence of a mobile insertion sequence (IS, e.g. ISS1, ISS981) (Sanlibaba and Çakmak, 2016).

Aromatic activity

Co-metabolism of citrate, fermentable sugar is very important in lactic bacteria since it is closely related to the aromatic activity. The strains HMTK2, HMTK4, HMTK8, HMTK10, HMTK20, HMTK21, HMTK24, HMTK50, HMTK51 and HMTK58 were found to produce citratase (Figure 6).

Lactic bacteria using citrate play an important role in many dairy processes. They are responsible for the production of aromatic compounds (diacetyl and acetoin). Diacetyl is essential for establishing the flavor of dairy products such as butter and buttermilk and sometimes, young cheeses. Because of these properties, these lactic bacteria are often called aroma bacteria. During the citrate metabolism, CO₂ is also produced, which leads to eye formation in certain types of cheese.

The strains HMTK 8, HMTK20, HMTK21 and HMTK58 show a positive result for the production of acetoin (Figure7).

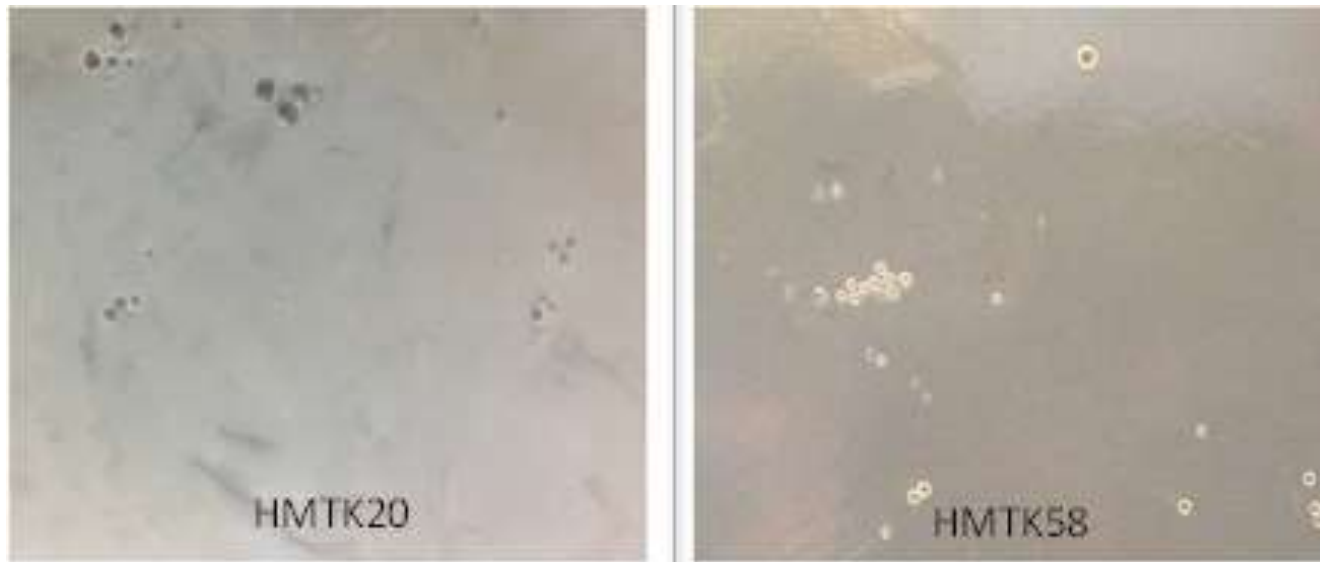


Figure 6. Citratase production in semi-solid agar with citrated milk.

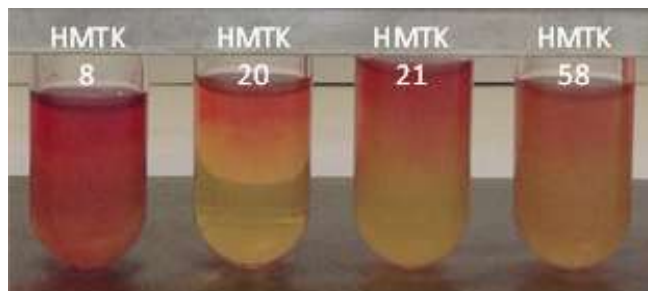


Figure 7. Production of acetoin revealed by Voges-Proskauer colored reaction.

Conclusion

This study shows the interesting technological properties presented by strains of *L. lactis* ssp *lactis*, *L. plantarum* and *L. brevis* isolated from hamoum. Some of them produce amounts of lactic acid of up to 9.7 g/L. They express proteolytic activity of milk proteins and lipolytic of milk fat. Ten strains possess citratase and four strains produce acetoin. These strains are good candidates for use in the food industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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