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Characterization of dominant cultivable lactic acid bacteria isolated from West Algerian raw camel's milk and assessment of their technological properties

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Abstract

In Algeria arid regions, camel milk is considered as one of the most important source of dairy products for human diet with potential therapeutic effects. The aim of the study was to characterize isolates of lactic acid bacteria from Algerian raw camel's milk and to study some of their technologically important properties. Microbiological diversity of Algerian raw camel's milk was determined by phenotypical, physiological, biochemical and genotypic characteristics. Only 134 Gram-positive and catalase-negative non-spor forming isolates were retained. Theses isolates were chosen for identification using API50CHL and 16S rDNA sequencing. From a total of 134 isolated lactic acid bacteria, 5 presumptive genera were determined, 47 *Enterococcus*, 29 *Lactobacillus*, 26 *Weissella*, 19 *Lactococcus* and 13 *Leuconostoc*. All the isolates were characterized by the determination of some technological aptitudes showing interesting features to be used as a starter in the production of fermented dairy in term of proteolytic activity, acetoin and dextran production. Acidification and growth kinetic during 48 h was carried out for the isolates, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Lactobacillus rhamnosus* and *Leuconostoc mesenteroides*, allowing to subdivide them in three groups: fast acidifying isolate, medium acidifying isolate and slow acidifying isolate.

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Introduction

In Algeria arid regions, camel's milk is considered as one of the most important source of dairy products for human diet with potential therapeutic effects. Lactic Acid Bacteria (LAB) are generally associated to habitats rich in nutrients like many food products (milk, meat, vegetables, drinks) (Boumehira *et al.*, 2011; König and Fröhlich, 2017; Salminen and Von Wright, 2004). Recent studies showed that camel's milk is a natural source for probiotics (Al-Otaibi *et al.*, 2013, Fguiri *et al.,* 2015). The dominant and beneficial microflora in camel's milk represented by LAB is a potential source of biological materials to be used in dairy technology (Khedid *et al.*, 2009). LAB comprise a heterogeneous group of non-sporulating Gram-positive organisms which ferment sugars and produce lactic acid. Their ability to lower pH by producing acid from sugar leads to the development of desirable organoleptic properties prevents the growth of pathogens and ensures the stability and safety of the final product.

The monograph of Orla-Jensen (1919) represents the reference in the LAB studies. The morphological characteristics, the fermentation way of glucose, the growth at certain temperature and fermentation of carbohydrates were used to identify these bacteria. All these characteristics are considered as important in the classification of the LAB (Axelsson, 2004; Boumehira *et al.,* 2011). Phenotypic identification and biochemical tests have been considered for a long time the conventional procedure for routine identification of bacteria. Several methods have been developed in the last few years and many commercial multi-test kits such as the analytical profile index (API) test kits are widely used. Recently, new molecular tools have been applied for the routine identification of microorganisms, and had led to an increase in the number of identified bacteria (Bittar and Rolain, 2010, Fguiri *et al.,* 2015). Most of the advance molecular methods are based on 16S ribosomal DNA sequences, complete or partial genomes (Ben Amour *et al.,* 2007).

They are also the object of intensive international researches for their ability to produce several antimicrobial compounds such as bacteriocin (De Vuyst and Leroy, 2007, Benmechernene *et al.*, 2013), for their essential role in the food fermentation and degradation of protein that lead to the synthesis of a wide range of compounds such as : organic acids, peptides, aromatic compounds and exopolysaccharides (Caplice and Fitzgerald, 1999; Leroy and De Vuyst, 2004). These products are involved in the organoleptic, technological and nutritional characteristics of these fermented foods (Widyastuti and Febrisiantosa, 2014).

If the cow and the goat's milk were widely studied and investigated, the camel's milk is still not well-known talking about its bacterial ecosystem especially the LAB. In the present investigation, LAB isolated from Algerian camel's milk were characterized by phenotypic and genotypic criteria and some of their technological relevant properties were screened in order to be evaluated as a new starters in controlled fermentations.

Material and methods

Sampling

Twelve (12) samples of raw camel's milk have been collected from different regions of south western of Algeria. The samples have been immediately cooled and transported to the laboratory in an isotherm container and analysed upon arrival.

Isolation of strains

10 mL of camel's milk were homogenized with 90mL of 0.1% (w/v) sterile peptone water to obtain a 1:10 dilution. Successive decimal dilutions were carried out with sterile 0.1% (w/v) peptone water (Oxoid). From each dilution 0.1mL volumes were surface plated in MRS agar (De Man *et al.*, 1960) and M17 agar (Terzaghi & Sandine, 1975) and incubated at 30°C for 24–48h. After growth, the colonies were randomly picked from plates and several representative strains displaying the general characteristics of LAB were chosen from each plate for further studies to apply the conventional tests for identification (Nguyen *et al.*, 2007, Marroki *et al.*, 2011). The transfers of retained colonies were repeated until to get pure colonies.

Working cultures were kept on MRS agar or M17 agar slant at 4°C and streaked every 4 weeks (Herrero *et al.*, 1996). For long-term storage of isolates, stock cultures were stored at -20°C in 30% (v/v) glycerol, with 70% (v/v) skim milk (Badis *et al.*, 2004).

Isolates characterization

All the isolates were initially examined for Gram staining and catalase reaction. Only Gram-positive and catalase-negative isolates were considered. They were tested for morphological aspect, production of CO_2 from glucose, hydrolysis of arginine (ADH) on M16BCP, growth at two different temperature, 15°C and 45°C, growth at 4 and 6.5% of NaCl, growth at pH 5.4 and 9.6 (Boumehira *et al.,* 2011; Marroki *et al.,* 2011). The hydrolysis of esculin is shown in the Bile Esculin Agar (BEA) where a positive reaction results is a blackening of the medium due to soluble Fe^{3+} and esculetine (Zarour *et al.*, 2012).

Molecular identification

The genomic DNA of ten strains was extracted using the DNA extraction and purification kit according to the manufacturer instructions (Fermentas, UK). The PCR reaction mixture contained 0.5µL of template DNA, 2.5µL of reverse primer (10mM), 2.5µL of forward primer (10mM), 2µL of dNTP (25mM), 4µL of $MgCl_2$ (25mM), 5µL of PCR buffer (10X) and 1µL Taq polymerase, in a 50µL final volume. The primers sequences used were U 27F (5'TCAACCGGGGAGG GT3') and 1432R (5'ATTACTAGCGATTCCGG3') used by (Deasy *et al.*, 2000). The cycling program was 94°C for 3 min, 29 cycles at 94°C for 40 sec, 55°C for 50 sec and 72°C for 2 min. Sequencing of PCR product was made by the sequencing facility offered by Eurofins (Germany). The obtained nucleotide sequences, displayed by BioEdit software, were analyzed using the blast tool of the NCBI site in order to research identity percentages with the sequences present in databases (http://blast.ncbi.nlm. nih.gov/ Blast.cgi).

Technological aptitudes

Citrate test

The KMK medium (Kempler and McKay, 1980), containing ferric citrate and potassium ferrocyanide is used to perform this test. After incubation at 30°C or 45°C for 24 h to 48 h, strains using citrate give blue colonies (Moulay *et al.*, 2013).

Acetoin production

The retained strains were inoculated in 10mL of Clarck and Lubs broth and incubated at 30°C for 24 h. Then, the reaction of Voges-Proskauer was performed by adding 0.5mL of α-naphtol to 6% in absolute alcohol (VP1) and 0.5mL of sodium hydroxide to 16% in distilled water (VP2) to 1mL of the fresh culture. The tubes are then stirred, after 10 min of rest, the presence of a pink ring on the surface of the culture was associated to a positive reaction (Guiraud, 1998; Moulay *et al.,* 2013).

Dextran production

Dextran production was performed on MSE agar medium (10% sucrose) (Mayeux *et al.*, 1962). Dextrane producing strains give viscous colonies (Zarour *et al.,* 2012).

Extracellular proteolytic Activity

To determine extracellular proteolytic activity of the isolated strains, Plate Count Agar (PCA) supplemented with 2% of skim milk was used. The presence of clear zones around the colonies indicates a proteolytic activity (Guessas *et al.*, 2012; Moulay *et al.,* 2013).

Growth kinetics of selected strains

The growth kinetic was performed on 3 strains chosen according to their acidifying ability. 10mL of skim milk already prepared and sterilized by autoclaving at 110°C for 10 min, was inoculated with overnight culture of these isolates and incubated at 30°C until coagulation. These pre-cultures are then distributed in tubes and incubated at 30°C. Samples were taken aseptically to carry out pH, total acidity (°D) following observation points: 0, 2, 4, 6, 8, 10, 12, 16, 20, 24 and 48 h (Boumehira *et al.,* 2011; Moulay *et al.,* 2013; Zarour *et al.*, 2013). The variation of pH is followed by a pH meter, total acidity (°D) was determined by titration of 10mL of each sample with NaOH 1/9 N and reported as a Degree Dornic of lactic acid per liter (Kihal *et al.*, 2009; Zarour *et al.,* 2013) and bacterial growth is followed by counting on solid medium containing

between 25 and 250 colonies (MRS or M17) and growth characteristics (growth rate, generation time) are calculated according to (Hassan *et al.*, 1989, Fu and Mathews, 1999, Rao *et al.*, 2004).

Results

Phenotypic identification of isolates

From 12 samples of camel's milk, we retained 134 Gram +, catalase – and non-spore forming isolates considered as LAB. After microscopic observation, we have determined 78% as cocci, 29% shaped as coccobacilli and 22% as rods. Among the 66 cocci, using physiological, biochemical properties, based on: production of CO_2 from glucose, growth at different temperature and different concentration of NaCl we were able to subdivide them into 2 genera: 47 isolates determined as presumptive *Enterococcus* which were homofermentative LAB that were able to grow at 45°C, in a presence of 6.5% of NaCl, able to grow at a pH of 9.6 and hydrolysis esculin. The two representative species are *Enterococcus faecalis* (32) and *Enterococcus durans* (15). The second group of LAB were determined as presumptive *Lactococcus* :19 homofermentative isolates subdivided into 2 presumptive species and subspecies, 15 isolates determined as *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (ADH^+, $acetoine^+$, NaCl 4%$^+$) (Badis *et al.*, 2004; Khedid *et al.*, 2009), 4 isolates *Lactococcus lactis* subsp. *lactis* (ADH^+, $acetoin^-$, NaCl 4%$^+$) (Badis *et al.*, 2004; Khedid *et al.*, 2009).

Among these lactococci, about 50% strains was considered as wild one and could resist to a pH of 9.6 and/or a temperature of 45°C which is in correlation with what was previously described in Drici *et al.* (2010). The coccobacilli shaped isolates, regarding to the hydrolysis of Arginine, they were divided into two genera, ADH^-: 26 isolates were considered as *Weissella paramesenteroides*, ADH^+: 13 isolates producing dextran from hypersaccharosis media, identified as Leuconostoc mesenteroides (Kihal *et al.*, 2007; Zarour *et al.*, 2013). And finally, 29 rods were classified up to their physiological and biochemical characteristics and were referred to the genus *Lactobacillus* where, 19 thermophilus isolates able to grow at 45°C, ADH^-, CO_2^- (from glucose), determined as presumptive *Lactobacillus rhamnosus* (Badis *et al.*, 2004) and 10 mesophilic isolates not able to grow at 45°C ADH^-, CO_2^- (from glucose), determined as presumptive *Lactobacillus plantarum* (Badis *et al.*, 2004; Zergui *et al.*, 2015). The general results of physiological, biochemical properties tested on the isolates are summarized in the table 1.

Table 1. Biochemical and physiological criteria of presumptive Lactic Acid Bacteria isolated from camel milk.

Presumptive strains	Biochemical and physiological properties									
	Citrate hydrolysis	Esculin hydrolysis	ADH production	Dextrane production	Acetoin production	CO_2 production	Growth at 45°C	growth at pH 9.6	Growth at 4% NaCl	Growth at 6.5% NaCl
Lactococcus genus (19)										
Lactococcus lactis subsp. lactis (4)	-	+	+	-	-	-	+	+	+	-
Lactococcus lactis subsp. *lactis* biovar. *diacetylactis* (15)	+	+	+	-	+	-	V	V	V	-
Enterococcus genus (47)										
Enterococcus faecalis (32)	+	+	+	-	+	-	+	+	+	+
Enterococcus durans (15)	-	+	+	-	+	-	+	+	+	+
Leuconostoc genus (13)										
Leuconostoc mesenteroides subsp. *dextranicum* (7)	+	+	-	+	+	+	+	-	+	-
Leuconostoc mesenteroides subsp. *mesenteroides* (6)	+	V	-	+	+	+	-	-	+	-
Weissella genus (26)										
Weissella paramesenteroides (26)	V	+	+	-	+	+	-	-	V	-

Presumptive strains	Biochemical and physiological properties									
	Citrate hydrolysis	Esculin hydrolysis	ADH production	Dextrane production	Acetoin production	CO_2 production	Growth at 45°C	growth at pH 9.6	Growth at 4% NaCl	Growth at 6.5% NaCl
Lactobacillus genus (29)										
Lactobacillus rhamnosus (19)	+	+	-	-	+	-	+	-	+	-
Lactobacillus plantarum (10)	V	+	-	-	V	-	-	-	+	-

-: negative result, +: positive result, V: variable result

Distribution of LAB

Distribution of genera

From the obtained results, there is a clear dominance of *Enterococcus* genus with 35% followed by *Lactobacillus* with nearly 22% and *Weissella* with more than 19%, *Lactococcus* came in the forth position with less than 15% and finally the genus *Leuconostoc* with a frequency less than 10% (Fig. 1).

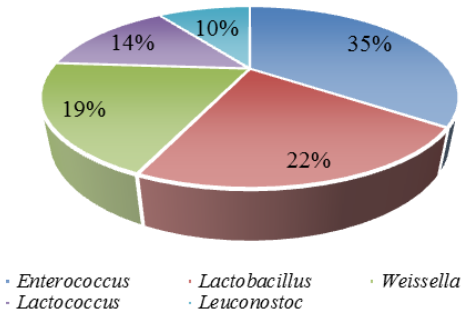

Fig. 1. Distribution of genera of Lactic Acid Bacteria isolated from raw camel's milk.

Distribution of species

Table 2 shows the frequency of the dominants each species found in the Algerian raw camel's milk. Regarding to the obtained results, the most frequent specie found is *Enterococcus faecalis* (23.80%). The genus *Enterococcus* was represented by two species *Enterococcus faecalis* and *Enterococcus durans*, they are communally met in the raw milk and its derivative (Aguilar-Galvez *et al*., 2012; Ismaili *et al*., 2016; Pedro Nieto-Arribas *et al*., 2011). The second most frequent specie is *Weissella paramesenteroides* reaching 19.50%, then *Lactobacillus rhamnosus* with 14.15%, followed by *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Enterococcus durans* reaching 11.21% and 11.20% respectively. The isolates with the lowest frequencies were *Lactobacillus plantarum* (7.45%), *Lactococcus lactis* subsp. *lactis* (7.01%) and *Leuconostoc* species with 5.22% for *Leuconostoc mesenteroides* subsp. *dextranicum* and 4.48% for *Leuconostoc mesenteroides* subsp. *mesenteroides*.

Table 2. Distribution of Lactic Acid bacteria species isolated from raw camel's milk.

Genus	Species	Number	Frequency (%)
Enterococcus		47	35.00
	Enterococcus feacalis	32	23.80
	Enterococcus durans	15	11.20
Lactobacillus		29	21.60
	Lactobacillus rhamnosus	19	14.15
	Lactobacillus plantarum	10	7.45
Weissella		26	19.50
	Weissella paramesenteroides	26	19.50
Lactococcus		19	14.20
	Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	15	11.21
	Lactococcus lactis subsp. lactis	4	7.01
Leuconostoc		13	9.70
	Leuconostoc mesenteroides subsp. *dextranicum*	7	5.22
	Leuconostoc mesenteroides subsp. *mesenteroides*	6	4.48

Leuconostoc which was represented by two species: *Leuconostoc mesenteroides* subsp. *dextranicum* and *Leuconostoc mesenteroides* subsp. *mesenteroides* which have nearly the same number (7 and 6 respectively) had the lowest frequency among all.

Technological properties

Acetoin and dextran production

Table 1 shows the result for the citrate use, acetoin and dextran production for all isolates tested. Regarding to the obtained results, a large number of the isolated LAB (more than 82%) were able to produce acetoin almost all the isolate of *Lactococcus* species were able to produce acetoin except the species of *Lactococcus lactis* subsp. *lactis* and some of the *Lactobacillus plantarum*.

All isolates of *Leuconostoc mesenteroides* were able to produce viscous colonies which mean a positive result for the dextran production. The species of *Leuconostoc mesenteroides* (13 isolates) and *Weissella paramesenteroides* (26 isolates) are heterofermentary and produce CO_2 gas.

Extracellular proteolytic activity

Growth on PCA medium supplemented with skim milk was recognizable by the presence of a clear halo around the colony (Fig. 2). All isolated LAB were tested for their proteolytic activity. More than the half of the strains was non-proteolytic bacteria (55%). The highest number of proteolytic isolates was found in the cocci (60%) and particularly in presumptive lactococci isolates where more than 80% had this ability. Less than the half of rods had this property. Unlike for the coccobacilli, whom the majority (more than 70%) wasn't able to express a proteolytic activity (Fig. 2).

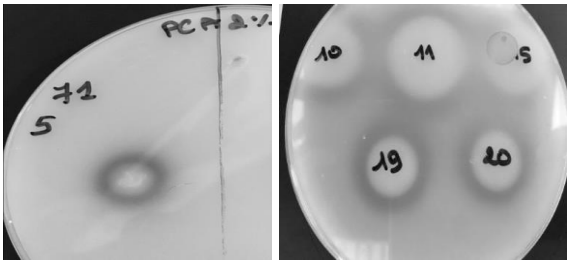

Fig. 2. Proteolytic activity of some lactococci isolates in PCA medium supplemented with 2% of skim milk (clear zone around the colonies).

Identification by Molecular and Sequencing Analyses

On the basis of technological properties, 03 representative bacteria from the 134 isolates were chosen for sequencing study. In fact, using 16S rRNA genes and the two primers 27F and 1432R, only one band at almost 1500 bp was observed for all strains (Fig. 3).

The isolates were identified to species level by 16S rDNA gene sequencing. The determined sequences were directly compared to those available at the NCBI Genebank database. The table (3) shows that the similarity higher than 99% was obtained for the isolates with references strains.

Table 3. The genotypic identification of the retained strains.

Isolate	similarity	The nearest strain (16S rDNA sequence)	Accession number NCBI database
MEC12	100%	*Leuconostoc mesenteroides* subsp. *dextranicum* strain SKB3032	MH844904.1
ADR9	100%	*Lactobacillus rhamnosus* strain NBRC 3425	NR_113332.1
BAB1	100%	*Lactococcus lactis* subsp. *lactis* XT7-4	MH891690.1

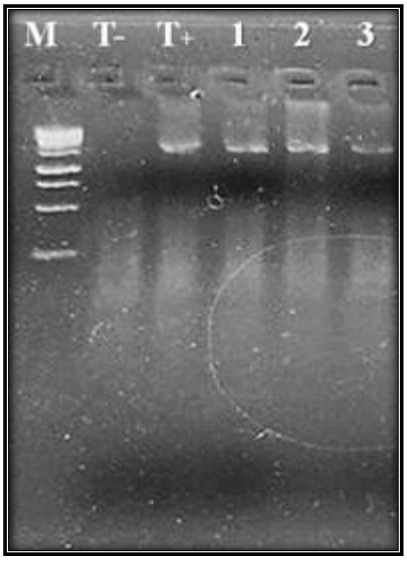

Fig. 3. Profile of amplification products migration of the encoding region 16S rRNA on agarose gel 1.5%. (M : gene ruler 1Kb, T- : negative test, T+ : positive test, 1 : ADR9, 2 : BAB1, 3 : MEC12).

Growth kinetic

The characterisation of isolates having a technological interest goes through a study of their growth in milk, 3 isolates were retained to carry out the assay: one strain *Leuconostoc mesenteroides*, one strain *Lactobacillus rhamnosus*, one strain *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*. The pH and titrable acidity (°D) were measured at regular interval time to calculate the acidification rate of the culture and the bacterial growth was calculated using the specific growth rate (μ) and generation time (G).

For the *Leuconostoc mesenteroides* subsp. *dextranicum* (MEC12) isolate, the exponential phase lasts 12 h, where the biomass production reaches 9.41 Log cfu/mL with a growth rate of 0.62 h^{-1} and a

generation time of 97 min, the pH during this period decreased by 0.65 Unit, and the amount of lactic acid after 12 h reached 32.20mM (Fig. 4).

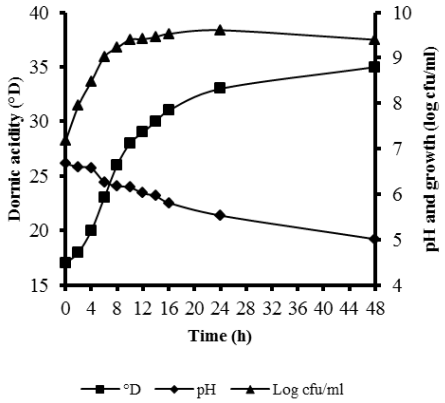

Fig. 4. Growth and acidification kinetic of *Leuconostoc mesenteroides* (MEC 12) isolated from raw camel's milk, at 30°C in skim milk.

For the *Lactobacillus rhamnosus* isolate, the exponential phase lasts 20 h, where the biomass production reached 8.80 Log cfu/mL with a growth rate of 0,33 h^{-1} and a generation time of 181 min , the pH during this period decreased by 1.34 unit, and the amount of lactic acid after 20 h reached 48.88mM (Fig. 5). For the *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* isolate, the exponential phase lasts 12 h, where the biomass production reaches 9.88 Log cfu/mL with a growth rate of 0,80 h^{-1} and a generation time of 75 min, the pH during this period decreased by 2.57 unit, and the amount of lactic acid after 12 h reached 80.00mM (Fig.6).

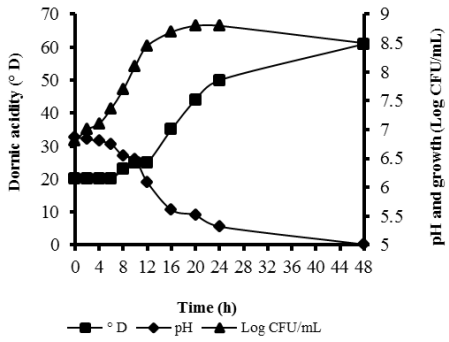

Fig. 5. Growth and acidification kinetic of *Lactobacillus rhamnosus* (ADR9) isolated from raw camel's milk incubated at 30°C in skimmed milk.

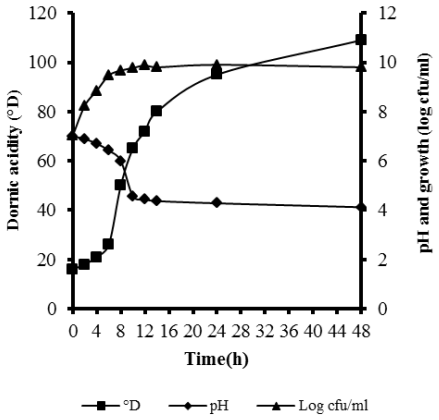

Fig. 6. Growth and acidification kinetic of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (BAB1) isolated from raw camel's milk at 30°C in skim milk.

Discussion

Raw camel's milk is a good additional source of new dairy LAB isolates (Khedid *et al.,* 2009; Quigley *et al.*, 2013). It showed a certain diversity of species that are frequently described in various dairy products. In the obtained results, there is a clear dominance of the genus *Enterococcus*, same results have been reported in Karam and Karam (2006) where it has been described a dominance of *Enterococcus* genus with 34.60% and in Ismaili *et al.* (2016), who have found a clear domination of *Enterococcus* (53.60%), both in camel's milk. For a long time the presence of enterococci isolates has been considered as a sign of a faecal contamination, but nowadays they are considered to be a part of the usual microflora in dairy products and other fermented foods (Morandi *et al.*, 2006). This presence is probably due to their tolerance to salt, heat and acid conditions (Pedro Nieto-Arribas *et al.,* 2011). Lactobacillus genus represented more than 20% of the isolates (table 3), represented by two species *Lactobacillus rhamnosus* and *Lactobacillus plantarum*, the *Lactobacillus* genus has been reported as a Non Starter LAB playing an important role during the cheese ripening producing typical cheese flavor (P Nieto-Arribas *et al.*, 2009; Sánchez *et al.*, 2006).

Weissella genus was represented by more than 19% (Tab. 3), they contribute in dairy products by the

production of dextran playing an important role in the rheological properties of fermented food products (Benhouna *et al.*, 2019), their potential probiotic effects and antimicrobial activity (Fusco *et al.*, 2015).

The Genus of *Lactococcus* described in literature, usually does not grow in temperature higher than 40°C, but the strains isolated from the camel milk had the capacity to support such high temperature and this was matching with what was described in Drici *et al.* (2010) and in Bendimerad *et al.* (2012). In the obtained results, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* seem to be the main specie in the *Lactococcus* genus (Tab.3) and this is similar with the works of (Saidi *et al.*, 2005).

Leuconostoc isolates were weakly present in raw camel's milk with less than 10% (Tab. 3), this may be due to the fact that they poorly grow in milk (Alegría *et al.*, 2013; Pedro Nieto-Arribas *et al.*, 2010; Server-busson *et al.*, 1999), although they are known to produce acetaldehyde, acetoin and diacetyl contributing to the organoleptic properties of dairy products (Hemme & Foucaud-Scheunemann, 2004).

Technological aptitudes of LAB strains isolated from raw camel's milk is a main condition to select new starter cultures to be used in the standardised production of dairy products (Fguiri *et al.*, 2016) and LAB are known to have several metabolic properties which contribute to the biochemical events of glycolysis, proteolysis, which have a crucial importance in cheese-making and dairy food fermentation in general (Pérez *et al.*, 2003). In the obtained results, a large number of the isolated LAB was able to produce acetoin (Tab. 2). This compound is considered as an important flavour and aroma in fermented milk products (Caplice and Fitzgerald, 1999; Domingos-Lopes *et al.*, 2017).

In this work only *Leuconostoc* isolates produced dextran with its ability also to produce acetoin (Tab. 2), they could be promising candidates as adjunct cultures in dairy fermentation (Server-busson *et al.,* 1999, Pedro Nieto-Arribas *et al.,* 2010). This characteristic is also considered as a relevant feature for LAB in dairy industry mainly in fermented food such as yoghurt, cheese and dairy-based desserts (Benhouna *et al.,* 2019) by having, not only the advantage to improve organoleptic behaviour such as rheological properties and the substitution of additive products but also by producing foods with potentially valuable properties for human health (immunogenic properties, protection against gastric ulcers, improvement of digestive transit, hypocholesterolaemic activity, antiviral, antitumor, etc.) and thus providing food with a quality label and a health-promoting properties (Leroy & De Vuyst, 2004; Ruas-Madiedo *et al.*, 2010; Zarour *et al.*, 2018, Benhouna *et al.,* 2019).

Proteolysis is a critical process for LAB to grow in milk (Jamaly *et al.*, 2010, Alegría *et al.,* 2013), producing organic acids, mainly lactic acid (Leroy & De Vuyst, 2004), peptides and aromatic compounds essential in dairy fermentation (Zhang *et al.*, 2014). In this work among all the isolates, *Lactococcus lactis* isolates gave the best result as judged by PCA supplemented with 2% of skim milk method. In other works (Centeno *et al.*, 1996; Pérez *et al.,* 2003), lactococci isolates are found to be frequently used as starter cultures thanks to their high proteolytic activity.

Three isolates (*Leuconostoc mesenteroides, Lactobacillus rhamnosus* and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*) were submitted to kinetic and growth acidification in skim milk. In view of the obtained result after 48 h of incubation, the isolate that gave the highest acidification was the *Lactococcus* isolate reaching an acidity of 109°D (121.00mM of lactic acid), followed by *Lactobacillus* isolate reaching an acidity of 61°D (67.78mM of lactic acid) then *Leuconostoc* isolate reaching an acidity of 35°D (38.89mM). This result allow us to classify the isolates in three groups, fast acidifying group for *Lactococcus* isolate the medium acidifying group for *Lactobacillus* isolate and slow acidifying group for *Leuconostoc* isolates. These results are in accordance with other authors (Alegría *et al.*, 2010; Ayad *et al.*, 2004; P Nieto-Arribas *et al.,* 2009). Thus, fast acidifying isolates are therefore good candidates for dairy fermentation process as primary starter culture, while poor acidification strains can be used as adjunct

cultures depending on other properties (Ayad *et al.,* 2004). The ability to acidify milk by LAB is a critical factor, the fast acidification of the raw material prevents growth of undesirable microorganisms and is also essential for aroma, texture of dairy products (Akabanda *et al*., 2014).

Conclusion

In conclusion, Algerian raw camel's milk could be considered as a good source of new strains of LAB to be used in the production of fermented dairy food by showing interesting and desirable technological abilities such as proteolytic activity, production of acetoin responsible of flavouring of fermented food and production of dextran involved in the natural texturing of dairy products. Some of the obtained isolates have demonstrated such characteristics like *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* to be used as starter culture and *Lactobacillus rhamnosus* or *Leuconostoc mesenteroides* as adjunct in starter culture. LAB isolates with interesting biotechnological profiles may influence the quality and variety of dairy products, if they are used as starters, and their cheese-making characteristics seem promising.

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