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Phenotypic study of lactic acid bacteria isolated from camel meat and milk

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Abstract

The dromedary, a symbol of man's survival in the desert, produces high-quality milk and meat. Because of their technological properties, lactic acid bacteria are used in a variety of food transformation processes. This study focused on the phenotypic identification of lactic acid bacteria isolated from camel meat, and milk in order to gain a better understanding of these lactic bacteria and to compare the bacterial genera found in the two other food products.

Camel meat and milk samples were collected under sterile conditions. On MRS and M17 agar, the lactic flora is counted. The enumeration results revealed a bacterial richness of the milk samples compared to the meat samples, as well as a strong lactic load on M17 agar and MRS for both types of products examined. On M17 agar, the contamination rates of camel meat and milk are 4.43±0.80 logufc/g and 4.93±10.77 logufc/ml, respectively, and 4.40±0.96 logufc/g and 3.89±7.07 logufc/ml, respectively on MRS. The 11 lactic isolates of camel meat were obtained after phenotypic identification and were linked to five genera listed in order of dominance: *Lactobacillus* (36.36 %), *Lactococcus* (27.27%), *Streptococcus* (18.18%), *Enterococcus*, and *Pediococcus* (9.09 %). While the 11 milk isolates were linked to five genera, they were listed in descending order: *Lactococcus* (54.55%), *Lactobacillus* (18.18%), *Leuconostoc*, *Enterococcus*, and *Streptococcus* (9.09 %). The *Pediococcus* genus has only been isolated from meat, whereas *Leuconostoc* has only been isolated from milk.

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Introduction

Lactic acid bacteria are prokaryotes cells, heterotrophs, and chemoorganotrophs (De Roissart, 1986). They are non-pathogenic in general and are regarded as "GRAS" (Generally Recognized As Safe) (Mozzi *et al.,* 2015; Mozzi *et al.,* 2016). They are common microorganisms that can be found in a variety of foods, including milk and its derivatives, meat, fruits, and vegetables (Leonard, 2013). For centuries, they have been associated with human and animal food. Humans and animals both tolerate these microorganisms (Klaenhammer *et al.*, 2005). These bacteria have complex growth factor requirements, including vitamin B, amino acids, nitrogenous bases, and peptides. They are linked to nutrient-rich environments, such as milk and meat (Salminen *et al.,* 2004; Trias, 2008). These bacteria are very interesting in the development of food products, especially fermented dairy products (Ghozlane, 2020). Meat has a high nutritional value because it is made up of digestible proteins that are high in essential amino acids. It is also high in iron and water-soluble vitamins. Camel meat is becoming increasingly popular among arid-area consumers, both economically and dietarily (Faye *et al.,* 2013). Camel milk has been widely consumed by nomadic populations for centuries due to its nutritional value and medicinal properties. This observation was scientifically supported by demonstrating that camel milk has the highest antimicrobial activity when compared to other animal milks (Konuspayeva *et al.,* 2009).

The purpose of this study is to determine the quantitative and qualitative lactic acid bacteria load of two commonly consumed food products in the study area: Ouargla, camel milk and meat. In addition, the genus composition of lactic acid bacteria in these two products was compared. In order to provide more information about the flora found in camel milk and meat.

Materials and methods

Camel meat and milk were used as biological materials in our study. The meat samples were collected immediately following the slaughter and *post-mortem* examination of the slaughtered animals. The carcasses' samples were taken from the same anatomical areas: the thigh and the shoulder. The carcasses were selected at random, with no regard for the animal's age or gender. Each sample, weighing about 250 g, was individually wrapped in a sterile bag. The transport was carried out in an isothermal cooler under cold conditions. The 18 samples were immediately cut aseptically into 10 g pieces in the laboratory. Each sample was placed individually in a sterile bag of Stomacher, and the whole was placed in a refrigerator at a temperature of around +4°C. Milk samples were taken from whole milking of healthy camels. These samples were collected aseptically in 250ml glass bottles and transported to the laboratory in an isothermal cooler.

Stock solution preparation and tenfold dilutions

The first dilution prepared from a product solid was the stock solution (meat). Each 10g of dromedary meat was placed in a stomacher bag containing 90ml of peptone water. Homogenization was accomplished by grinding each test portion in the stomacher for 2 minutes. The ground material obtained thus formed the basis of the solution 1/10. (10^{-1}). The decimal dilutions were prepared in accordance with the French standard ISO 6887-2. (ISO, 2004). As a result, one milliliter of this solution was aseptically transferred into a test tube containing 9 ml of sterile peptone water 0.1% (the 10^{-2} dilution). We continue in this manner until the 10^{-4} dilution is reached (Guiraud, 2003). A liquid product (milk) was used to make a stock solution. A series of decimal dilutions was achieved by transferring 1 ml of milk straight into tubes containing 9 ml of sterile physiological water to generate a dilution of 10^{-1}. After that, it's utilized to make a series of dilutions up to 10^{-4}. (Khedid *et al.*, 2009).

Enumeration of lactic acid bacteria

The meat and milk samples were subjected to enumeration of the lactic flora according to standard NF EN 15787 (V18-231) and NF EN 15786 (V 18-230) on solid medium MRS (De Man, Rogosa and Sharpe)

was used for the culture and enumeration of *Lactobacillus* and the M17 medium was used for the research and enumeration of *Lactococcus*, *Streptococcus* and *Enterococcus* in the food industry by mass inoculation. The reading of the plates thus inoculated was carried out after 72 hours of incubation at 37°C (Guiraud, 1998).

Characterization and phenotypic identification of strains

The purification of isolated lactic acid bacteria was carried out by several subcultures successively on liquid MRS or M17 broth and solid MRS or M17 media (by the streak method), and incubation at 37°C, until very distinct and homogeneous colonies are obtained (Ghozlane, 2020). The purity of the strains is revealed by homogeneous colonies having the same appearance (color, size and shape) (Guiraud, 2003). These colonies thus obtained were subjected to characterization: macroscopic based on visual observation of the colonies and of the culture medium, microscopic by Gram staining of the bacterial cells and by observation in the fresh and biochemical state, by tests catalase, oxidase and the search for the fermentation of glucose and lactose and the production of gas on the TSI medium and of the respiratory type on the liver meat medium.

Results

Enumeration of the lactic flora of camel meat and milk

Lactic acid bacteria were recorded in all analyzed samples of meat and milk. Their number varied according to the sample studied, the nature of the product analyzed and the culture medium used (Table 1).

Table 1. Average lactic acid bacteria loads in meat and milk, depending on the culture medium used.

Culture medium	M17		MRS	
	Meat	Milk	Meat	Milk
Average BL loads (logufc/g) or (logufc/ml)	4.43±0.80	4,93±10,77	4.40±0.96	3,89±7.07

The average loads of these bacteria sampled were 4.43±0.80 logufc/g and 4.40±0.96 logufc/g for the meat and 4.93±10.77 logufc/ml and 3.89±7.07 logufc/ml for the milk on M17 and MRS media respectively (Table 1).

The percentage of lactic flora counted differed according to the culture medium (M17 or MRS) and according to the origin of the sample (milk or meat). M17 showed the highest percentages for the two studied products (51.90% for meat and 91.68% for milk). This difference was very remarkable for milk. According to the cellular form, the cocci form was the most abundant for the two products, for the 11 strains isolated from each product, 4 strains (36.36%) and 2 strains (18.18%) showed the bacillus form, whereas 2 strains (63.64%) and 9 strains (81.82%) in cocci, for meat and milk respectively (Table 2).

Table 2. Percentage of lactic acid bacteria in meat and milk, depending on the culture medium used.

Culture medium	% lactic flora		% Cell forms	
	M17	MRS	Bacillus	Cocci
Meat	51,90	48,10	36,36	63,64
Milk	91,68	8,32	18,18	81,82

Macroscopic characterization of lactic acid bacteria

Camel meat: A total 11 strains of lactic acid bacteria were isolated from camel meat samples, of which 5 strains were cultured on MRS medium and 6 on M 17 medium. These bacteria exhibited a wide range of appearances (smooth shiny and creamy, jagged to rough and dry, rough and dry) and sizes (small, medium and large), colors white, beige and yellow) with regular or irregular contours and circular or eroded shape, was recorded on the M17 and MRS media (Table 3 and Fig. 1).

Table 3. Macroscopic appearance of lactic acid bacteria colonies isolated from camel meat on M17 and MRS.

Isolates	Culture medium /Forme	Contour	Color	Elevation Against the medium	Opacity	area and Consistency	Size
S1 M	M17 Circular	Regular	White	Convex	Opaque	Shiny and creamy smooth	Average
S2 M	M17 Circular	Regular	White	Plate	Opaque	Shiny and creamy smooth	Small
S3 M	MRS Circular	Regular	White	Convex	Opaque	Shiny and creamy smooth	Big
S4 M	M17 Circular	Irrégulier	Beige to White	Convex	Opaque	Shiny and creamy smooth	Big
S5 M	MRS Eroded	Irregular	Beige	Plate	Transparent	Shiny and creamy smooth	Big
S6 M	MRS Eroded	Irregular	Beige	Plate	Transparent	Jagged to rough and dry	Big
S7 M	M17 Eroded to Circular	Irregular	White	Plate	Transparent	Rough and dry	Small
S8 M	M17 Circular	Regular	White	Convex	Opaque	Shiny and creamy smooth	Average
S9 M	MRS Circular	Irregular	White	Concave	Translucent	Rough and dry	Average
S10 M	M17 Circular	Irregular	Yellow	Convex	Opaque	Shiny and creamy smooth	Big
S11 M	MRS Circular	Irregular	White	Convex	Opaque	Shiny and creamy smooth	Big

Légend : S M: Strain isolated from camel meat.

Table 4. Macroscopic appearance of lactic acid bacteria colonies isolated from camel milk on M17 and MRS.

Isolates	Culture medium /Forme	Contour	Color	Elevation Against the medium	Opacity	area and Consistency	Size
S1 K	M17 Circular	Regular	White	Convex	Opaque	Smooth	Small
S2 K	M17 Circular	Regular	White	Convex	Opaque	Smooth and creamy	Average
S3 K	M17 Circular	Regular	White	Convex	Opaque	Smooth and creamy	Petite
S4 K	MRS Circular	Regular	White	Convex	Opaque	Smooth and creamy	Average
S5 K	MRS Eroded	Irregular	White	Convex	Opaque	Smooth and creamy	Big
S6 K	MRS Wavy	Irregular	White	Raised center	Opaque	Smooth and creamy	Big
S7 K	M17 Wavy	Irregular	Beige	Convex	Translucent	Smooth and creamy	Big
S8 K	M17 Punctif to Circular	Irregular	Beige	Raised center	Translucent	Smooth and creamy	Big
S9 K	M17 Circular	Regular	White	Convex	Opaque	Smooth and creamy	Small
S10 K	M17 Circular	Irregular	Yellow	Convex	Opaque	Rough and dry	Small
S11 K	M17 Circular	Irregular	Yellow	Convex	Opaque	Smooth and creamy	Average

Legend: S K: Strain isolated from camel milk.

Macroscopic characterization of lactic acid bacteria from camel milk

In all, 11 lactic strains were isolated from camel milk samples, of which 3 strains were obtained on the MRS medium and 8 on the M 17 medium (Table 4).

The isolates showed different macroscopic appearances. round, wavy or eroded colonies with regular or irregular outline of white, beige or yellow color, creamy or dry, smooth or rough surface and small, medium or large size (Table 4 and Fig. 1).

Table 5. Characterization of lactic acid isolates from meat.

Isolates	Mob	Cell form	Gr	Cat	Ox	V .f	L / S	G	Gas	H_2S	Genus
S1 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S2 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Streptococcus*
S3 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S4 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S5 M	_	Bacillus	+	_	_	Optional Anaerobic	+	+	_	_	*Lactobacillus*
S6 M	_	Bacillus	+	_	_	Optional Anaerobic	+	+	_	_	*Lactobacillus*
S7 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Micro-aerophilic	+	+	_	_	*Pediococcus*
S8 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Enterococcus*
S9 M	_	Bacillus	+	_	_	Optional Anaerobic	+	+	_	_	*Lactobacillus*
S10 M	_	Bacillus	+	_	_	Optional Anaerobic	+	+	+/_	_	*Lactobacillus*
S11 M	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Streptococcus*

Legend: SM: meat strain, Mob: Motility, Gr : Gram**.** Cat: Catalase, Ox: Oxidase, V.f: liver meat, L/S: Lactose / Sucrose, G: Glucose, H_2S: Hydrogen sulphide.

Microscopic and biochemical characterization of lactic isolates from meat

The microscopic observation in the fresh state, of lactic acid bacteria isolated from camel meat, shows that they all show the immobile character and after Gram staining, all the cells were Gram positive, presenting themselves in two forms. Cellular: shells or bacilli with different modes of association, arranged in diplococci or in chains for the bacteria cultured on the M17 medium and bacilli for those isolated on MRS medium. These bacteria lack the catalase enzyme, and do not produce the cytochrome oxidase enzyme. On TSI, all these strains were able to ferment the three sugars (lactose, sucrose and glucose) with the absence of H_2S and gas production, with the exception of the S10M strain which presented a slight gas release. The respiratory type: anaerobic was noted in all these bacteria except S7 M which proved to be micro-aerophilic (Table 5). Based on these results, the lactic acid isolates were assigned to the genera: *Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus* and *Lactobacillus* (Table 5).

Microscopic and biochemical characterization of milk lactic isolates

The 11 lactic strains isolated from camel milk did not show the activity of the catalase enzyme nor of the cytochrome oxidase enzyme. On the other hand, they had the ability to degrade the three sugars on the TSI medium, with the absence of production of H2S and gas except the S4 K strain which presented a slight gas evolution. On the Liver Meat medium, these bacteria expressed an anaerobic respiratory metabolism with the exception of the S3 K and S5 K strains which developed near the surface of the

culture medium, being micro-aerophilic (Table 6). According to the macro and microscopic and biochemical results, these lactic acid bacteria were attributed to the genera: *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Enterococcus* and *Lactobacillus* (Table 6).

Table 6. Characterization of lactic isolates from camel milk.

Isolates	Mob	Cell form	Gr	Cat	Ox	V .f	L / S	G	Gas	H_2S	Genus
S1 K	_	Cocci in pair or chain	+	_	_	Optional Anaerobic	+	+	_	_	*Streptococcus*
S2 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S3 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Micro-aerophilic	+	+	_	_	*Lactococcus*
S4 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Anaerobic	+	+	+/-	_	*Leuconostoc*
S5 K	_	Bacilli	+	_	_	Micro-aerophilic	+	+	_	_	*Lactobacillus*
S6 K	_	Bacilli	+	_	_	Optional Anaerobic	+	+	_	_	*Lactobacillus*
S7 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S8 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S9 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Enterococcus*
S10 K	_	Cocci in pair, and in a bunch of grapes	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*
S11 K	_	Cocci in pair,	+	_	_	Optional Anaerobic	+	+	_	_	*Lactococcus*

Legend: SK: milk strain, Mob: motility, Cat: Catalase, Ox: Oxidase, V .f: liver meat, L/S: Lactose / Sucrose, G: Glucose, H_2S: Hydrogen sulphide.

Table 7. Percentage of genera of lactic acid bacteria isolated from camel meat and milk.

Product	Meat	Milk
Bacterial genus (%)		
Lactobacillus	36.36	18.18
Lactococcus	27.27	54.55
Streptococcus	18.18	9.09
Pediococcus	9.09	00
Enterococcus	9.09	9.09
Leuconostoc	00	9.09

Percentage of genera of lactic acid bacteria isolated from camel meat and milk

Bacteria of the genus *Lactobacillus* were the most abundant in the camel meat samples with a rate of 36.36% followed by those of the genus *Lactococcus* with 27.27% then come those of the genus *Streptococcus* with 18.18%, while the least represented were those belonging to the genera *Pediococcus* and *Enterococcus* with each 9.09%. For the milk samples, a dominance of the *Lactococcus* genus with a rate of 54.55% followed by the *Lactobacillus* genus with 18.18% while the

Streptococcus, *Leuconostoc* and *Enterococcus* genera each represented 9.09% (Table 7).

Discussion

According to Djeghri *et al.* (2010), lactic acid bacteria have nutritional requirements in nitrogenous matter and especially in growth factors (vitamins, amino acids, minerals). Bacteria of the genera *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Micrococcus* are original flora of milk. Lactic bacteria are a group of Gram positive, anaerobic to aerotolerant, oxidase and catalase negative bacilli or coccobacilli (Alexson, 2004 ; Saidi, 2020). These criteria agree with those shown by the strains isolated from the milk and camel meat studied. According to Dicks and Van Vuvren (1987) and Axelsson, (2004), homofermentative lactic acid bacteria produce two molecules of lactic acid from glucose, while heterofermentative strains synthesize lactic acid, oxygen dioxide and ethanol (or acetic acid) as end products. So, the difference between these two groups lies in the release of CO_2.

All of the strains isolated from the two products analyzed did not generate the presence of CO_2. So, they were homofermentative, except S4 K and S10 M which showed a very low release of CO_2 in their tubes.

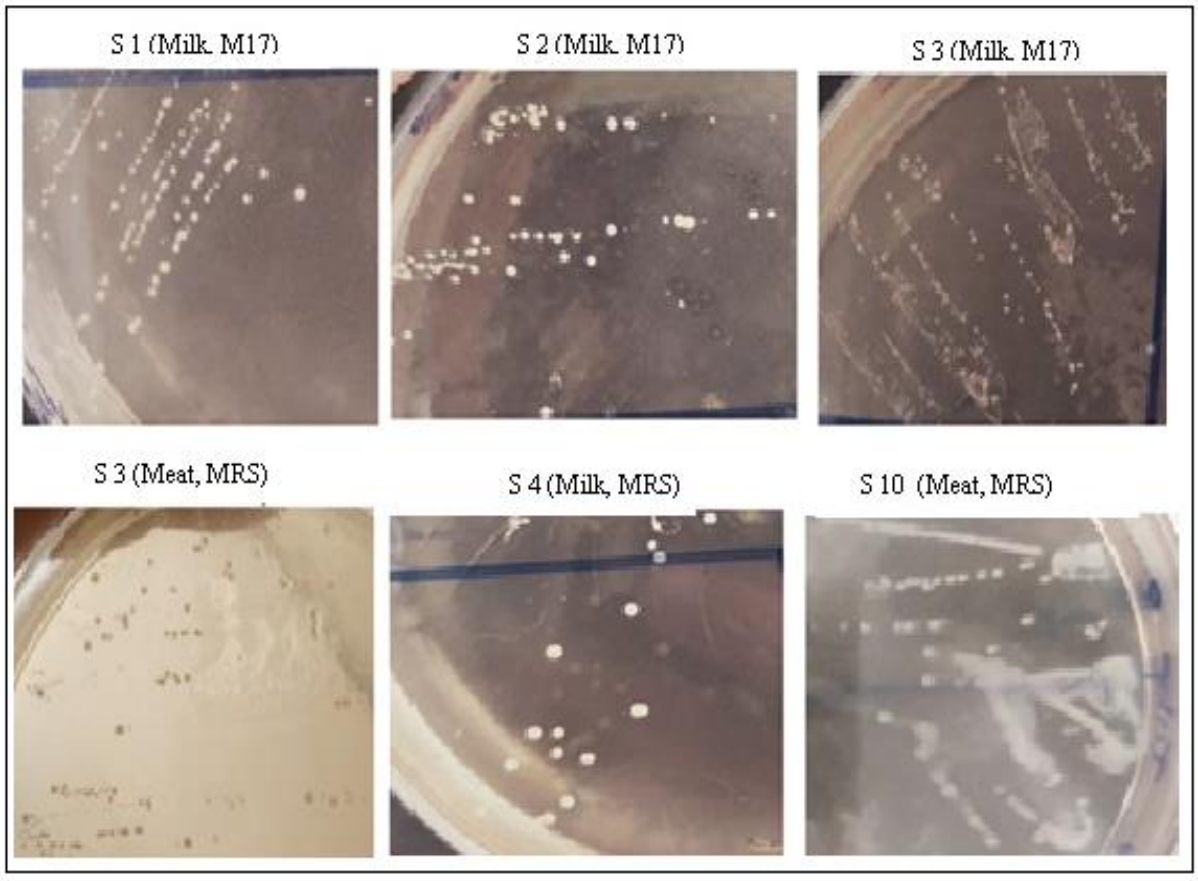

Fig. 1. Macroscopic appearance of some strains on M17 and MRS media.

Through phenotypic identification, it appears that the contamination flora of camel meat and milk were made up of a diversity of lactic acid bacteria represented by five genera in meat: *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Enterococcus* and *Pediococcus*, the percentage of which varied from one genus to another: 36.36% for *Lactobacillus*, 27.27% for *Lactococcus*, 18.18% for *Streptococcus* and 9.09% for *Enterococcus* and *Pediococcus*. While in milk the five genera: *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Enterococcus* were noted and their percentages were 54.55% for *Lactococcus*, 18.18% for *Lactobacillus* and 9.09% for each genus of *Leuconostoc*, *Streptococcus* and *Enterococcus*. Bouguerra, (2021), isolated the same genera of lactic acid bacteria from camel milk from M'sila, Biskra and

Oued Souf. Stiles *et al.,* (1997), state that lactic acid bacteria colonize many food products such as dairy products, meat, vegetables.

The presence of the genera *Lactococcus, Lactobacillus, Streptococcus* and *Enterococcus* was noted in the two food products analyzed. While the genus *Pediococcus* has only been demonstrated in meat and the genus *Leuconostoc* only in milk. Similar results were reported by work by Bouguerra in 2021 on camel milk and Bouzaid *et al.,* in 2016 with regard to camel meat.

Saidi *et al.* (2005), Zaidi-Karam *et al.* (2006) and Drici *et al.* (2010) reported a dominance of cocci in raw camel milk (more than 50%), this agrees with our results (81.82%). This dominance was also noted for the camel meat studied whose percentage of shells was around 63.64%. The predominance of the genera *Lactococcus* (27.27% and 54.55%) and *Lactobacillus* (36.36% and 18.18%), respectively recorded on meat and milk, is justified according to Raynaud (2006), who announces that lactic acid bacteria of the genus *Lactobacillus* and *Lactococcus*, are homofermentative which colonize dairy and meat products. These results are consistent with those taken by Ouadghiri *et al.,* (2009) on camel milk. According to Hassaïne *et al.* (2008) and Ashmaig *et al.,* (2009), the genus *Lactococcus* is associated with milk and dairy products. Also Liu *et al.,* (2011) announce that bacteria of the *Lactobacillus* genus are naturally present in milk and fermented products. According to Lairini *et al.,* (2014), genus *Streptococcus* is abundant in milk and dairy products.While the presence of strains of the genus *Enterococcus* may be due to direct contamination of these food stuffs, from animal faeces, or indirectly from a source of contaminated water or poorly cleaned equipment (De Vos *et al.,* 2009).

Conclusion

The enumeration of the lactic flora revealed the presence of these bacteria in the two products, with a significant load in the milk. A total of eleven lactic acid bacteria strains isolated from each product were phenotypic identified. The lactic isolates were Gram-positive cocci and bacilli and were assigned to the genera: *Lactobacillus, Lactococcus, Streptococcus, Enterococcus* and *Pediococcus* for meat, and to the genera: *Lactobacillus, Leuconostoc, Lactococcus, Streptococcus* and *Enterococcus* for milk. The absence of the genus *Leuconostoc* in meat and of the genus *Pediococcus* in milk has been recorded. These results indicate that the lactic flora of camel meat and milk is very diverse, knowing that the proportions have varied from one bacterial genus to another.

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