Characterization of Algerian raw camel's milk : identification of dominant lactic acid bacteria and proteins analysis

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SUMMARY

The chemical composition of Algerian raw camel's milk is slightly rich in fat 34.4 ± 2.8 g.l^{-1}, proteins 33.1 ± 2.1 g.l^{-1}, lactose 45.1 ± 3.1 g.l^{-1}, ash 8.15 ± 0.15 g.l^{-1} and total solids 122.6 ± 0.12 g.l^{-1}. This composition varied by several factors such as feeding, breeds, milk yielding and the health of the animal. Our results showed that total casein proteins were higher than whey proteins. SDS-PAGE showed that casein proteins of camel's milk and cows' milk have the same molecular weight, about 24 kDa. The whey proteins of camel's milk were presented by 5 bands. Compared with cow's milk, camel's milk contents a little amount of β-lactoglobuline. The pH of raw milk decrease from 6.5 to 5.4 after 72 h of incubation at 30°C. The total count of lactic acid bacteria can reach 200 x 10^{6} cfu ml^{-1} in MRS medium. Fourty strains of lactic acid bacteria were isolated from camel milk. The mesophilic group were represented by Lactococcus lactis subsp. lactis biovar. diacetylactis which is the dominant strain, L. lactis subsp. Lactis, L. lactis subsp. cremoris and L. lactis subsp. lactis biovar. diacetylactis produce more than 100 mM of lactic acid after 24 h of incubation at 30°C. The thermophilic strains were represented by two species, Streptococcus thermophilus and Enterococcus faecalis. From a technological point of view, the camel milk can be used as a source of new starters in the development of the industrial manufacture of these strains.

Key words: Camel's milk. Lactic Acid Bacteria. Proteins. Acidification.

RESUME

La composition chimique du lait cru de chamelle algérien est légèrement riche en graisse 34.4 ± 2.8 g.l^{-1}, en protéines 33.1 ± 2.1 g.l^{-1}, en lactose 45.1 ± 3.1 g.l^{-1}, en cendre 8.15 ± 0.15 g.l^{-1} et en matière solide totale 122.6 ± 0.12 g.l^{-1}. Cette composition varie en fonction de plusieurs facteurs tels que l'alimentation, la période de lactation, de la race et de la santé de l'animal. Nos résultats ont montré que le taux des caséines totales de étaient plus élevé que des protéines sériques. L'analyse par la SDS-PAGE a montré que les protéines de caséine du lait du chamelle et du lait de vache ont le même poids moléculaire d'environ 24 kDa. 5 bandes de protéines du lactosérum lait du chamelle ont été détectées. Comparé au lait de la vache, le lait de chamelle contient un taux faible en β - lactoglobuline. La diminution de pH du lait cru de chamelle atteint une valeur de 6.5 à 5.4 après 72 h d'incubation à 30°C. La microflore lactique atteint une densité de 200 x 10^{6} cfu ml^{-1} sur milieu MRS. Cinquante souches de bactéries lactiques ont été isolées a partir du lait cru de chamelle. Le groupe de bactéries mésophiles sont représentés par la sous-espèce de Lactococcus lactis biovar. diacetylactis qui est la souche dominante. Les sous-espèces de L. lactis, L. cremoris et de L. lactis biovar. diacetylactis produisent plus de 100 mM d'acide lactique après 24 h d'incubation à 30°C. Les espèces thermophiles sont représentées par deux espèces, Streptococcus thermophilus et Enterococcus faecalis. Sur le plan technologique, le lait de chamelle, de part sa richesse en différentes espèces de bactéries lactiques, peut servir comme source dans la sélection de nouvelles souches d'intérêt industriel.

Mots clés : Le lait cru de chamelle. Bactéries lactiques. Protéines. Acidification.

1- INTRODUCTION

In many arid areas, camels (*Camelus dromedarius*) play a central role as a milk supplier. The highest camel density in Algeria is found in the south especially the hot climate. He can live under inhospitable conditions that are otherwise very difficult for other domestic animals (GHOZAL *et al*. 1981). It has been reported that the Arabian camel can survive for up to 20 days without food and water (SIEBERT and MACFARLANE, 1975). The estimated number of camels in Algeria is 150 000 animals (WARDEH *et al*. 1990).

Several countries, such as Saudi Arabia and Mauritania, started to sell pasteurised, homogenised and carton packed camel milk in the market. This milk is very popular in these countries. The average total milk production of different camel breed was 2211.7 kg and the average lactation period was 12 months for the whole herd (SAOUD *et al.,* 1988). Only fragmentary data are available on composition of camel milk. The most complete data are those reported by BEG *et al.* (1987) and FARAH (1993). Camel's milk production in Algeria is estimated to be about 40.5 thousand tones annually (WARDEH *et al.,* 1990).

Little information is found about raw camel's milk. Only, several studies had treated the hygienic quality of raw camel's milk. The bacteriological studies had revealed that *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* were the major pathogens responsible for the intramammary infection (Mastitis) (ABDURAHMAN, 1995; and TEFERA and GEBREAH, 2001).

Great international attention has been recently focused on the need of new isolates of lactic acid bacteria for dairy industries (GASSON, 1993 and KIHAL *et al.*, 2000). The selection of lactic acid bacteria used as starter is based on the acid lactic and the aromatic compounds production, the stability of the strains during the fermentation, the production of anti-microbial substances and the resistance to bacteriophages (DESMAZEAUD, 1983 and KIHAL *et al.,* 1996).

This investigation was undertaken in order to determine some biochemical and the characterization of dominant lactic acid bacteria strains isolated from raw camel's milk, as well as the selection of bacteria that could be used in the manufacture of a more specific starter.

2- MATERIALS AND METHODS

2-1- Samples.

A total of 15 samples of raw camel's milk were collected from south-west of Algeria, in Béchar and Tindouf localities. The animals were fed throughout the year exclusively by grazing. After being taken, the samples (500 ml) were immediately cooled and brought to the laboratory in an isotherm container. For the comparison of spontaneous fermentation by native microflora, a part of crude milk from camel and cow were incubated at 30°C and 45°C.

A camel milk casein protein and whey protein have been isolated by precipitation and were lyophilized.

2-1-1 Protein and α-NH_2 assays

Protein concentration of raw camel's milk was measured by the method of BRADFORD (1976) and the fractions of α-NH_2-terminal residues were determined by the procedure described by DOI *et al*. (1981). The Cd-Ninhydrin method was modified as follows: water content in the reagent was reduced to 1ml. Cd-ninhydrin reagent contained 0.8 g of ninhydrin dissolved in a mixture of 80 ml of 99.5 % ethanol and 10 ml of acetic acid, followed by the addition of 1 g of $CdCl_2$ dissolved in 1 ml of water. 0.5 ml of sample and 1 ml of the Cd-ninhydrin reagent were heated in a tube for 5 min at 84 °C for colour development. After cooling, absorbance was read at 507 nm.

2-1-2 SDS-PAGE

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed according to the method of BOTHE *et al.* (1985). Five hundred µg of lyophilized samples of camel's milk, casein and whey protein were solubilized in 500 µl buffer (Tris-HCl 1 mole, pH 6.8, β-mercaptoethanol 5%, glycerol 10%, bromophenol blue 0.1%). Eighty µl of the samples were loaded into each slab electrophoresis. The gel was stained by the Coomassie blue R 250 (Merck, USA). SDS VII (Sigma, USA) was used as an inner standard.

2-2-1 Isolation of bacterial strains and culture conditions

Total microflora has been determined by plating various dilution according to standard methods of the INTERNATIONAL DAIRY FEDERATION (1981). Lactic acid bacteria count were made on MRS and M17 solid media supplemented with 1.5% agar (DE MAN *et al., 1960;* TERZAGHI and SANDINE*,* 1975). Predominant types of colonies were picked randomly and some representative strains displaying the general characteristics of lactic acid bacteria were chosen from each plate for further studies.

Long-term conservation of lactic acid bacteria strains, without appreciable loss of properties, was achieved by maintaining in skim milk with glycerol 7/3 (v/v) at - 20 °C. Working cultures were also kept on MRS agar slant at 4°C and re-streaked every 4 weeks (SAMELIS *et al.,* 1994). Details of the incubation conditions will be described for each set of experiments.

2-2-2 Physiological and biochemical tests.

All isolates were initially tested for Gram reaction, catalase production and presence of spores. Cell morphology and colony characteristics on MRS and M17 agar were also examined and a separation into phenotypic groups was undertaken. Only the Gram positive, cytochrome-oxydase and catalase negative isolates were further identified by using Sherman test. Growth at different temperatures was observed in MRS broth after incubation for 5 days at 15°C, 37°C and 45°C; 12 days at 4°C and 10 °C and the resistance to 60°C for 30 min. Gas production from glucose was determined in MRS broth containing inverted Durham tubes. Hydrolysis of arginine was tested on M16BPC medium (THOMAS, 1973). Growth in the presence of 40 $g.l^{-1}$ and 65 $g.l^{-1}$ NaCl was observed in MRS broth at 30 °C for 2 days. The ability to growth at pH 3.9 and pH 9 was tested on MRS broth. Citrate utilisation, in the presence of carbohydrates, was performed on the media of KEMPLER and MC KAY (1980). Production of dextrane (slime) from sucrose was determined on MRS agar in which glucose was replaced by 50 $g.l^{-1}$ sucrose (MAYEUX *et al.,* 1962). Production of acetoin from glucose was determined by using Voges-Proskauer test (SAMELIS *et al.* 1994).

2-2-3 Carbohydrate fermentation assays

The fermentation of carbohydrates was determined on MRS broth containing bromocresol purple (0.04 $g.l^{-1}$) as a pH indicator, and supplemented with 10 $g.l^{-1}$ of the following carbohydrates: lactose, sucrose, xylose, arabinose, rhamnose, sorbitol, fructose, galactose, mannitol, cellobiose, raffinose and maltose. To ensure anaerobic conditions, each tube was supplemented with two drops of sterile liquid paraffine after inoculation. Hydrolysis of aesculin was also tested using the MRS broth supplemented with 2 $g.l^{-1}$ (w/v) aesculin (SAMELIS *et al.,* 1994).

2-2-4 Growth and acid production in milk.

Skim milk medium was prepared from reconstituted skim milk powder (110 $g.l^{-1}$ distilled water) and sterilized by autoclaving at 110°C for 10 min. Sterilized milk medium (100 ml) was inoculated with active culture (2 ml) of each strain to obtain approximately 10^7 cfu/ml and incubated at 30°C for 24 h. Total acidity

was determined by titration 10 g of sample cultures with 0.11 N NaOH and reported as a mM of lactic acid per litre (KIHAL *et al*., 1996).

Statistical analysis

Group means were compared by one-way analysis of variance. Means were analysed for significant differences ($p< 0.05$) using a Student's *t*-test.

3-RESULTS AND DISCUSSION

Composition of raw camel's milk

The chemical analyses of raw camel's milk indicated 34.4 ± 2.8 $g.l^{-1}$ fat, 33.1 ± 2.1 $g.l^{-1}$ protein, 45.1± 3.1 $g.l^{-1}$ lactose, 8.15 ± 0.15 $g.l^{-1}$ ash, 6.57 pH and 873.7 $g.l^{-1}$ moisture. The differences among the various sets of data undoubtedly reflect variation in breed and state of lactation of animal sampled, feeding, milk yield, milking interval and the health of the animal (FARAH, 1993). Recently GORBAN and IZZIDIN (2001) had revealed that total fat of Saudian camel milk (32.8 $g.l^{-1}$) were composed by a proportion of 2/3 (w/w) of unsaturated fatty acid. The raw camel's milk of local breed show to be slowly rich on total solids, fat, and protein.

The results showed that total protein in casein were higher than whey proteins and represented 231.5 ± 11.90 µg/ml and 174.4 ± 56.13 µg/ml respectively. Whereas, the α-NH_2 fraction were also higher in total casein than whey proteins and represented 83.42 ± 5.93 µg/ml and 41.76 ± 6.3 µg/ml respectively. Examination of the composition of camel's milk proteins revealed 74.1 % of casein and 25.9 % of whey proteins of the total content. The values of casein and whey protein expressed as a percentage of the total milk protein, lay within the ranges 71-76% and 17-23% respectively (FARAH, 1993). The studies of GIRARDET *et al*. (2000) indicated that in the total amount of whey proteins, albumin constitute 18.8 %, globulin 13%, and proteose-peptone 17.8%, and the tryptophan is a limiting amino acids.

From their mobility in a gradient SDS-PAGE, the proteins were found to have molecular masses ranged from 13 kDa to 64 kDa. The SDS-PAGE results showed that casein proteins of camel's milk and cows' milk have the same molecular weight about 24 kDa. Whereas, whey proteins of camel's milk are presented by five proteins bands in which the molecular weight were 66, 60, 32, 24 and 15 kDa (Fig. 3). Our results indicated that the band of β-lactogobulin was found in little amount in whey proteins.

Figure I showed the spontaneous pH evolution in camel's milk and cows' milk by the native microflora in two different temperatures of incubation (30 °C and 45°C for 100 h). The pH decreases slowly in camel's milk after 48 h of incubation and reaches 5.5 at 30 and 45 °C. This pH value remain stable after one week of incubation at 30 and 45 °C and no coagulation had been observed. Whereas, the pH decreases drastically from 6.7 to 4.8 and 4.4 at 30 and 45°C in cow's milk after 8 h of incubation. The final pH reaches 3.7 after 48 h of incubation and remain stable. Several autors had been studied about the pH evolution in cow's milk (DESMAZEAUD 1983; SORENSEN and PETERSEN 1993). YAGIL *et al*., (1984) mentioned that camel's milk did not sour at 4°C for up 3 months. Our results suggest that camel's milk contains less casein proportion than cows' milk, rich in whey proteins as show in SDS-PAGE, and the microflora is less efficient. Similar results had been obtained by URBISINOV *et al*. (1981).

Camel's milk did not sour at 4°C for up 3 months. This means that camel's milk is mainly good only for drinking (YAGIL *et al.* 1984). Our results showed a contribution to the overall knowledge of camels as a food source, but much still needs to be learned if efficient improvement programs are to be initiated.

Microbiological analysis

The total count of lactic acid bacteria in crude camel's milk was 200 ± 31 x 10^6 cfu.ml^{-1} and 160 ± 13 x 10^6 cfu.ml^{-1} at 30°C in M17 and MRS media respectively. Whereas, the number was 18 ± 5 x 10^4 cfu.ml^{-1} and 95 ± 12 x 10^3 cfu.ml^{-1} at 45 °C in M17 and MRS, respectively. The number of lactic acid bacteria was significantly higher in M17.

From seven samples, all strains were isolated from M17 and MRS agar plates from different dilutions (10^{-5} and 10^{-6}). On these media, the colonies were circular, convex and non pigmented. A total of 40 single colonies of lactic acid bacteria were selected randomly from pinpoint colonies in MRS and M17 agar, restreaked on the same solid media and examined for purity. The morphological studies had shown that the total bacteria were dominated by the coccoid lactic acid bacteria. These isolates were representative and they have the following characteristics: Gram positive, catalase and cytochrome-oxydase negative, non spores forming, lacking nitrate reductase and aero-anaerobic facultative. All coccoid isolates produced acid with no apparent gas production from glucose. Also, some of them hydrolysed arginine and used citrate. Morphological characteristics, incubation temperature at 45°C and gas production were used for identification. The isolates were separated in two groups, mesophilic (group 1) and thermophilic (group 2) (Table I).

Group 1 consisted of 34 mesophilic homofermentative cocci lactic acid bacteria. They do not grow at 65 g.l^{-1} NaCl and can not produced dextrane. 24 of them utilize citric acid and arginine and belong to *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*. Four isolates which can not utilize citrate and produce NH_3 from arginine belong to *Lactococcus lactis* subsp. *lactis*. Six later isolates of *Lactococcus* sp. Which can not utilize arginine, used citrate and utilize a limited number of sugars, belong to *Lactococcus lactis* subsp. *cremoris* (Table I).

Group 2 was formed by the thermophilic strains (six isolates). A total of 4 isolates were identified to *Enterococcus faecalis* which grow at 45°C, 15°C, pH 9.6, resist to 65°C for 30 min, used arginine and does not ferment arabinose. Also, two isolates belong to *Streptococcus thermophilus* which grow at 45°C and does not grow at 15°C, at 65 g.l^{-1} NaCl and at pH 9.6.

The dominant lactic acid bacteria of camel's milk were identified to *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* that can produce over than 100 mM of lactic acid in 24 h of incubation at 30 °C (Fig 2). This species of lactic acid bacteria can be exploited in some dairy products for the production of flavour and lactic acid from citric acid and lactose. Its dominance is layed on the presence of high ascorbic acid in camel's milk (ABU-TARBOUCHE *et al.,* 1998). The works of DE ROISSART (1986) and CHEKROUN *et al.* (1998) allow us to identify the all isolates.

Streptococcus thermophilus is a Gram positive cocci grows in chains. It ferments a limited number of carbohydrates. It generally considered among the most heat resistant and survives at least 30 min at 65°C. In this strain, lactose is cleaved by β-galactosidase to yield glucose and galactose. The glucose moiety enters the glycolytic pathway with the fermentation end product being lactic acid. It is via this pathway that this organisms are able to derive the metabolic energy required for growth. The galactose moiety is excreted from the cells and accumulated in fermented milk or cheese.

The role of *Enterococcus faecalis* in the manufacture of cheese has been extensively investigated in the last few years due to the consistent presence of this strain in different varieties of cheese. This strain play a significant role in the development of a good quality cheese (HERRANZ *et al*. 2001).

At the begining in raw milk *Lactococcus* and *Enterococcus* were dominant and *Leuconostoc* and *Lactobacillus* were present in low proportions (LOPEZ-DIAZ *et al*., 1999). In our research the absence of *Leuconostoc, Lactobacillus, Pediococcus* and *Bifidobacterium* can be explained by their low concentration in raw camel's milk, similar results were observed by KIHAL *et al*. (2000).

As indicated by our results, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* may be considered as a predominant indigenous and ubiquitous species of camel's milk flora, well adapted, able to compete and to outnumber other micro-organisms in raw camel's milk in arid regions.

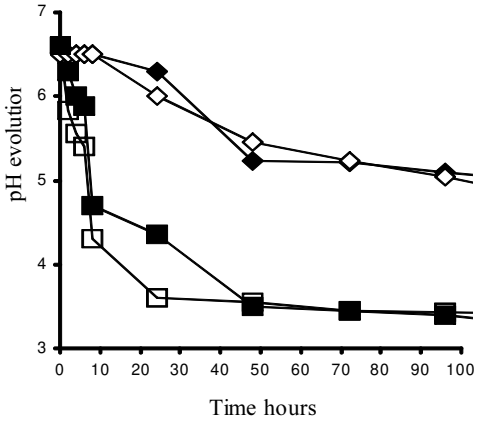

Figure 1. pH evolution of raw camel's milk (◆, ◇) and raw cow's milk (■,□) incubated at 30°C and 45°C, respectively and fermented by their native spontaneous microflora.

Figure 1. Evolution du pH des laits crus de chamelle (◆, ◇) et de vache (■,□) incubés à 30 et 45 °C, respectivement et fermentés par la flore lactique endogène.

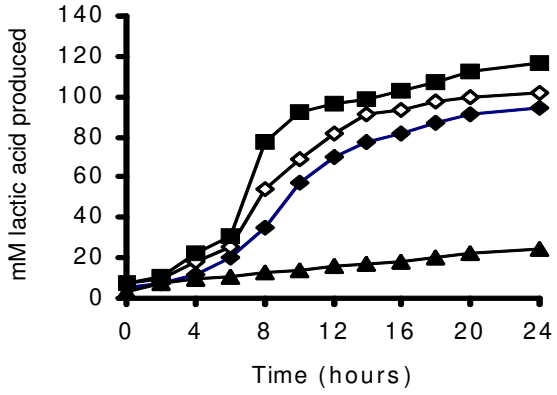

Figure 2. Kinetics of lactic acid produced by different strains of lactic acid bacteria isolated from raw camel's milk, *Lactococcus lactis* subsp. *lactis* (CH8, ■), by *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (CH1, ▲*) Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* (CH20, ◊) and *Lactococcus lactis* subsp.*cremoris* (CH9, ◆), growth in skim milk at 30°C.

Figure 2. Cinétique de production d'acide lactique par les différents souches de bactéries lactiques isolées du lait cru de chamelle, *Lactococcus lactis* subsp. *lactis* (CH8, ■), *Lactococcus lactis* subsp. lactis biovar. *diacetylactis* (CH1, ▲) *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (CH20, ◊) et *Lactococcus lactis* subsp. *cremoris* (CH9, ◆), sur lait écrémé à 30°C.

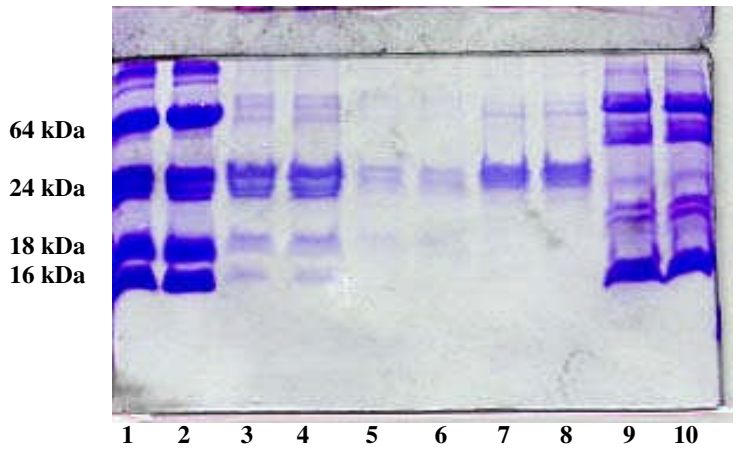

Figure 3: Electrophoretic SDS-PAGE patterns for whole casein and whey proteins of cows' and camel's milk on polyacrylamide gel. Lane 1 and 2: Indicators of molecular weight, bovin serum albumin (64 kDa), caseins (24 kDA), β-lactoglobulin (18 kDa) and α-lactalbumin (13 kDA). Lane 3 and 4: total casein of cow milk. Lane 5 and 6: total whey proteins of cows' milk. Lane 7 and 8: total casein of camel's milk. Lane 9 and 10: total whey proteins of camel's milk.

Figure 3 : Profil électrophorètique des protéines du lait de chamelle et du lait de vache sur gel de polyacrylamide (SDS-PAGE). Lignes 1 et 2: Marqueurs de poids moléculaire, sérum albumine bovine (64 kDa), caséines (24 kDA), β- lactoglobuline (18 kDa) et α- lactalbumine (13 kDA). Lignes 3 et 4 : caséines totales de lait de vache. Lignes 5 et 6 : protéines totales de lactosérum de vaches. Lignes 7 et 8 : caséines totales du lait du chamelle. Lignes 9 et 10 : protéines totales du lactosérum de chamelle.

Cell morphology	*Coccis*				
Group	*1*			*2*	
CO_2 from glucose	-			-	
Number of isolates	*4*	*24*	*6*	*2*	*4*
ADH	+	+	-	-	+
Growth at 15°C	+	+	+	-	+
37°C	+	+	+	+	+
45°C	-	-	-	+	+
Growth at 4% NaCl	+	+	-	-	+
6.5% NaCl	-	-	-	-	+
pH 9.6	-	-	-	-	+
Production of dextran	-	-	-	-	-
Production of acetoïn	-	+	-	-	-
Sugar fermentation					
Arabinose	*3+*	-	-	-	-
Cellobiose	+	+	-	-	+
Fructose	+	+	-	+	+
Galactose	+	+	+	-	+
Lactose	+	+	*5+*	+	+
Maltose	+	+	-	-	+
Mannitol	+	+	-	-	+
Mellibiose	-	+	-	-	-
Raffinose	+	-	-	-	+
Ribose	+	+	-	-	-
Saccharose	+	+	-	+	+
Sorbitol	-	-	-	-	+
Trehalose	*2+*	-	-	-	+
Xylose	*3+*	-	-	-	-
	2+	-	-	-	+
	3+	-	-	-	-

Table I: Physiological and biochemical properties of lactic acid bacteria isolated from Algerian raw camel's milk.

Tableau 1. Propriétés physiologiques et biochimiques des bactéries lactiques isolées à partir du lait cru de chamelle d'Algérie.

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