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BACTERIAL COMPOSITION OF ALGERIAN RAW CAMEL MILKS AFTER COMMERCIAL-LIKE STORAGE, AS REVEALED BY TTGE AND DGGE

Habiba Drici[1], Françoise Berthier[2], Marina Aigle[3], Claire Darrigo[3] and Agnès Delacroix-Buchet[3]
[1]Laboratoire des Sciences et Environnement « Bio-ressources, Géochimie-Physique, Législation et développement Socio-Economique», Centre Universitaire de Tamanghasset-Sis, Serssouf, Tamanrasset Algérie
[2]INRA, UR342 Technologie et Analyses Laitières, Poligny, France
[3]INRA, UMR1319 Micalis, Jouy-en-Josas, France
E-Mail: habiba.drici@laposte.net

ABSTRACT

This study describes the physico-chemical parameters and the bacterial dominant communities in three (03) Algerian raw camel's milks during their six (06) days' storage in conditions prevailing in the region of Tamanrasset. The storage included namely a first stage (milking then transportation to the local store) without cooling for less than 24h and then successive phases at 4°C and at ambient temperature during their commercialization. Spontaneous fermentation of the milks occurred during the first four days of storage, as shown by their acidification over time and their high microbial load at day 5 (> 8 log). Two molecular methods, Denaturating Gradient Gel Electrophoresis (DGGE) and Temporal Temperature gradient Gel Electrophoresis (TTGE), that do not require microorganism cultivation, were used to fingerprint the bacterial communities at the end of storage. The TTGE fingerprints allowed to detect, presumably identify and semi-quantify five (05) low GC taxa. The DGGE fingerprints revealed the presence of subdominant populations belonging to at least eight (08) high GC taxa.

Keywords: raw camel's milk, bacteria, acidification, culture-independent analysis, TTGE, DGGE.

1. INTRODUCTION

Raw camel's (*Camelus dromedarius*) milk is an important and vital food source. It traditionally plays an essential role in the nutrition of rural communities living in the arid and semi-arid regions of many countries including Algeria. In the last years, its consumption in raw, fermented [1] or pasteurized states [2] has increased among the urban population of Africa and Asia countries due to its potential therapeutic properties and to its hypoallergenicity for newborn infants not tolerant to bovine milk [3]. Therefore, accurate knowledge of the microbiological composition of raw milk or non bovine mammals milk in raw state, especially after storage at 4°C as usually made in the stores, are required.

Most microbiological analysis of such milks have exploited culture-dependent approaches to describe the diversity of predominant lactic acid bacteria (LAB) to select [4] LAB species that have technological value for proteolysis and citrate fermentation [5], and to detect bacteria that constitute food-borne disease or human health risk [6]. However culture-dependent approaches cannot allow detecting non cultivable microflora. By contrast, culture-independent methods based on direct analysis of DNA (or RNA) without prior microorganism cultivation can detect them. They are also useful to rapidly fingerprint the microbial diversity in different complex ecosystems including varied range of fermented food. For example both Denaturating Gradient Gel Electrophoresis (DGGE) and Temporal Temperature gradient Gel Electrophoresis (TTGE) methods are now widely used to assess the microbial diversity of dairy products such as bovine, ovine and caprine raw milks or to describe the dynamics of microbial community during commercial cheese manufacturing [7].

To our knowledge, microflora analysis of camel milk or its fermented products has been only investigated by culture-dependent methods. This conventional culturing technique was usually followed by phenotypic and/or by genotypic
(e.g. sequencing, genetic fingerprinting) identification of a subset of purified isolates randomly selected [8].

The purpose of this study was to describe the bacterial communities of three Algerian camel milks, after their storage in commercial-like conditions, without using microorganism cultivation. This was achieved by amplification of the DNA extracted directly from milk by Polymerase Chain Reaction (PCR), followed by DGGE for the high G+C bacteria or TTGE for the low G+C bacteria PCR products, as previously described [9].

2. MATERIAL AND METHODS

2.1. Milk sampling and storage

Raw camel's milks were sampled from three different breeds, Ouled Sid Echik (OSE), Reguibi (R) and Tergui (T) breeds, at a private dairy farm located in Southern Algeria (in the region of Ghardaïa). The samples (2 x 250 mL per breed) were collected directly in sterile screw capped flasks at the farm by the shepherd in charge of the herd and of milking. They were stored first at ambient temperature (≥ 30 °C) between 16 h and 24 h, depending on the breed, from milking to their arrival to the University of Oran, then at 4 °C for 24h at the University

of Oran; then at ambient temperature for 12h during their transportation to France, and finally at 4°C for 108 h. This storage condition mimics conditions used for commercial purpose that include the minimum number of period at ambient temperature intercalated between period at 4°C from the beginning of commercialization.

2.2. Gross physical, chemical and microbiological characteristics

The pH value was determined at the sample arrival in Oran using a pH meter (Hanna Instruments), and after 3, 4 and 23 days of storage in France using another pH meter (digital pH meter). At day 3 of storage, the fat matter content (FM, in g/L) was determined by the Gerber method, the total protein content (TP, in g/kg) by the Amido black assay (IDF Norm 098A) and the dry matter content (DM, in %) was measured using an halogen moisture analyzer (Precisa XM 60, Precisa Instrument Ltd., Dietikon, Switzerland). The total bacterial counts (TBC, in log cfu.mL^{-1}) were assessed at day 3 and day 5 of storage. The milk samples were diluted in 1 % (wt/v) peptone solution, 52.5 µl of each dilution were plated on PCA agar medium (Oxoid, Basingtoke,United Kingdom) with a spiral system (Interscience) and plates were incubated for 72 h at 30 °C.

2.3. DNA fingerprints

Bacterial fingerprints were obtained at day 6 of milk storage. The techniques used were those previously described by [9]. Briefly, Whatman FTA card was used for DNA extraction [10]. Then, one or two card discs were placed in a 0.2 ml microcentrifuge tube as DNA template. Amplicons of V3 region within 16S rRNA gene were obtained by performing two successive PCR runs using a Gene Amp system model 2400 (PerkinElmer, France). Then, amplicons were subjected to TTGE (low G+C bacteria) and DGGE (high G+C bacteria) electrophoresis. The GelCompar software (Applied-Maths, Belgium) was used to normalize and analyze TTGE and DGGE fingerprints TTGE and DGGE gels were normalized by using a ladder made up of four bands. Band identifications were performed by comparison to a fingerprints database, which includes TTGE and DGGE fingerprints of about 170 bacterial species isolated from dairy ecosystems [11].

3. RESULTS AND DISCUSSIONS

3.1. Gross physicochemical and bacterial composition

The gross chemical composition of the three milks was similar to that previously reported for camel milks [1, 3, 12]. Results are shown in Table-1; fat content and dry matter varied moderately between the three milks. After storage of less than 24 h at ambient temperature, the pH values were quite similar for the three milks. They were in the range of those reported for other Algerian raw camel milks after their transportation to the laboratory without cooling [13], or under cooling conditions [14], and for many fresh market-sold or partly fermented Kenyan raw camel milks frozen after their sampling [6]. But the T and OSE values were already lower than the values 6.60-6.67 usually recorded after storage in cool tight container or at 4°C immediately after sampling [3, 12, 15]. During the subsequent storage 24 h at 4°C, followed by 12 h at ambient temperature and finally 24 h at 4°C (during the second and third days of storage) the pH values decreased twice as much in milk OSE than in characteristics milks R and T (0.55, 0.48 and 1.26 pH units for milks R, T and OSE, respectively). Finally, during the subsequent 20 days of storage at 4°C, the pH values continued decreasing, still twice as much in milk OSE than in milks R and T, (-0.43, -0.33, -0.63 for milks R, T and OSE, respectively), but slower than the days before as shown during day 4 of storage (-0.08, -0.07, +0.01 for milks R, T and OSE, respectively). Values at day 4 of storage were similar to values previously obtained during the spontaneous fermentation of Kenyan camel raw milk at 30°C [16]. The time course of pH values suggests that a spontaneous fermentation was initiated in the three milks during the first day of storage and continued at least during the subsequent three days of storage, probably at higher rate during the period at ambient temperature than at 4°C. Acidification occurred at two different rates according to the milks (R, T, OSE).Counts of total aerobic mesophilic bacteria from day 3 to day 5 of storage were rather high (>8 log cfu.mL^{-1}).

Table-1. Gross physico-chemical and bacterial during the storage of camel milks R, T and OSE.

Time after milking	Type of analysis	Milk sample		
		R	T	OSE
Between 16 h and 24 h[a]	pH	6.60	6.40	6.50
03 days[b]	pH Fat (g/l) Protein (g/kg) Dry matter (%) Total bacterial count (log cfu mL^{-1})	6.05 37.5 24.6 10.7 >8.0 [c]	5.92 35.0 24.6 10.6 >8.0[c]	5.24 42.5 26.0 11.5 >8.0[c]
04 days	pH	5.97	5.85	5.25
05 days[d]	Total bacterial count (log cfu mL^{-1})	8.38	8.26	8.78
23 days[e]	pH	5.62	5.69	4.61

[a] depending on the sample

[b] including 16 h-24 h at ambient temperature, then 24 h at 4°C, followed by 12 h at ambient temperature and finally 24 h at 4°C

[c] the sample were not diluted enough

[d] including 16 h-24 h at ambient temperature, then 24 h at 4°C, followed by 12 h at ambient temperature and finally 72 h at 4°C

[e] including 16 h-24 h at ambient temperature, then 24 h at 4°C, followed by 12 h at ambient temperature and finally 480 h at 4°C

At day 5, they were approximately three times higher in milk OSE than in milks R and T. They were thus in accordance with the spontaneous acidification observed. Indeed, they were at least 3 log units higher than in unprocessed camel milks from the transport container immediately after milking and after their transportation to the laboratory without cooling [13].

But, they were similar to those recorded in Kenyan and Somalia raw camel milks stored 24h to 36h [17] without cooling that turned sour in less than 24 hours at 25ºC or in less than 12 hours at 35ºC after milking [18], and in fermented camel raw milks. The total counts were higher as acidification rates observed between milks were lower.

3.2. Fingerprints of bacteria present in camel milks spontaneously fermented

The TTGE (Figure1-A.) and DGGE (Figure1-B.) fingerprints allowed to evaluate the taxonomical diversity within the dominant bacteria and to semi quantify (Table-2) the different taxa present at day 6 of storage, i.e. a relative early spontaneous fermentation stage. Most of the bands were presumably assigned to a species by the comparison of their electrophoretic position with the positions of 170 reference bands corresponding to 170 bacterial dairy species. The species assignation is therefore presumed because band sequencing was lacking to confirm it.

3.2.1. TTGE fingerprints

TTGE fingerprints of milks R, T and OSE showed five major bands (bands *a-e*; Figure-1 A); each milk exhibited two to three of them. Band d was common to the three milks OSE, R and T, band c was common to the two milks R and T, bands "a" and band "e" were specific to milk OSE and band b was specific to milk R (Table- 2.)

TTGE revealed the presence of at most five species of lactic acid bacteria, probably including *Lc. lactis* and *Ln. mesenteroides*. But they may have revealed also *Staphylococcus* species. Only two bands were assigned to a single species, band c to *St. hyicus* and band e to *Lc. lactis*. The other bands (a, b and d) were assigned to several co-migrating species, including *Leuconostoc*, *Staphylococcus* and/or *Streptococcus* species.

Species associated to the TTGE bands may have all participate to milk fermentation. *Lc. lactis* and *Ln. mesenteroides* may thus have participated to the acidification of milk OSE, but not to those of milks R and T. Bands *b* and *e* were also encountered in cow raw milks, fresh or refrigerated for 24 h at 4 °C [9, 11].

3.2.2. DGGE fingerprints

DGGE fingerprints of milks R, T and OSE revealed eight bands (bands f-m; Figure-1 B) which may be assigned to *Corynebacterium*, *Micrococcus* and/or *Clostridium* species. Such species are commonly encountered in bovine unprocessed raw milk [19]. Each

milk sample exhibited two to four of them. Band i was common to the three milks, band f was common to the two milks R and T. Bands h and m were specific to milk T. Bands g, j, k and l were specific to milk OSE. Bands (f, m and l) could not been assigned, indicating they were affiliated to species not included in the reference database. Compared to the TTGE bands, the DGGE bands exhibited an intermediate intensity (bands f, g, i and j) or a low intensity (bands h, k, l and m) (Table-2). Their presumed nature and their level strongly suggest that they did not multiply from the milking on. The question of their possible metabolic activity during the storage requires further investigations.

4. CONCLUSIONS

When stored less than one day at ambient temperature from milking, raw camel milks spontaneously fermented. This fermentation continued under the storage conditions used for commercialization leading to mesophilic total counts higher than 8 log cfu mL^{-1} in the course of acidification.

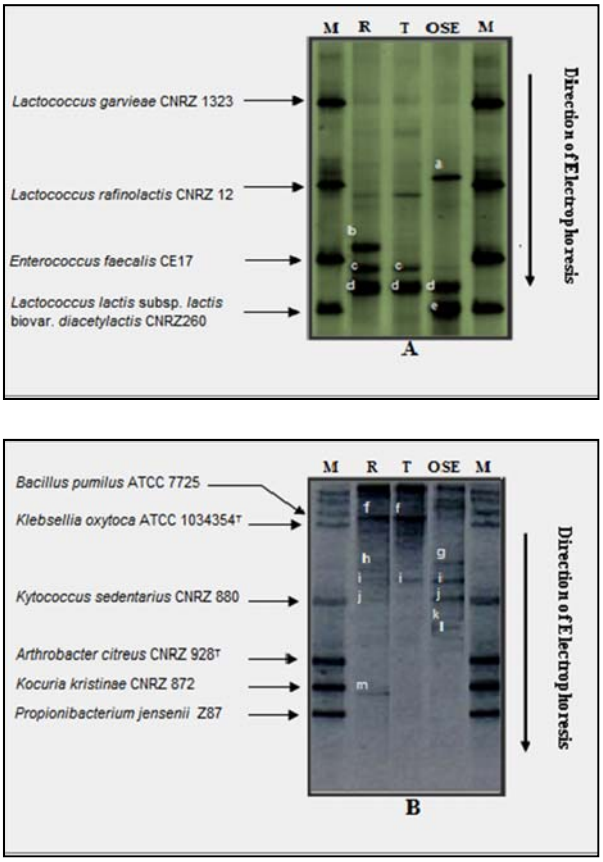

Figure-1. TTGE (A) and DGGE (B) fingerprints of V3 16S rDNA for the R, T and OSE fermented camel milks.

Table-2. Putative bacterial species present in spontaneous fermented camel milks R, T and OSE at day 6 of storage, as revealed by TTGE and DGGE.

Milk sample[a]	Band name	Band Intensity[b]	Method	Putative species or groups
OSE	a	5	TTGE	*Leuconostoc mesenteroides* susbsp. *dextranicum Staphylococcus aureus Staphylococcus simulans*
R	b	6		*Streptococcus uberis Bacillus circulans*
R/T	c	6/5		*Staphylococcus hyicus*
R/T/OSE	d	6/7/6		*Streptococcus bovis Streptococcus dysgalactiae Streptococcus equinus*
OSE	e	7		*Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis Lactococcus lactis* subsp. *Lactis Lactococcus lactis* subsp. *cremoris*
R/T	f	4	DGGE	Unidentified
OSE	g	3		*Mycobacterium spp Corynebacterium casei Serratia spp*
R	h	1		*Micrococcus sedentarius Micrococcus luteus*
OSE/R/T	i	3/1/1		*Micrococcus lilae Pantoea spp Corynebacterium ssp*
OSE/R	j	3/2		*Clostridium phytofermentans Corynebacterium aurimucosum*
OSE	k	1		*Clostridium phytofermentans Pantoea spp Corynebacterium ssp*
OSE	l	2		Unidentified
R	m	2		Unidentified

[a] R, breed Reguibi; T, breed Tergui; OSE, breed Ouled Sid Echikh
[b] on a 1-7 scale (1, the lowest intensity; 7, the highest intensity)

Though the storage was principally done at 4 °C, the acidification was rather high, suggesting counts as high 10^6 cfu mL^{-1} already in the unprocessed milks.

The TTGE and DGGE fingerprints allowed detecting thirteen different taxa in the raw camel milks R, T and OSE spontaneously fermented. The populations of low GC bacteria dominated largely the populations of high GC bacteria. They show that milks collected at the same time from different part of the herd in a single farm can have quite different microbial composition. The TTGE fingerprints allowed detecting lactic acid bacteria taxa that may have been involved in the spontaneous fermentation. The DGGE fingerprints revealed the presence of subdominant populations belonging to at least eight high GC taxa. These populations were probably not directly involved in the spontaneous fermentation. But they could have interesting technological potential, if their affiliation to corynebacteria and micrococci is confirmed.

It would be interesting to increase the number of available culture-independent bacterial fingerprints of camel milks, fermented or not to provide further insights into the taxonomical diversity of camel milk microflora. It would be interesting to explore the presence and taxonomical diversity of eukaryotes (yeasts and moulds) in such milks, using also culture-independent fingerprints.

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REFERENCES

[1] Konuspayeva G, Faye B, Loiseau G. 2009. The composition of camel milk: A meta-analysis of the literature data. J Food Compos Anal. 22: 95-101.

[2] Al Haj OA, Metwalli AA, Ismail EA. 2011. Heat stability of camel milk proteins after sterilisation process. J Camel Pract Res. 18: 277-282.

[3] Al Haj OA, Al Kanhal HA. 2010. Compositional, technological and nutritional aspects of dromedary camel milk. Int Dairy J. 20: 811-821.

[4] Shori AB. 2012. Comparative study of chemical composition, isolation and identification of micro-flora in traditional fermented camel milk products: Gariss, Suusac and Shubat. J Saudi Soc Agric Sci. 11: 79-88.

[5] Drici H, Gilbert C, Kihal M, Atlan D. 2010. Atypical citrate-fermenting Lactococcus lactis strains isolated from dromedary's milk. J Appl Microbiol. 108:647–657.

[6] Jans C, Kaindi DWM, Böck D, *et al.* 2013. Prevalence and comparison of Streptococcus infantarius subsp. infantarius and Streptococcus gallolyticus subsp. macedonicus in raw and fermented dairy products from East and West Africa. Int J Food Microbiol. 167: 186-195.

[7] Quigley L, O'Sullivan O, Stanton C, *et al.* 2013. The complex microbiota of raw milk. FEMS Microbiol Rev. 37: 664.-698.

[8] Verraes C, Claeys W, Cardoen S, *et al*. A review of the microbiological hazards of raw milk from animal species other than cows. Int. Dairy J. 39: 121-130.

[9] Ogier J-C, Lafarge V, Girard V, *et al*. 2004. Molecular fingerprinting of dairy microbial ecosystems by use of temporal temperature and denaturing gradient gel electrophoresis. Appl Environ Microbiol. 70: 5628-5643.

[10] Tilsala-Timisjärvi A, Alatossava T. 2004. Rapid DNA preparation from milk and dairy process samples for the detection of bacteria by PCR. Food Microbiol. 21: 365-368.

[11] Lafarge V, Ogier J-C, Girard V, *et al*. 2004. Raw cow milk bacterial population shifts attributable to refrigeration. Appl Environ Microbiol. 70: 5644-5650.

[12] Ramet J-P. 2001. The technology of making cheese from camel milk (Camelus dromedarius). Animal Production and Health Paper, Rome, Italy: F.A.O.

[13] Merzouk Y, Chahrour W, Zarour K, *et al*. 2013. Physico-chemical and Microbiological Analysis of Algerian Raw Camel's Milk and Identification of Predominating Thermophilic Lactic Acid Bacteria. J Food Sci Eng. 3:55-63.

[14] Siboukeur O. 2008. Etude du lait camelin collecté localement: caractéristiques physico-chimiques et microbiologiques; aptitudes à la coagulation. Doctorat, Université de Ouargla.

[15] Abbas S, Ashraf H, Nazir A, Sarfraz L. 2013. Physico-Chemical analysis and composition of camel milk. Int Res. 2: 83-98.

[16] Lore TA, Mbugua SK, Wangoh J. 2005. Enumeration and identification of microflora in suusac, a Kenyan traditional fermented camel milk product. LWT - Food Sci Technol. 38: 125-130.

[17] Sela S, Pinto R, Merin U, Rosen B. 2003. Thermal inactivation of Escherichia coli in camel milk. J Food Prot. 66: 1708-1711.

[18] Farah Z, Matthias M, Younan M, Dahir R. 2007. Camel dairy in Somalia: Limiting factors and development potential. Livest Sci. 187-191.

[19] Montel M-C, Buchin S, Mallet A, *et al*. 2014. Traditional cheeses: Rich and diverse microbiota with associated benefits. Int J Food Microbiol. 177: 136-154.