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Short communication: Survival of the characteristic microbiota in probiotic fermented camel, cow, goat, and sheep milks during refrigerated storage

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ABSTRACT

The objective of this study was to monitor the viability during storage of *Lactobacillus acidophilus* LA-5 (A), *Bifidobacterium animalis* ssp. *lactis* BB-12 (B), and *Streptococcus thermophilus* CHCC 742/2130 (T) in probiotic cultured dairy foods made from pasteurized camel, cow, goat, and sheep milks fermented by an ABT-type culture. The products manufactured were stored at 4°C for 42 d. Microbiological analyses were performed at weekly intervals. *Streptococcus thermophilus* CHCC 742/2130 was the most numerous culture component in all 4 products both at the beginning and at the end of storage. The viable counts of streptococci showed no significant decline in fermented camel milk throughout the entire storage period. The initial numbers of *Lb. acidophilus* LA-5 were over 2 orders of magnitude lower than those of *Strep. thermophilus* CHCC 742/2130. With the progress of time, a slow and constant decrease was observed in lactobacilli counts; however, the final viability percentages of this organism did not differ significantly in the probiotic fermented milks tested. The cultured dairy foods made from cow, sheep, and goat milks had comparable *B. animalis* ssp. *lactis* BB-12 counts on d 0, exceeding by approximately 0.5 $\log_{10}$ cycle those in the camel milk-based product. No significant losses occurred in viability of bifidobacteria in fermented camel, cow, and sheep milks during 6 wk of refrigerated storage. In conclusion, all 4 varieties of milk proved to be suitable raw materials for the manufacture of ABT-type fermented dairy products that were microbiologically safe and beneficial for human consumption. It was suggested that milk from small ruminants be increasingly used to produce probiotic fermented dairy foods. The development of camel milk-based probiotic cultured milks appears to be even more promising because new markets could thus be conquered. It must be emphasized, however, that further microbiological and sensory studies, technology development activities, and market research are needed before such food products can be successfully commercialized.

Key words: camel, fermented milk, *Bifidobacterium*, *Lactobacillus*

Short Communication

The regular consumption of lactic acid bacteria through fermented milks has long been associated with improved health and longevity (Metchnikoff, 1907). Over the last few decades, several strains of various *Bifidobacterium* and *Lactobacillus* spp. have received attention as probiotic organisms (Klein et al., 1998; Biavati et al., 2000; Zacarchenco and Massaguer-Roig, 2006). These bacteria are known to have health-promoting effects and, for this reason, they have been incorporated into a wide variety of dairy foods throughout the world (Alm, 1991; Ashraf and Shah, 2011; Karimi et al., 2012).

The numerous health benefits reported for lactobacilli and bifidobacteria include reduction in lactose intolerance in some individuals, treatment and prevention of diarrhea, alleviation of constipation, contribution to faster recolonization of the intestinal microbiota after administration of antibiotics, possible treatment of inflammatory bowel disease, reduction in serum cholesterol level, increased resistance to microbial infections, effect on immune function, and potential role in cancer prevention (Möller and de Vrese, 2004; Leahy et al., 2005; Zavisic et al., 2012). However, the therapeutic effects exerted by probiotic bacteria are dependent on the number of viable microbial cells reaching the human gut (Leahy et al., 2005; Ghoddusi and Hassan, 2011). Although no worldwide consensus exists as to the minimum concentration of bacteria that need to be consumed to produce a beneficial effect on human health (Farnworth, 2008), various authors argue that numbers of at least 10^6 cfu/mL should be present at the time of consumption if a health claim is to be made (Sanders and Huis in't Veld, 1999; Shah, 2000; Kechagia et al., 2013).

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Unlike classic yogurt bacteria, probiotic organisms grow slowly in milk and, therefore, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* are often added to cultured milks to speed up the fermentation process (Tharmaraj and Shah, 2003; Ashraf and Shah, 2011).

Although bovine milk production represents ca. 83% of the total world milk production, milking animals are not limited to cows in many parts of the world and, thus, buffalo (13% of world production), goat (2.2%), sheep (1.3%), and camel (0.3%) milks are also available in significant quantities (El-Salam, 2011; IDF, 2011). Every mammal species has a unique milk composition in terms of both major (proteins, fats, and lactose) and minor (e.g., vitamins, oligosaccharides, free amino acids, assimilable peptides, trace minerals, and so on) milk constituents (Hashim et al., 2009; Mayo et al., 2010; Fukuda, 2013), and this may influence the growth and survival rates of lactobacilli and bifidobacteria.

The objective of this research was to monitor the viability during refrigerated storage of *Lactobacillus acidophilus* LA-5 (**A**), *Bifidobacterium animalis* ssp. *lactis* BB-12 (**B**), and *Streptococcus thermophilus* CHCC 742/2130 (**T**) in probiotic cultured dairy foods made from different varieties of milk fermented by an ABT-type culture. To our knowledge, this is the first study evaluating the survival of probiotic lactobacilli and bifidobacteria in fermented camel milk.

Dromedary camel milk was received from Emirates Industry for Camel Milk and Products (Dubai, United Arab Emirates), cow milk was kindly supplied by Lajta Hanság Inc. (Mosonmagyaróvár, Hungary), goat milk was obtained from Tebike Inc. (Győr-Ménfőcsanak, Hungary), and sheep milk was provided by Pharma-Gene-Farm Inc. (Mosonmagyaróvár, Hungary). The chemical composition of raw materials is shown in Table 1. Raw milks were heated to 80°C and held for 10 min in a Pearl M water bath (Julabo Labortechnik GmbH, Seelbach, Germany).

The ABT-5 culture, which consisted of *Lb. acidophilus* LA-5, *B. animalis* ssp. *lactis* BB-12, and *Strep. thermophilus* CHCC 742/2130, was purchased from Chr. Hansen (Hørsholm, Denmark) in freeze-dried direct vat set form. It was added to the heat-treated process milks cooled to 40°C at the rate of 0.2 U/L corresponding to 2.0% (vol/vol) conventional bulk starter.

Table 1. Chemical composition (n = 2) of raw milks used for the manufacture of fermented dairy foods

Component (%, wt/wt)	Milk source			
	Camel	Cow	Goat	Sheep
Fat	2.31	3.71	3.29	5.95
Protein	2.85	3.25	2.95	5.42
Lactose	4.38	4.57	3.93	4.35
TS	10.30	12.21	10.98	16.88
SNF	8.17	8.56	7.74	10.88
Moisture	89.70	87.79	89.02	83.12

Milks were fermented at 37°C until a pH value of 4.6 was reached. Thereafter, the fermented ABT milks were cooled to 15°C in ice water and were each separated into 21 fractions that were transferred in sterile tightly capped centrifuge tubes (50 mL; Greiner Bio One Hungary Inc., Mosonmagyaróvár, Hungary). After 24 h of cooling at 8°C (d 0), the samples were stored at refrigeration temperature (4°C). The entire experimental program was repeated twice.

Three tubes of all 4 products were taken at each sampling time (i.e., following 0, 7, 14, 21, 28, 35, and 42 d of storage). Samples were aseptically removed from centrifuge tubes and diluted by mixing 10 mL with 90 mL of 0.1% peptone water. Further dilutions were made as required. The pour-plate method was used to enumerate microorganisms.

M17 agar (Merck KGaA, Darmstadt, Germany) was used to enumerate *Strep. thermophilus* CHCC 742/2130. The pH of the medium was 6.8 ± 0.2 at 25°C. The inoculated plates were incubated at 45°C for 24 h under aerobic conditions. *Streptococcus thermophilus* CHCC 742/2130 formed lenticular colonies with a diameter of 1 to 2 mm. Colony-forming units, expressed as logarithm (base 10) per milliliter, were used to report survival of streptococci.

Lactobacillus acidophilus LA-5 was enumerated in de Man, Rogosa, and Sharpe (**MRS**)-clindamycin-ciprofloxacin agar (ISO-IDF, 2006). Commercial MRS agar (Merck KGaA) was rehydrated in distilled water according to the manufacturer's instructions. It was distributed in portions of 200 mL into bottles of 250 mL capacity and sterilized in a Varioklav 500E autoclave (H+P Labortechnik AG, Oberschleißheim, Germany) set at 121°C for 15 min. The final pH was 6.2 ± 0.2 at 25°C. Simultaneously, 2.0 mg of clindamycin hydrochloride (Sigma-Aldrich, St. Louis, MO) and 20.0 mg of ciprofloxacin hydrochloride (Sigma-Aldrich) were dissolved separately in two 10.0-mL aliquots of distilled water. Both solutions were then sterilized by filtering through a 0.22-μm membrane filter (Millipore Corp., Bedford, MA). Immediately before pouring, 0.1 mL of clindamycin stock solution and 1.0 mL of ciprofloxacin stock solution were added to 200 mL of MRS agar cooled to between 44 and 47°C. Thus, the complete MRS-clindamycin-ciprofloxacin agar had final clindamycin and ciprofloxacin concentrations of 0.1 and 10.0 mg/L, respectively. The plates were incubated at 37°C for 72 h. Anaerobic culture jars (2.5 L) were used to generate anaerobic conditions, atmospheric oxygen being absorbed by means of AnaeroGen AN 25 sachets