Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

Original Research Doi:10.22103/Vcbr.2024.23216.1010

Antibacterial activity of dromedary camel milk fermented with probiotics against some pathogenic bacteria

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Article history:

Received: 03 March 2024
Revised: 22 April 2024
Accepted: 04 May 2024
Published: 08 May 2024

Keywords:

Antibacterial
Camel
Heat treatment
Lactic antagonism,
Milk
Pathogen
Probiotic

Abstract The production of natural dairies with antimicrobial properties represents a significant advancement in the context of food biopreservation. This study aims to explore the antibacterial properties of a novel standard probiotic-fermented camel milk (PFCM) and assess the impact of product heat treatment and dilution on these properties. A standard PFCM was prepared using a probiotic starter culture (ABT-10) containing *Lactobacillus acidophilus* La-5, and Bifidobacterium animalis subsp. lactis BB-12® probiotics. The PFCM subjected to heat treatment to produce two subgroups of heated (H-PFCM) and non-heated (N-PFCM) products. The products were then subjected to chemical and bacteriological evaluation within ten days. The antagonistic activity of N-PFCM against *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA) was investigated by comparison between antibacterial activity and minimum inhibitory level (MIL) of N-PFCM with its lactic acid content and H-PFCM ($p<0.05$). The assessment of the chemical and bacteriological properties of PFCM demonstrated an augmented antibacterial effect. The antibacterial activity of camel milk was enhanced 2- to 4-fold after fermentation. The study additionally assessed the antibacterial efficacy of N-PFCM and H-PFCM, comparing it to their lactic acid content, in order to investigate lactic antagonism within PFCM. In this context, N-PFCM demonstrated effective bacterial inhibition at its minimum inhibitory level (MIL), while the lactic acid concentration alone within the MIL did not exhibit antibacterial activity. Furthermore, heat treatment of PFCM at 85°C for 2 minutes reduced the antibacterial activity by 1- to 2-fold in MIL assay. Except for MRSA, the thermal process reduced the antibacterial activity of PFCM to its lactic acid level. These findings reveal the antagonistic impact of lactic acid bacteria (LAB) within N-PFCM. The study concludes that non-thermally abused PFCM retains significant antibacterial properties even at 8-times dilution, suggesting its potential as a natural antibacterial compound for the bio-preservation of foods.

Introduction

Dromedary camel milk is white and opaque with an acceptable taste ranging from sweet to salty [1]. The main components of camel milk are relatively close to bovine milk, whereas its viscosity and pH are lower than cow milk [2, 1]. Camel milk is the starting material of choice for fermented milk [3, 4]. Probiotic supplements are an insightful way of producing functional

fermented camel milk with enhanced health benefits [5, 6].

One of the therapeutic effects of fresh camel milk originates from its antibacterial activity [7-9]. The natural antimicrobial agents of camel milk include lysozyme, hydrogen peroxide, lactoferrin, lactoperoxidase, peptidoglycan recognition protein short variant (PGRP-S), and immunoglobulins [1, 10]. In addition to milk, fermented camel milk shows antibacterial activity. The antibacterial activity of fermented camel milk is most likely due to a phenomenon known as lactic antagonism. The antagonistic effect of LAB against other bacteria is probably related to the type of starter culture and food matrix [11]. In the context of fermented camel milk, the metabolites produced by lactic acid bacteria (LAB), including proteins, peptides, and bacteriocins, play a crucial and multifaceted role in shaping the outcome of bacterial interference. These bioactive compounds within fermented milk play a multifaceted role: LAB-derived proteins directly interact with other bacteria, influencing their growth and viability. Additionally, LAB generate peptides through proteolytic processes during fermentation, exhibiting diverse effects such as antimicrobial properties, immunomodulation, and gut health enhancement. Notably, bacteriocins—small antimicrobial peptides secreted by LAB—selectively target other bacteria, inhibiting their growth and inducing cell lysis [12-15].

Camel milk and its fermented products are ingredients in various cooked or uncooked food, appetizers, and desserts. From a nutraceutical perspective, the direct topical application of unadulterated fresh and fermented camel milk for antibacterial therapy is prudent. However, contrary to this, the enteral administration route via the gastrointestinal tract or the presence of base ingredients in topical delivery systems leads to dilution of the bioactive antibacterial components present in fresh and fermented camel milk [16].

Bio-preservation of such foods by fermented camel dairy products is achievable regarding the antibacterial metabolites of starter bacteria or the competitive exclusion against undesired bacteria [17]. Here, the thermal process or dairy dilution during food preparation may diminish this bioprotective activity. Assessing antibacterial activity in a functional food spiked with pathogens does not guarantee its efficacy as a nutraceutical or natural food preservative, given the dilution effects within food, the body, and drug delivery systems.

To elucidate the antibacterial effect of PFCM, we studied the susceptibility of some pathogenic bacteria to PFCM featuring a refreshing lactic tang. In PFCM, we assessed the impacts of thermal inactivation of probiotics, antimicrobial agents' dilution, and lactic acid production on antibacterial activity.

Materials and Methods

Milk sample

Dromedary camel milk was collected in October from Kerman (southeast of Iran) and transferred to the laboratory on ice. Milk vat pasteurization was performed at 63-65°C for 30min and then quickly cooled [18]. The pasteurized samples were kept in 4±1°C as “P” samples until use.

Production of PFCM

The heated pasteurized camel milk (95°C/15min) with 9% dry matter and 5% solid non-fat was cooled to 43°C and added 0.5% probiotic starter culture (ABT-10 Chr. Hansen, Denmark). The samples were incubated at 43°C until the pH dropped to 4. The fast fermented probiotic product was gently mixed and stored as the N-PFCM at 4±1°C for ten days [5, 4]. Another aliquot of N-PFCM was heat-treated in a water bath (85°C/2min) and then quickly cooled to 4±1°C to produce H-PFCM. The experiment was repeated on three separate occasions.

Titratable acidity (TA) and pH of PFCM

The TA and pH of PFCM were measured at room temperature on days one, six, and ten. The pH was evaluated by a calibrated pH meter (J. P. Selecta, Spain). For TA of PFCM, each sample (ten mL) was titrated with NaOH (0.1 N)

in the presence of phenolphthalein [19]. Titratable acidity (g/L) was calculated as follows where "V" is the volume of NaOH (mL) used for titration.

Eq. (1) $\mathrm{TA\ (g/L)} = \mathrm{V} \times 0.9$

The lactic acid content of PFCM was roughly estimated from the TA of PFCM, as TA-based lactic acid, for the control groups of antibacterial assays.

The Probiotic bacterial count of PFCM

Bifidobacteria and lactobacilli counts of PFCM were performed on days one, six, and ten through a double-layered pour plate technique. *Bifidobacterium* selective medium (BSM) was used to enumerate *Bifidobacterium (B.) animalis.* To prepare BSM, De Man, Rogosa, and Sharpe (MRS) agar (Merck-Germany) was supplemented with L-cysteine hydrochloride 0.5 g/L (30120; Sigma) and lithium mupirocin supplement 50 mg/L (69732; Fluka). The inoculated BSM plates were incubated (37°C/72h) anaerobically (Anaerocult®A, Merck). Colonies with a diameter of ≥1 mm were enumerated as bifidobacteria. Selective enumeration of *Lactobacillus (Lb.) acidophilus* was performed on MRS-bile (MRSB) agar containing bile salt 1.5 g/L (Liofilchem, Italy) under aerobiosis (37°C/72h) [20].

Preparation of the bacteria

Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853), *Listeria monocytogenes* (ATCC 7644), and *Escherichia coli* O157:H7 (ATCC BAA-460) were obtained from Kerman Faculty of Veterinary Medicine. The bacteria were inoculated in brain heart infusion (BHI) broth and subsequently on BHI agar to isolate pure colonies (37°C/24h).

Antibacterial activity of PFCM

The antibacterial activity of PFCM was determined by the agar well diffusion assay [21]. The turbidity of bacteria suspension in Mueller-Hinton broth (90922; Fluka) was adjusted to a 0.5 McFarland standard. The bacterial suspension was swabbed on Mueller-Hinton agar (70191; Merck), and six mm-diameter wells were bored on agar. 100 µL of thoroughly homogenized PFCM or camel milk was added into the wells and allowed to diffuse for 15min. The negative (sterilized distilled water), positive (cefixime, 5 µg/well, CDS021590, Sigma; azithromycin dihydrate, 15 µg/well, PZ0007, Sigma), and lactic acid (1.1 mg/well equivalent to PFCM titratable acidity; L6661, Sigma) controls were also placed in wells. After incubation (37ºC/16-18h), the diameter of bacterial inhibition zones was measured by calipers. Each experiment was carried out in triplicate.

Determination of MIL of PFCM

The MIL of PFCM and camel milk was measured *via* an agar dilution assay [22]. Aseptically, 2-fold serial dilutions of camel milk, N-PFCM, and H-PFCM with molten Mueller-Hinton agar (45°C) were prepared through vigorous mixing to obtain the final milk and PFCM levels in Petri plates (15.6 to 500 mg/mL). The agar concentration was adjusted to 17 g/L with bacteriological agar (A5306; Sigma). Positive (cefixime, 0.37 to 48 µg/mL and azithromycin, 0.094-12 µg /mL) and lactic acid (0.17 to 5.6 mg/mL which are equivalent to the titratable acidity of PFCM) control plates were also prepared. The plates were inoculated by one µL of the bacterial suspension having a 0.5 McFarland turbidity standard. The minimum inhibitory level (MIL) or concentration (MIC) was the lowest camel milk, PFCM, and control level that inhibits visible bacterial growth (37ºC/16-20h). Each experiment was carried out in triplicate.

Statistical analysis

Probiotic bacteria count, pH, and acidity of the PFCM and the antibacterial inhibition zones were analyzed using a One-Way ANOVA followed by Duncan's post hoc test. The Kruskal-Wallis H and Mann-Whitney U tests were applied to compare the differences between the MIL or

MIC values. All the differences were considered significant at $p<0.05$.

Results

Table 1 and Figure 1 represent probiotic bacterial counts of N-PFCM samples at days one, six, and ten. The Bifidobacteria cell count declined by 1.06 log CFU/mL ($p<0.001$), and the *Lactobacilli* count dropped by 0.26 log CFU/mL after ten days ($p=0.038$). Despite the decreasing trend in probiotic counts over time, each milliliter of N-PFCM contained more than 10^7 CFU of the probiotics after ten days (Table 1).The tested bacteria exhibited different levels of susceptibility to camel milk and PFCM. The pasteurized camel milk showed significant listerial inhibition zones with a mean diameter of 19.53 mm, whereas it did not illustrate antibacterial activity against other bacteria (Table 2). The MIL of camel milk against *L. monocytogenes* was 250 mg/mL (Table 3).

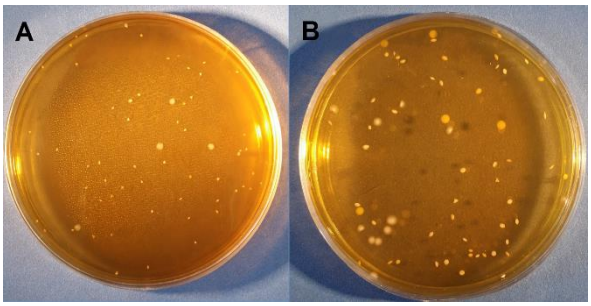

Fig 1. *Lactobacilli* (A) and bifidobacteria (B) colonies of N-PFCM in BSM and MRSB agar.

The N-PFCM showed higher antibacterial activity than camel milk. The N-PFCM generated inhibition zones against both Gram-positive and Gram-negative bacteria. Here, the sizes of N-PFCM originated inhibition zones were *L. monocytogenes*> *E. coli* O157:H7> MRSA> *S. aureus*> *P. aeruginosa* (Figure 2, Table 2). Here, the mean diameter of azithromycin induced inhibition zones for MRSA, *L. monocytogenes*, and *S. aureus* were 15.5, 23.5, and 30.5 mm, respectively. Furthermore, cefixime made inhibition zones with a mean diameter of 12, 14.5, 17.5, and 19.5 mm against MRSA, *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7.

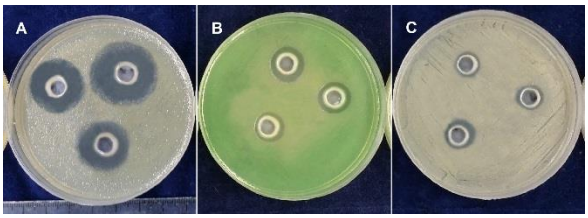

Fig 2. Zones of inhibition formed by N-PFCM against *L. monocytogenes* (A), *P. aeruginosa* (B), and MRSA (C).

The MIL evaluation also showed that the N-PFCM has a higher antibacterial activity than camel milk. The MILs of N-PFCM against *L. monocytogenes* were 2-fold lower than camel milk, while in the case of *E. coli* O157:H7, this difference was at least 4-fold ($p<0.05$). Notably, the MILs of N-PFCM were at least 3-fold smaller than camel milk for other bacteria ($p<0.05$). (Table 3; Figures 3).

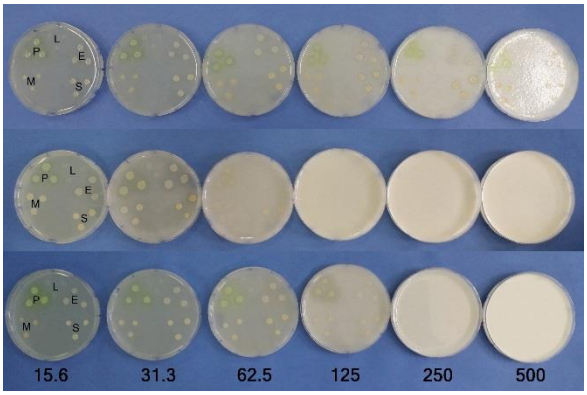

Fig 3. The MIL evaluation of pasteurized camel milk (top row), N-PFCM (middle row), and H-PFCM (bottom row) against *E. coli* (E), *L. monocytogenes* (L), MRSA (M), *P. aeruginosa* (P), and *S. aureus* (S) through 2-fold serial agar dilution (15.6 to 500 mg/mL) technique.

The N-PFCM could inhibit *L. monocytogenes* at a minimum of 62.5 mg/mL. The MIC study of lactic acid revealed that 62.5 mg/mL of N-PFCM does not have enough TA-based lactic acid content as the MIC of lactic acid for *L. monocytogenes* ($p=0.034$). The N-PFCM could inhibit *S. aureus* and *P. aeruginosa* at a minimum of 125 mg/mL. Nonetheless, the TA-

based lactic acid of 125 mg/mL of N-PFCM could not inhibit the bacteria (p=0.025). The inhibition of these bacteria genuinely needed a 1-fold higher lactic acid concentration. Similarly, the N-PFCM showed higher anti-MRSA and anti-*E. coli* O157:H7 activity than their equivalent lactic acid control groups. Interestingly, the inhibition of *E. coli* O157:H7 and MRSA literally required a 2-fold higher lactic acid concentration than the N-PFCM TA-based lactic acid content (p=0.025) (Figure 4).

The MIL of H-PFCM against *L. monocytogenes* was statistically similar to camel milk (p=0.114), while for other bacteria, it was at least 2-fold lower than camel milk (Table 3; Figure 3). Intriguingly, the H-PFCM showed smaller bacterial inhibition zones than the N-PFCM. This dramatic effect of the thermal process was statistically significant for *S. aureus*, MRSA, *E. coli* O157:H7, and *L. monocytogenes* (Table 2).

It is noteworthy that the N-PFCM showed lower MIL values than the H-PFCM (p<0.05). The thermal process of PFCM caused a 2-fold increase in MIL for *E. coli* O157:H7 and at least a 1-fold increase for the other bacteria (Table 3; Figure 3). For all the bacteria, except MRSA, the concentration of TA-based lactic acid in H-PFCM MILs was similar to the MIC of lactic acid in the control groups (p>0.05). However, the MIC of lactic acid for MRSA was 1-fold higher than the lactic acid concentration already existing in the MIL of H-PFCM.

Concerning positive controls, cefixime showed MIC values of <0.375 and 12 µg/mL for *E. coli* O157:H7 and *S. aureus*, respectively. The inhibition of other bacteria did not occur even at 48 µg/mL of cefixime. The MIC values of azithromycin for *L. monocytogenes*, *S. aureus*, and *E. coli* O157:H7 were 0.375, 0.75, and 6 µg/mL, respectively. Azithromycin did not inhibit the growth of MRSA and *P. aeruginosa* even at 12 µg/mL.

Discussion

The natural antibacterial activity of camel milk depends on the stage of lactation. lysozyme, lactoferrin, and lactoperoxidase are reported to act against *Listeria* [23]. Camel milk antimicrobial factors are more heat resistant than cow milk proteins. Heating camel milk at 65°C/30min has no significant effect on lysozyme and lactoferrin [24]. Based on this rationale, vat pasteurization of camel milk (63-65°C/30min) could not inactivate lysozyme and lactoferrin. In this study, camel milk showed anti-bacterial activity against listeria. Most of the anti-bacterial effects against listeria constituents of camel milk are water-dispersible since they have generated substantial bacterial inhibition zones in a water-based medium (Table 2).

When raw camel milk undergoes heat processing, its flavor profile undergoes

Table 1. Probiotic bacterial counts, titratable acidity, and pH values (Mean±SD; n=3) of N-PFCM during storage up to ten days

Product characteristics	**Days**		
	1	**6**	**10**
Probiotic bacterial counts (Log CFU/mL)			
***B. animalis subsp.lactis* BB-12**	8.48±0.18^{a}	8.02±0.09^{b}	7.42±0.39^{c}
***Lb. acidophilus* La-5**	7.97±0.09^{a}	7.88±0.004^{ab}	7.71±0.10^{b}
pH	3.89±0.19	3.97±0.31	3.98±0.19
Titratable acidity (g/L)	11.5±0.10^{a}	11.26±0.05^{ab}	11±0.20^{b}

Values with the different superscripts in each row are significantly different (p<0.05).

Table 2. The diameter of bacterial inhibition zones (mm; Mean ± SD) produced by camel dairy products

Dairy products	*S. aureus*	MRSA	*L. monocytogenes*	*E. coli* O157:H7	*P. aeruginosa*
P	0^{b}	0^{b}	19.5 ± 0.93^{b}	0^{c}	0^{c}
N-PFCM	9.7 ± 0.32^{a}	11.9 ± 0.85^{a}	25.2 ± 0.96^{a}	12.5 ± 0.36^{a}	8.75 ± 0.32^{b}
H-PFCM	0^{b}	0^{b}	15.9 ± 0.61^{c}	11.5 ± 0.51^{b}	8.48 ± 0.46^{b}
L	0^{b}	0^{b}	0^{d}	0^{c}	10.1 ± 0.14^{a}

P: pasteurized camel milk; N-PFCM: non-heated probiotic-fermented camel milk; H-PFCM: heated probiotic-fermented camel milk; L: lactic acid (equivalent to PFCM titratable acidity); MRSA: methicillin-resistant *Staphylococcus aureus*. Values with the different superscripts in each column are significantly different ($p<0.05$)

Table 3. The median MIL values (mg/mL) of camel dairy products against gram-positive and gram-negative bacteria

Dairy products	*S. aureus*	MRSA	*L. monocytogenes*	*E. coli* O157:H7	*P. aeruginosa*
P	$>500^{a}$	$>500^{a}$	250^{a}	$>500^{a}$	$>500^{a}$
N-PFCM	125^{c}	125^{c}	62.5^{b}	62.5^{c}	125^{c}
H-PFCM	250^{b}	250^{b}	125^{a}	250^{b}	250^{b}

P: pasteurized camel milk; N-PFCM: non-heated probiotic-fermented camel milk; H-PFCM: heated probiotic-fermented camel milk; MRSA: methicillin-resistant *Staphylococcus aureus*. Values with the different superscripts in each column are significantly different ($p<0.05$)

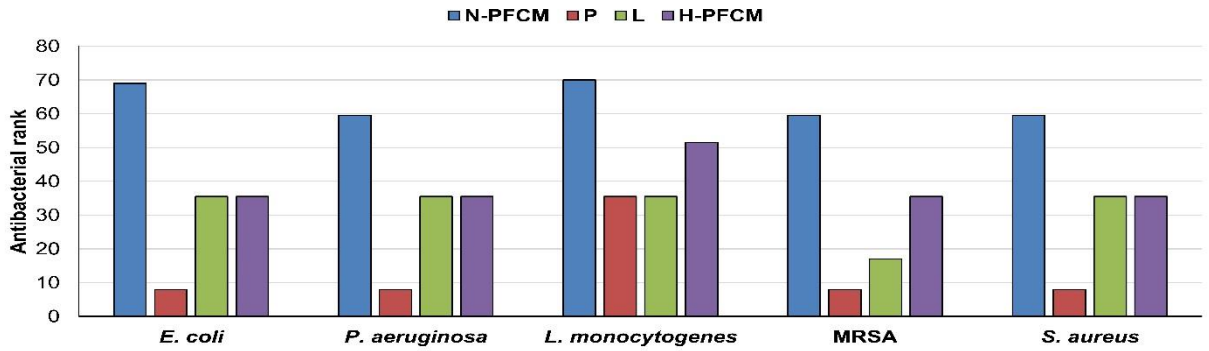

Fig 4. The mean antibacterial ranks of pasteurized camel milk (P), N-PFCM, H-PFCM, and their lactic acid constituent (L) obtained from MIC and MIL studies

alterations. After heating, the sour and umami tastes decrease, while saltiness increases. During fermentation, a refreshing lactic tang dominates the product [25].

Antibacterial lactoproteins of camel milk, including lactoferrin and IgG, are susceptible to hydrolysis during fermentation [26]. Moreover, the remnants of antibacterial compounds from camel milk are conceivably far from their optimal pH of activity in the acidic condition of PFCM. In contrast, the production of PFCM from camel milk enhanced its antibacterial activity. The MIL of PFCM against all the Gram-positive and Gram-negative bacteria was lower than camel milk (Table 3). In reports, various pathogenic bacteria show susceptibility to fermented camel milk [27, 8]. Lactic acid and lactates are among the general antibacterial agents of fermented milk [28, 29]. Generally, the antibacterial activity of lactic acid is due to the penetration of undissociated lactic acid through the cytoplasmic membrane into the cytoplasm where the dissociated form reduces intracellular pH and results in proton motive forces failure and finally decreases the available energy for bacterial growth [30]. Intriguingly, the assessment of MILs of PFCM revealed that most of the bacteria, except *L. monocytogenes*, were

more vulnerable to N-PFCM than its TA-based lactic acid constituent (Table 3). Lactic antagonism is a phenomenon imparts the antagonistic effects of LAB against closely related or food-poisoning and food spoilage organisms. Frequently, mixed starter cultures show this antagonism [31, 15]. Hydrogen peroxide and bacteriocins, as the antibacterial metabolites of LAB, and nutrient depletion are some of the other presumed mechanisms of lactic antagonism [32, 33].

The lactic acid producing cultures in the current study contained *Streptococcus thermophilus*, *Lb. acidophilus* La-5, and *B. animalis* subsp. *lactis* BB-12. Bacteriocins are antimicrobial peptides produced by bacteria to inhibit the growth of other bacterial strains. Thermophilins are the bacteriocins produced by *Strep. thermophilus* that inhibit *S. aureus* and *L. monocytogenes* [34]. Bifidobacteria and *Lb. acidophilus*, as synergistic probiotics, show antagonistic effects towards pathogens [35, 31]. In milk, *Lb. acidophilus* produces lactic and acetic acid, H_2O_2, and bacteriocin [31, 33]. Several bacteria such as *E. coli*, *S*. Typhimurium, and *P. aeruginosa* are susceptible to *Lb. acidophilus* La-5 [36]. When *Lb. acidophilus* La-5 grows in co-cultures with *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, it produces a class II bacteriocin called Lactacin B [33, 37]. The so-called class II bacteriocins induce membrane permeabilization in sensitive Gram-positive bacteria. As reported, *L. monocytogenes* is vulnerable to this class of bacteriocin [38, 33]. Bifidobacteria are lactic and acetic acid-producing anaerobes. In a study, *B. animalis* subsp. *lactis* BB-12 inhibited *S*. Typhimurium and *P. aeruginosa* [31, 36]. Notably, *B. animalis* subsp. *lactis* produces a bacteriocin-like inhibitory substance (BLIS) that inhibits *L. monocytogenes* [39]. Furthermore, a study showed that the bacteriocin of *B. animalis* subsp. *lactis* BB-12 inhibits *S. aureus*, *S.* Typhimurium, and *E. coli* [40]. In the current study, *L. monocytogenes* and *E. coli* O157:H7 were more eminently affected by lactic antagonism of N-PFCM (Table 2 and 3). As mentioned, *L. monocytogenes* is a common target by the bacteriocins of *Strep. thermophilus*, *Lb. acidophilus*, and *B. animalis* subsp. *lactis*. Finally, it is noteworthy that proteins changes, such as limited proteolysis of whey proteins [41] or PGRP digestion [42], along with the proteolytic activity of probiotics [43] may propose some unknown aspects of lactic antagonism.

We further evaluated the effect of heat treatment (85°C/2min) on the antibacterial activity of PFCM. The production of PFCM degrades natural antibacterial lactoproteins of camel milk but produces new proteinous antibacterial metabolites that are nearly heat resistant. The antibacterial activity of H-PFCM is mostly higher than camel milk due to the active remnants of antimicrobial metabolites from the fermentation process (Table 2 and 3). Lactacin B, the bacteriocin produced by *Lb. acidophilus*, is stable at 100°C for 60min [44]. Moreover, thermophilins of *Strep. thermophilus* show heat stability at 100°C for 45min [34]. Accordingly, heating PFCM for 85°C/2min does not fully disable the abovementioned bacteriocins. Nonetheless, the thermal process may affect the antibacterial activity of lactic acid in PFCM by the relative polymerization [45]. The starter bacteria in this study are relatively heat-sensitive organisms. *Lactobacillus acidophilus* does not tolerate 60°C for 30min and *B. animalis* subsp. *lactis* BB-12 can be inactivated at 62.3°C in 6min [46, 31]. Heat treatment of N-PFCM at 85°C for 2min presumably inactivates the starter bacteria especially considering the hurdle effect of heat with the acidic condition of PFCM. Despite the presence of active antibacterial metabolites of starter culture in H-PFCM, the destructive action of thermal process on the starter bacteria and thus losing the competition exclusion impairs the lactic antagonism. The heat-induced antibacterial reduction rate of PFCM was more distinct regarding *E. coli* O157:H7 and *L. monocytogenes* (Table 2 and 3).

Application of PFCM as a natural food preservative, nutraceutical, or functional food is always associated with dilution through food constituents, gastrointestinal contents, or base ingredients of topical delivery systems. An 8-times diluted N-PFCM could still inhibit the growth

of *S. aureus*, MRSA, and *P. aeruginosa*, while *L. monocytogenes* and *E. coli* O157:H7 inhibition can be obtained through a 16-times dilution. Conspicuously, the H-PFCM should not be diluted more than 4-times to demonstrate antibacterial activity against most bacteria (Table 3).

Fermented camel milk combines the advantages of regular camel milk with the added benefits of fermentation. So camel milk is a compelling choice for those looking to improve nutrient intake, digestion, or benefit from its antibacterial effects through fermentation [47, 48].

Conclusion

In conclusion, we found that *L. monocytogenes* is a sensitive bacterium to pasteurized camel milk. The PFCM showed antagonistic activities against all the Gram-positive and Gram-negative bacteria. A substantial increase in *E. coli* O157:H7 inhibition from camel milk to N-PFCM was an exposition of this lactic antagonism. The MIL of N-PFCM against bacteria was 1- to 2-fold lower than TA-based lactic acid. These results supported the theory of multifactorial nature for this bacterial interference originating from PFCM. The bioactive compounds responsible for the commendable antibacterial effect of fermented camel milk could be identified through additional constitutional analysis. The heating of PFCM reduced its antibacterial activity by 1- to 2-fold. For most bacteria, the antibacterial activity of N-PFCM was downscaled to its lactic acid equivalent level by the heat treatment showing the role of live probiotics in lactic antagonism. The findings suggest that a less thermally abused PFCM can be a candidate for nutraceutical formulations or natural food preservatives.

Abbreviations

PFCM: probiotic-fermented camel milk, N-PFCM: non-heated PFCM, H-PFCM: heated PFCM, MIL: minimum inhibitory level, MIC: minimum inhibitory concentration, LAB: lactic acid bacteria, MRSA: Methicillin-resistant *Staphylococcus aureus*, BSM: Bifidobacterium selective medium, MRS: De Man, Rogosa, and Sharpe, TA: Titratable acidity, BLIS: bacteriocin-like inhibitory substance

Acknowledgements

This work was supported financially by a Grant for Scientific Research from Vice Chancellor of Research of Shahid Bahonar University of Kerman.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

Not applicable

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How to cite this article:
Ebrahimnejad, H., Mansouri-Najand, L., Nikvarz A. Ahmadi, A. Antibacterial activity of dromedary camel milk fermented with probiotics against some pathogenic bacteria. Veterinary and Comparative Biomedical Research, 2024, 1(1): 61 – 71. http://doi.org/10.22103/Vcbr.2024.23216.1010