

Study of Potential Probiotic Properties of Lactic Acid Bacteria Isolated from Raw and Traditional Fermented Camel Milk

M. Mahmoudi¹, M. Khomeiri^{1*}, M. Saeidi², M. Kashaninejad¹, and H. Davoodi³

ABSTRACT

In the present research work, the potential probiotic properties of *Lactococcus lactis* KMCM3 and *Lactobacillus helveticus* KMCH1 isolated from raw camel milk and traditional fermented camel milk (Chal), respectively, were studied. The probiotic properties of isolates that were investigated included the hemolysis, antibiotic resistance, antibacterial features, resistance to low pH and bile salts, survival under simulated GastroIntestinal Tract (GIT) conditions, adhesion ability to hydrocarbon, and their auto-aggregation and co-aggregation rates. None of isolates exhibited hemolytic activity. They were susceptible against tetracycline, penicillin, ampicillin, chloramphenicol, erythromycin and vancomycin. *Lac. lactis* KMCM3 and *L. helveticus* KMCH1 retained their viability at pH 3.0 (8.68 and 8.6 log cfu mL⁻¹, respectively), 0.3% w/v bile salts (8.23 and 8.58 log cfu mL⁻¹, respectively) and under simulated GIT conditions (8.31 and 8.46 log cfu mL⁻¹, respectively). Both of these isolates inhibited the growth of *E. coli*, *S. aureus*, *L. monocytogenes*, *B. cereus* and *S. enterica* subsp. *enterica* serovar Typhimurium with MIC values of 6.25 to 25 mg mL⁻¹. In addition, They exhibited an ability to adhere to hydrocarbon (xylene), and possessed a high auto-aggregation and co-aggregation rate (more than 40%).

Keywords: *Lactococcus lactis*, *Lactobacillus helveticus*, Chal, Auto-aggregation rate, Co-aggregation rate

INTRODUCTION

Camel Milk (CM) and traditional Fermented Camel Milk (FCM) are widely consumed as important sources of human nutrition in Africa and the Middle Eastern countries (Fguiri *et al.*, 2015). CM contains low amounts of cholesterol. CM can be considered as a nutritious product with high stability due to high contents of antimicrobial agents such as lysozyme, lactoperoxidase, lactoferrin, and immunoglobulin (Khalesi *et al.*, 2017). It is easily digestible due to soft coagulum formation after milk ingestion in the gastrointestinal tract (Shamsia, 2009).

Chal is an Iranian traditional FCM; it involves spontaneous fermentation without the addition of a starter culture and is produced in Turkmen Sahra and AqQala, Golestan Province, Iran (Soleymanzadeh *et al.*, 2016). Traditional products of FCM are consumed under different names in other countries such as Gariss in Sudan (Ashmaig *et al.*, 2009), Susaac in Kenya (Fguiri *et al.*, 2015), and Shubat in Kazakhstan (Akhmetsadykova *et al.*, 2015), and Ititu in Ethiopia (Seifu *et al.*, 2012). It is known as a functional food because of claimed health benefits such as its traditionally anti-infective, anti-cancer, antidiabetic effects (Fguiri *et al.*, 2015). Also, Ayyash *et al.* (2018) reported that the proteolytic, antioxidant, anti-cancer activity and ACE-

¹ Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Islamic Republic of Iran.

*Corresponding author; e-mail: khomeiri@gau.ac.ir

² Stem Cell Research Center, Golestan University of Medical Sciences, Gorgan, Islamic Republic of Iran.

³ Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Islamic Republic of Iran.



inhibition of water soluble extracts from all FCMs were higher than those of fermented bovine milk. There has been a great interest towards the isolation of new probiotic strains with health promoting benefits in order to use these bacteria in industrial and pharmaceutical applications (Khan, 2014).

Different species of Lactic Acid Bacteria (LAB) such as *L. plantarum*, *L. kefir*, *L. paracasei*, *L. casei*, and *Enterococcus faecium* are involved in the fermentation of CM (Soleymanzadeh et al., 2016; Akhmetsadykova et al., 2015). CM and FCM are important sources for the isolation of LAB having a high probiotic potential (Abushelaibi et al., 2017).

However, studying or identifying the LAB isolates in traditional fermented dairy products can be useful for their application in the industrial production of functional dairy products with indigenous strains (Ashmaig et al., 2009).

The aim of this study was to identify the LAB isolated from CM and Iranian traditional FCM (Chal), and to investigate their probiotic potential, including the antibacterial features, resistance to low pH and bile salts, survival under simulated GastroIntestinal Tract (GIT) conditions, adhesion ability to hydrocarbon, and finally, their auto-aggregation and co-aggregation rates. Thus, using these parameters, the effectiveness of the probiotic cultures can be understood for development of functional products in the dairy industry.

MATERIALS AND METHODS

Isolation and Initial Screening of LAB

Ten samples of CM and Chal were collected in sterilized bottles from Turkman Sahra and AqQala, Iran. Briefly, 10 mL of raw milk and Chal samples were added to 90 mL of sterile NaCl solution (0.85% w/v). The suspensions were homogenized with a vortex, then, 100 μ L of an appropriate dilution (10^{-1} to 10^{-3}) was spread on MRS agar. The plates were incubated anaerobically using a gas pack system (Anaerocult A, Merck, Germany) at

37°C for 48 hours. After the incubation time, the isolated bacteria were selected based on microscopic characteristics, Gram staining, and catalase activity for molecular identification (Ashmaig et al., 2009).

Molecular Identification of Isolates

The genomic DNA extraction was performed according to the instructions of the DNA extraction kit (Yekta Tajhiz Azma, Iran). The amplification of *16S rDNA* gene (1500 bp) was performed using a universal primer pair: 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R 5'-GGTACCTTGTTACGACTT-3' (Leite et al., 2015). The thermal cycler program used for the PCR reaction consisted of an initial denaturation at 95°C for 5 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 2 minutes; and then final extension at 72°C for 10 minutes. The sequencing of PCR products was carried out by Macrogen Inc. (Seoul, Korea). Finally, to identify each isolate, the sequences were analyzed using the BLAST program of the NCBI and selected based on the highest percentage of identity.

Hemolysis

To determine the non-pathogenic bacteria, their hemolytic activity was evaluated according to the method by Tejero-Sariñena et al. (2012). Each isolate was cultured on a blood agar plate containing 5% sheep blood and incubated for 48 hours at 37°C. Hemolytic activity is characterized by the observation of either clear zones around the colonies (β -hemolysis), green-hued zones around the colonies (α -hemolysis), or absence of any zone around the colonies (γ -hemolysis).

Antibiotic Resistance

The disk diffusion method was applied to determine the antibiotic susceptibility of

isolates against commonly prescribed antibiotics such as tetracycline, penicillin, ampicillin, chloramphenicol, erythromycin, vancomycin, kanamycin, streptomycin and gentamycin (PadanTeb Co., Iran) as reported by Vijayakumar *et al.* (2015). The results were interpreted as susceptible, intermediate, or resistant based on CLSI (2013).

Tolerance to Acidic and Bile Salts Conditions

The acid and bile salts tolerance was tested as reported by Nami *et al.* (2014b). The 24-hour bacterial cultures were inoculated into MRS broth adjusted to pH 3.0 or MRS broth containing 0.3% (w/v) bile salts. Suspensions were then incubated at 37°C for 2 hours under acidic conditions and for 2 and 3 h under bile salts conditions.

Survival Assessment under Simulated GastroIntestinal Tract (GIT) Conditions

The survival of isolates during simulated GIT passage was investigated according to the method by Nami *et al.* (2014b) with some modifications. Briefly, the cultures in a stationary phase were centrifuged at 4,000 rpm for 10 minutes. Then, a cell pellet of each bacterium was resuspended in 1 mL of MRS broth, then inoculated into 9 mL of MRS broth adjusted to pH 3.0 (by adding 4 N HCl) and later supplemented with a filter-sterilized solution of pepsin (3 mg mL⁻¹). The simulated gastric juice was incubated for 2 hours at 37°C. Subsequently, to create a simulated intestinal condition, a sterile solution of 4 N NaOH was added to bring the pH to 6.5, supplemented by a filter sterilized bile salt solution (0.3% w/v) and pancreatin solution (0.1% w/v). The simulated intestinal environment was incubated at 37°C and the sampling was performed at an interval of 2 and 3 hours, as these periods represent the simulation of fast and slow digestion in the

intestine. The viable cell counts were done on MRS agar to determine the survival rate.

Preparation of Cell Free Supernatant (CFS)

Each bacterium was inoculated into a MRS broth and incubated at 37°C under anaerobic conditions to reach the end of the logarithmic phase. After centrifugation at 14,000×g for 15 minutes at 4°C, the pH of CFS was neutralized to 7.2 by adding 5N NaOH. The neutralized and acidic CFSs were sterilized by using a 0.22 µm sterile syringe filter and were frozen at -20°C followed by freeze drying. On the test day, the freeze dried CFS was reconstituted with 1 mL of deionized water (Nami *et al.*, 2014a).

Antibacterial Activity

The microdilution method was used to evaluate the antimicrobial properties of LAB CFSs against pathogenic bacteria. Briefly, 180 µL of diluted CFS in Muller-Hinton Broth and 20 µL of each bacterial suspension (10⁵ cfu mL⁻¹) were added to each well. After 20 h of incubation, the lowest concentration of CFS that completely inhibited the growth of pathogenic bacteria was reported as Minimum Inhibitory Concentrations MIC. To determine the Minimum Bactericidal Concentration (MBC), 10 µL of each well of MIC was spotted on MHA and incubated at 37°C for 24 hours (Ben Slama *et al.*, 2013).

Auto-aggregation and Co-aggregation

The auto-aggregation and co-aggregation ability of the isolates were evaluated according to the method described by Collado *et al.* (2008) with slight modifications. Briefly, bacterial suspension were prepared in PBS and adjusted to an absorbance (A) of about 0.25±0.05 at 600 nm. Then, the samples were



incubated at room temperature without agitation. The auto-aggregation percentage is calculated as $1-(A_t/A_0) \times 100$, where A_t represents absorbance at 600 nm at time $t=5$ h, and A_0 the absorbance at $t=0$ h.

For the co-aggregation assay, equal volumes of each LAB suspension and pathogenic bacteria were mixed. The percentage of co-aggregation was expressed as $1-(A_{mix})/(A_{pat}+A_{pro}/2) \times 100$, where A_{pat} and A_{pro} represent the absorbance of each bacterial suspension in control tubes including pathogenic and probiotic bacteria at 0 hour, and A_{mix} represents the absorbance of the two mixed bacterial suspensions at 5 hours.

Cell Surface Hydrophobicity

Briefly, equal volumes of bacterial suspension and solvent were transferred to the tube and the two-phase mixture was completely mixed. After 1 hour, the hydrophobicity was reported as: $[(A_0-A)/A_0] \times 100$, where A_0 and A are the Absorbance before and after separation of the aqueous phase, respectively (Collado et al., 2008).

Statistical Analysis

All experiments were performed in triplicates. Statistical analysis was done on the data in a randomized complete design by using the SAS 9.1.3. Software, and Duncan test was applied to compare the averages at 5% level. The values were presented as mean \pm standard deviation.

RESULTS AND DISCUSSION

Molecular Identification of LAB Isolated From Camel Milk and Chal

Based on the BLAST results of sequences, two bacterial isolates, namely, *Lac. lactis* KMCM3 and *L. helveticus* KMCH1 isolated from CM and Chal, respectively, with the highest

percentage of identity (97%) were selected for further studies. Based on previous researches, the *Lac. lactis*, *Lac. garvieae*, *L. reuteri* and *L. plantarum* were isolated from raw CM in Abu Dhabi (United Arab Emirates) (Abushelaibiet al., 2017). *Enterococcus*, *Lactobacillus* and *Lactococcus* genera were dominant in raw and fermented CM from Kazakhstan (Akhmetsadykova et al., 2015). Leite et al. (2015) also identified 34 isolates from four Brazilian kefir grains by *16S rDNA* gene sequencing. Eighteen isolates belonged to *Leuconostoc mesenteroides*, 11 to *Lactococcus lactis* and 5 to *Lactobacillus paracasei*. Also, Soleymanzadeh et al. (2016) isolated *L. kefir*, *L. gasser*, *L. paracasei*, *L. plantarum*, *L. paraplantarum*, *Leuconostoc lactis*, *Enterococcus faecium*, *Weissella cibaria* from Chal by *16S rRNA* gene sequences. LAB species isolated from spontaneously FCM were *Lac. lactis*, *Pediococcus acidilactici*, *Lactobacillus plantarum*, *Streptococcus lutetiensis* and *Weissella confuse* which were identified through *16S rRNA* gene sequencing (Fugl et al., 2017).

However, to our knowledge, no other researcher has reported the isolation of *L. helveticus* from traditional FCM. The sequencing results and the isolation origin of the bacteria are presented in Table 1.

Hemolysis Activity

According to the recommendation of FAO/WHO (2002), non-hemolytic activity is the first property for selection of a probiotic strain, since it indicates that the bacteria are not pathogenic. According to our results, none of the selected isolates was able to hydrolyze red blood cells when grown in blood agar. These results are in agreement with studies of Abushelaibi et al. (2017) and Tejero-Sariñena et al. (2012).

Antibiotic Susceptibility of LAB

According to the results presented in Table 2, *L. helveticus* KMCH1 is resistant to

Table 1. Sequencing results of PCR products for identification of LAB species isolated from raw and traditional FCM (Chal).

Initial identification code	Sequencing results	Identity	Source	Sample collection location
KMCM3	<i>Lactococcus lactis</i>	97%	CM	AqQala, Iran
KMCH1	<i>Lactobacillus helveticus</i>	97%	(Chal)	TurkmanSahra, Iran

Table 2. Susceptibility of isolates to different antibiotics.^a

Antibiotics	Concentration (μg disk ⁻¹)	<i>Lac. lactis</i> KMCM3	<i>L. helveticus</i> KMCH1
		Diameter of inhibition zone (mm)	
Cell wall inhibitors			
Vancomycin	30 μg	18 (S)	0 (R)
Penicillin	10 μg	24 (S)	23 (S)
Ampicillin	10 μg	20 (S)	17 (S)
Protein synthesis inhibitors			
Tetracycline	30 μg	15 (I)	17 (I)
Chloramphenicol	30 μg	23 (S)	22 (S)
Streptomycin	10 μg	0 (R)	0 (R)
Gentamicin	10 μg	10 (R)	0 (R)
Kanamycin	30 μg	11 (R)	0 (R)
Erythromycin	15 μg	19 (I)	20 (I)

^a S: Susceptible, I: Intermediate, R: Resistant.

vancomycin (a glycopeptide), kanamycin, gentamycin, and streptomycin (aminoglycosides), while *Lac. lactis* KMCM1 is resistant to aminoglycosides and susceptible to the other antibiotics. Generally, the LAB isolates tested were sensitive to tetracycline, ampicillin, penicillin, chloramphenicol, and erythromycin, which are commonly administered for the treatment of gastrointestinal infections (Tejero-Sariñena *et al.*, 2012). In particular, resistance to vancomycin is a major concern because it is one of the few antibiotics that have a broad effect against clinical infections caused by multi-drug resistant pathogens (Zhou *et al.*, 2005). In our study, it has been observed that *Lac. lactis* KMCM3 had good sensitivity to most of the tested antibiotics, particularly vancomycin. Our results are similar to those obtained by Tejero-Sariñena *et al.* (2012) and Nami *et al.* (2014b). The resistance to vancomycin is an intrinsic

property in many *Lactobacillus* species that could be attributed to the presence of D-Ala-D-lactate instead of the normal dipeptide D-Ala-D-Ala in their peptidoglycan (Ammor *et al.*, 2008). The resistance to aminoglycosides is attributed to the absence of a cytochrome-mediated electron transport system that mediates in the antibiotic uptake (Argyri *et al.*, 2013). In the intrinsic resistance to antibiotics, the resistance genes are chromosomally encoded and cannot be transmitted to other bacteria. Thus, the risk of transmission to other organisms can be minimized (Tejero-Sariñena *et al.*, 2012).

Acid and Bile Salts Resistance

A pH value below 3.0 is not the most common pH value in the human stomach (Argyri *et al.*, 2013). Resistance to acid stress is one of the essential properties for probiotic microorganisms. According to our



results in Figure 1-a, no significant reduction was observed in viable counts of the tested LAB at pH 3.0 at the 2 hours time-point. They maintained their survival at more than $8.0 \log \text{cfu mL}^{-1}$. Similar results are reported by Nami *et al.* (2014b) and Abushelaibi *et al.* (2017). The acid tolerance of LAB is attributed to F0F1-ATPase activity, which is activated when the extracellular pH is low, to increase the intracellular pH (Corcoran *et al.*, 2005).

The bile salts tolerance is an important property for survival of probiotics in the small intestine to survive and exert their health benefits in GIT (Argyri *et al.*, 2013). Based on our findings in Figure 1-b, *Lac. lactis* KMCM3 showed a significant

decrease ($P < 0.05$) in bile salts in comparison to *L. helveticus* KMCH1, however both of them retained their viability more than $8.0 \log \text{cfu mL}^{-1}$. Our results are consistent with the results of Nami *et al.* (2014b), Lee *et al.* (2015), and Bian *et al.* (2016).

Survival in GIT Conditions

Screening of potential probiotic strains is based on their tolerance under GIT conditions (Vera-Pingitore *et al.*, 2016). As shown in Figure 2, the LAB testing shows no significant difference in simulated gastric juice environment. Mahmoudi *et al.* (2016)

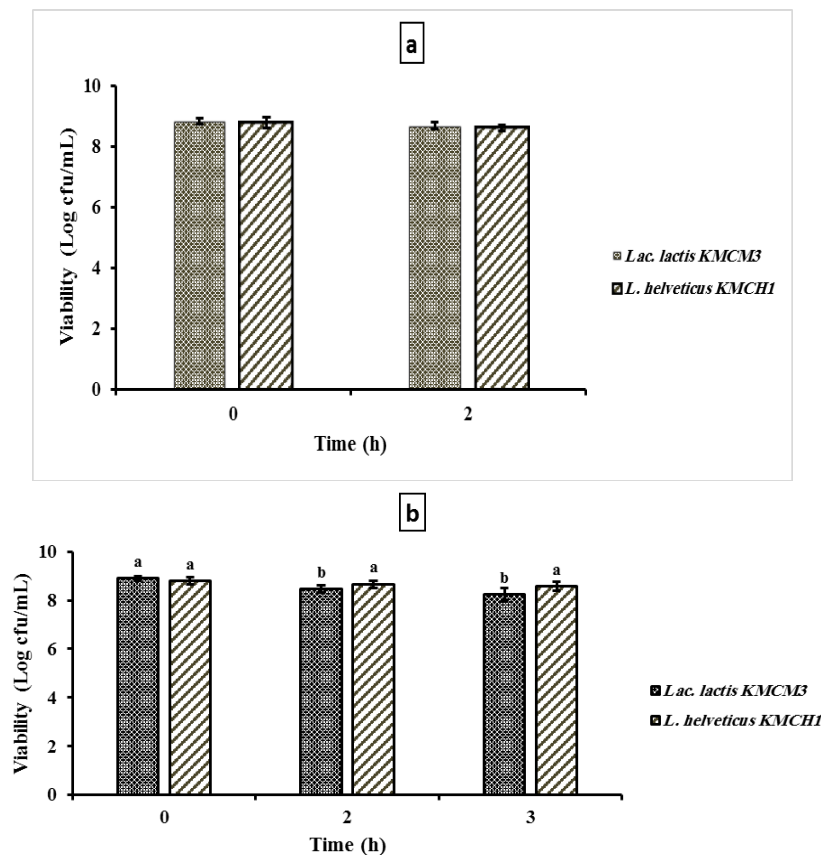


Figure 1. The viability of each bacterium (log cfu/ml) was measured with itself during time. Viability of *Lactobacillus helveticus* KMCH1 and *Lactococcus lactis* KMCM3 at MRS broth with pH 3.0 after 2 hours of exposure (a), MRS broth containing bile salts after 3 hours of exposure (b). Data of experiments in triplicate are expressed as Mean \pm SD. Mean values are not statistically significant in Duncan test ($P > 0.05$).

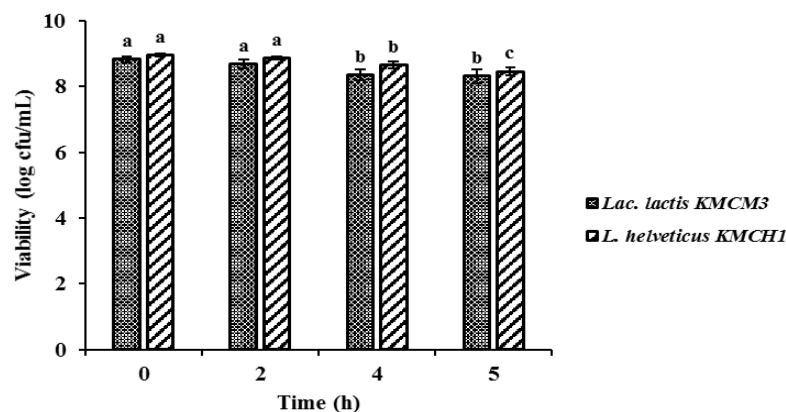


Figure 2. Viability of *Lactobacillus helveticus* KMCH1 and *Lactococcus lactis* KMCM3 in the simulated GastroIntestinal Tract (GIT). Experiments were performed in triplicate and data displayed as Mean \pm SD. Mean values with different lower case letters indicate significant differences between isolates in the Duncan test ($P < 0.05$) and means with the same letters are not significantly different ($P > 0.05$) by Duncan test.

reported that the action of pepsin on the cell membrane is not lethal to most LAB. This result is in accordance with Bian *et al.* (2016), whereas Lee *et al.* (2015) reported a significant decrease, which is approximately $3.0 \log \text{ cfu mL}^{-1}$ for *Lac. lactis* K24 after 2 hours of incubation in simulated gastric juice. This difference may be dependent on the strain. For probiotics to exert health benefits, they should be resistant to stomach acid stress and must survive the passage through the small intestine into the large intestine for subsequent colonization (Nami *et al.*, 2014b; Mahmoudi *et al.*, 2016). The tested LAB indicated a significant decrease ($P < 0.05$) while passing through the GIT, however, they retained their survival in the range of $8.0\text{--}9.0 \log \text{ cfu mL}^{-1}$. According to our findings, the resistance of *Lac. lactis* KMCM3 isolated from CM was remarkably more than *Lac. lactis* 2HL isolated from vaginal microflora by Nami *et al.* (2014b). This difference could be related to the origin of isolation and strain. Based on the data obtained from bile salts and GIT tolerance tests, the significant decrease observed for *L. helveticus* KMCH1 may be owing to the effect of pancreatin on the cell wall or membrane components (Ferrando *et al.*, 2016). In conclusion, the survival rate of the tested LAB was excellent in the simulated

GIT and could exert the expected health benefits.

Anti-Pathogen Features

Our results as seen in Table 3 indicate that the isolated LAB have MICs of 6.25 to 25 mg mL^{-1} against Gram-positive and Gram-negative pathogenic bacteria. This result reveals that the related

MIC values of CFS of the analyzed LAB have wide spectrum of anti-pathogenic activity. Considering that the neutralized CFSs exerted no antibacterial effect (data not shown), it can be concluded that antimicrobial activity of the CFSs is related to pH. Antimicrobial activity of LAB strains may be due to the production of organic acids, bacteriocins or other metabolites (Abushelaibi *et al.*, 2017). The effects of antimicrobial activity of *L. helveticus*, isolated from poultry waste (Ayantola and Oladunmoye, 2016), and *L. helveticus*, isolated from Sinkiang traditional cheese (Bian *et al.*, 2016), were attributed to the production of organic acids such as lactic and acetic acid. There is a hypothesis that organic acids, by neutralizing the cytoplasmic membrane's electrochemical potential, increase the membrane

**Table 3.** Antimicrobial activity of LAB species CFSs ^a against Gram-positive and Gram-negative pathogenic bacteria.

Pathogenic bacteria	ATCC No.	MIC ^b (mg mL ⁻¹)		MBC ^c (mg mL ⁻¹)	
		<i>Lac.</i> KMCM3	<i>Lactis</i> L. <i>helveticus</i> KMCH1	<i>Lac.</i> KMCM3	<i>Lactis</i> L. <i>helveticus</i> KMCH1
<i>Escherichia coli</i>	25922	12.5	6.25	25	12.5
<i>Staphylococcus aureus</i>	25923	25	6.25	25	12.5
<i>Listeria monocytogenes</i>	19115	12.5	6.25	50	12.5
<i>Bacillus cereus</i>	11778	12.5	6.25	12.5	6.25
<i>Salmonella enterica</i> subsp. <i>enterica</i> ser. <i>Typhimurium</i>	14028	12.5	6.25	25	12.5

^a Cell Free Supernatant; ^b Minimum Inhibitory Concentration, ^c Minimum Bactericidal Concentration.

permeability causing bacteriostasis and, subsequently, death of the susceptible cells (Dalié *et al.*, 2010). Other researchers have confirmed the inhibitory activity of organic acids and bacteriocins as produced by *Lac. lactis* strains (Hwanhlem *et al.*, 2017; Kruger *et al.*, 2013). The bacteriocins' mechanism of antimicrobial action could be related to the steps-adsorption of the bacteriocin on the cell wall, its transmission through the membrane and, finally, its activity within the cytoplasm (Garcha and Sharma, 2013).

Auto-aggregation, Co-aggregation, and Hydrophobicity Abilities

Cell adhesion is the process in which cells interact and attach to interacting surfaces or another cell (Kos *et al.*, 2003). It has been suggested that the bacterial strains' ability to adhere to epithelial cells and mucosal surfaces is another important property for selection of potential probiotic strains (Vijayakumar *et al.*, 2015). Based on several studies, it is observed that the aggregation ability is attributed to cell adherence properties (Kos *et al.*, 2003; Del Re *et al.*, 2000). The ability of microorganisms belonging to the same bacterial strain to aggregate is known as auto-aggregation; co-aggregation is associated with the aggregation between two different bacterial

strains. Cell surface hydrophobicity is defined as the ability of a strain to adhere to hydrocarbons (Collado *et al.*, 2008). A correlation has been reported between auto-aggregation and adhesion ability in *L. acidophilus* M92 (Kos *et al.*, 2003) as well as a relationship between adhesion and hydrophobicity (Del Re *et al.*, 2000) factors in some Bifidobacterium strains.

As seen in our results in Figure 3, the tested LAB show good auto-aggregation percentages, (more than 40%), indicating that such strains have a probiotic capacity (Peres *et al.*, 2014). These results are different from other *Lac. lactis* strains that indicated strain-dependence as reported by Abushelaibi *et al.* (2017).

Both LAB isolates showed co-aggregation ability with all the tested pathogenic bacteria (Figure 4). *L. helveticus* KMCH1 demonstrated a remarkable co-aggregation with all pathogens (more than 40%), but *L. monocytogenes* exhibited a high co-aggregation ability with both of the tested LAB (to a level of more than 50%). Moreover, *Lac. lactis* KMCM3 showed the lowest levels of co-aggregation toward *salmonella enterica* subsp. *entericas* erovar *Typhimurium*, and *S. aureus* (less than 40%) ($P < 0.05$). The co-aggregation of food-associated LAB with pathogens is of special interest for potential applications since it involves protecting the human gut from the

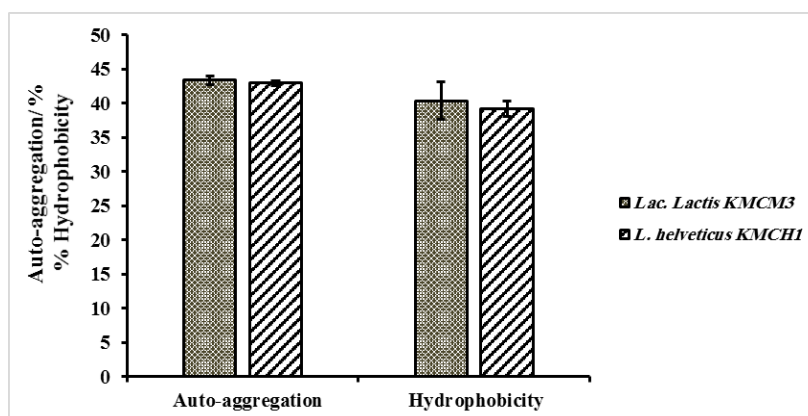


Figure 3. Auto-aggregation and cell surface hydrophobicity ability of *L. helveticus* KMCH1 and *Lac. Lactis* KMCM3 as a percentage. The results are represented as Mean of triplicates \pm SD. Values presented are not statistically significant in the Duncan test ($P > 0.05$).

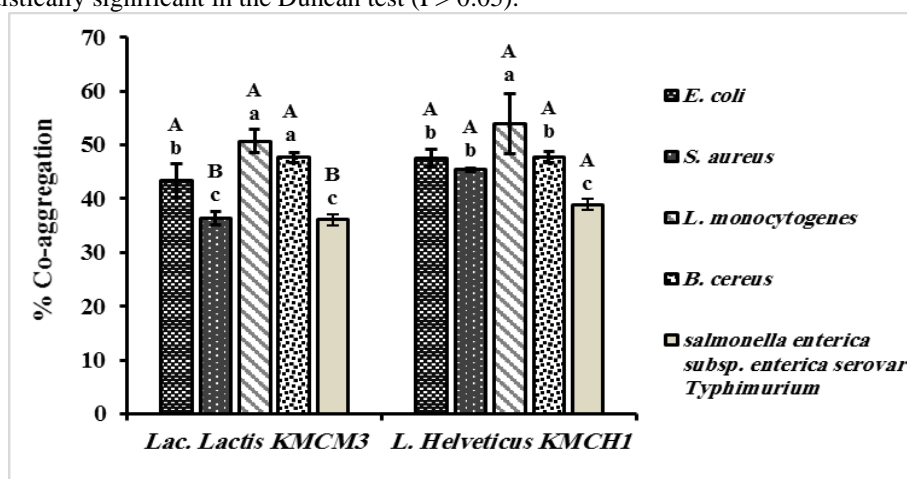


Figure 4. Percentage of co-aggregation of *L. helveticus* KMCH1 and *Lac. lactis* KMCM3 with pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* subsp. *Enterica* serovar *Typhimurium*, *Bacillus cereus*, and *Listeria monocytogenes*) after 5 hours co-incubation at room temperature. Bars with no common letter are statistically significant ($P < 0.05$) from each other; lower case letters show differences between pathogens for each *L. helveticus* KMCH1 and *Lac. lactis* KMCM3 strain and capital letters show differences of each pathogen between *L. helveticus* KMCH1 and *Lac. lactis* KMCM3 strains.

colonization of pathogens and infection of the gastrointestinal tract (Peres *et al.*, 2014).

The ability of bacteria to adhere to xylene reflects the cell surface hydrophobicity or hydrophilicity (Kos *et al.*, 2003). According to Figure 3, there is no significant difference between the two tested LAB in terms of affinity to the hydrocarbon. Both showed good adhesion capabilities towards xylene, indicating the hydrophobic property of the cell surface. It has been proposed that the probiotics possessing hydrophobic cell surface property are capable of adhering to

the intestinal mucosa. Although hydrophobicity may represent adhesion capability, it cannot be a prerequisite for strong adhesion to the human intestinal cells (Todorov *et al.*, 2008). From previous studies carried out on the microbial cell surface chemistry, it appears that hydrophobicity is related to the presence of (glyco-) proteinaceous material, whereas hydrophilicity is due to the presence of polysaccharides (Kos *et al.*, 2003).



CONCLUSIONS

In the present study, *Lactococcus lactis* KMCM3 and *Lactobacillus helveticus* KMCH1 isolated from camel raw milk and Chal, respectively, displayed the absence of hemolytic activity as well as sensitivity to antibiotics. Therefore, they are considered safe. Both of the LAB isolates showed a wide antibacterial activity spectrum. Furthermore, they displayed potential probiotic properties such as a remarkable survival rate under simulated GIT conditions. The tested LAB had high percentages of auto-aggregation, co-aggregation, and adhesion capabilities to the hydrocarbon xylene. These characteristics are related to the capability of isolates to adhere to the intestinal epithelial cells to compete with pathogens. Thus, we conclude that *Lac. lactis* KMCM3 and *L. helveticus* KMCH1 have all the necessary probiotic properties required for use as a probiotic culture in the development of functional dairy products.

REFERENCES

1. Abushelaibi, A., Al-Mahadin, S., El-Tarabily, K., Shah, N. P. and Ayyash, M. 2017. Characterization of potential probiotic LAB isolated from Camel Milk. *LWT - Food Sci. Technol.*, **79**: 316-325.
2. Akhmetsadykova, S. H., Baubekova, A., Konuspayeva, G., Akhmetsadykov, N., Faye, B. and Loiseau, G. 2015. LAB biodiversity in raw and Fermented Camel Milk. *Afr. J. Food Sci. Technol.*, **3**: 84-88.
3. Ammor, M. S., BélenFlórez, A., van Hoek, A. H. A. M., Reyes-Gavilan, C. G. D. L., Aarts, H. J. M., Margolles, A. and Mayo, B. 2008. Molecular characterization of intrinsic and acquired antibiotic resistance in LAB and bifidobacteria. *J. Mol. Microbiol. Biotechnol.*, **14**: 6-15.
4. Argyri, A. A., Zoumpopoulou, G., Karatzas, K. A. G., Tsakalidou, E., Nychas, G. J. E., Panagou, E. Z. and Tassou, C. C. 2013. Selection of Potential Probiotic LAB from Fermented Olives by In Vitro Tests. *Food Microbiol.*, **33**: 282-291.
5. Ashmaig, A., Hasan, A. and El Gaali, E. 2009. Identification of LAB Isolated from Traditional Sudanese Fermented Camel Milk (*Gariss*). *Afr. J. Microbiol. Res.*, **8**: 451-457.
6. Ayantola, K. J. and Oladunmoye, M. K. 2016. Antibacterial Activity of *Lactobacillus* Species Isolated from Poultry Waste (Droppings) against Poultry Pathogens. *Cur. Res. Poult. Sci.*, **1**: 7-12.
7. Ayyash, M., Al-Nuaimi, A. K., Al-Mahadin, S. and Liu, S. Q. 2018. In Vitro Investigation of Anticancer and ACE-Inhibiting Activity, α -Amylase and α -Glucosidase Inhibition, and Antioxidant Activity of Camel Milk Fermented with Camel Milk Probiotic: A Comparative Study with Fermented Bovine Milk. *Food Chem.*, **239**: 588-597.
8. Ben Slama, R., Kouidhi, B., Zmantar, T., Chaieb, K. and Bakhrouf, A. 2013. Anti-Listerial and Anti-Biofilm Activities of Potential Probiotic *Lactobacillus* Strains Isolated from Tunisian Traditional Fermented Food. *J. Food Safe.*, **33**: 8-16.
9. Bian, X., EtareriEviwie, S., Muhammad, Z., Luo, G. W., Liang, H. Z., Wang, N. N. and Huo, G. C. 2016. In Vitro Assessment of the Antimicrobial Potentials of *Lactobacillus helveticus* Strains Isolated from Traditional Cheese in Sinkiang China against Food-Borne Pathogens. *Food Function*, **7**: 789-797.
10. CLSI. 2013. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement*. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Collado, M. C., Meriluoto, J. and Salminen, S. 2008. Adhesion and Aggregation Properties of Probiotic and Pathogen Strains. *Europ. Food Res. Technol.*, **226**: 1065-1073.
12. Corcoran, B. M., Stanton, C., Fitzgerald, G. F. and Ross, R. P. 2005. Survival of Lactobacilli in Acidic Environments Is Enhanced in the Presence of Metabolizable Sugars. *Appl. Environ. Microbiol.*, **71**: 3060-3067.
13. Dalié, D. K. D., Deschamps, A. M. and Richard-Forget, F. 2010. LAB – Potential for Control of Mould Growth and Mycotoxins: A Review. *Food Control*, **21**: 370-380.
14. Del Re, B., Sgorbati, B., Miglioli, M. and Palenzona, D. 2000. Adhesion, Autoaggregation and Hydrophobicity of 13 Strains of *Bifidobacterium longum*. *Let. Appl. Microbiol.*, **31**: 438-442.
15. Ferrando, V., Quiberoni, A., Reinheimer, J. and Suárez, V. 2016. Functional Properties of *Lactobacillus plantarum* Strains: A Study In

- Vitro* of Heat Stress Influence. *Food Microbiol.*, **54**: 154-161.
16. Fguiri, I., Ziadi, M., Arroum, S., Khorchani, T., Gancel, F. and Dhulster, P. 2015. Selection of LAB Isolated from CM According to Production and Technological Criteria. *J.Chem. Biol.Physic. Sci.*, **2**: 1660-1671.
 17. Fugl, A., Berhe, T., Kiran, A., Hussain, S., Laursen, M. F., Bahl, M. I., Hailu, Y., Sørensen, K. I., Guya, M. E., Ipsen, R. and Hansen, E. B. 2017. Characterisation of LAB in Spontaneously FCM and Selection of Strains for Fermentation of CM. *Int. Dairy J.*, **73**: 19-24.
 18. Garcha, S. and Sharma, N. 2013. Use of Combination of Bacteriocins from *Lactobacillus plantarum* MTCC 1407 and *Bacillus coagulans* MTCC 492. *Afr. J. Microbiol. Res.*, **47**: 5338-5342.
 19. Hwanhlem, N., Ivanova, T., Haertlé, T., Jaffrès, E. and Dousset, X. 2017. Inhibition of Food-Spoilage and Foodborne Pathogenic Bacteria by a Nisin Z-Producing *Lactococcus lactis* subsp. *Lactis* KT2W2L. *LWT - Food Sci. Technol.*, **82**: 170-175.
 20. Khalesi, M., Salami, M., Moslehishad, M., Winterburn, J. and Moosavi-Movahedi, A. K. 2017. Biomolecular Content of CM: A Traditional Superfood towards Future Healthcare Industry. *Tren. Food Sci. Technol.*, **62**: 49-58.
 21. Khan, S. U. 2014. Probiotics in Dairy Foods: A Review. *Nut. Food Sci.*, **1**: 71-88.
 22. Kos, B., Šušković, J., Vuković, S., Šimpraga, M., Frece, J. and Matošić, S. 2003. Adhesion and Aggregation Ability of Probiotic Strain *Lactobacillus acidophilus* M92. *J. Appl. Microbiol.*, **94**: 981-987.
 23. Kruger, M. F., Barbosa, M. D. S., Miranda, A., Landgraf, M., Destro, M. T., Todorov, S. D. and Franco, B. D. G. D. M. 2013. Isolation of Bacteriocinogenic Strain of *Lactococcus lactis* subsp. *Lactis* from Rocket Salad (*Eruca sativa* Mill.) and Evidences of Production of a Variant of Nisin with Modification in the Leader-Peptide. *Food Control*, **33**: 467-476.
 24. Lee, N. K., Han, K. J., Son, S. H., Eom, S. J., Lee, S. K., Paik, H. D. 2015. Multifunctional Effect of Probiotic *Lactococcus lactis* KC24 Isolated from Kimchi. *LWT - Food Sci. Technol.*, **64**: 1036-1041.
 25. Leite, A. M. O., Miguel, M. A. L., Peixoto, R. S., Ruas-Madiedo, P., Paschoalin, V. M. F., Mayo, B. and Delgado, S. 2015. Probiotic Potential of Selected LAB Strains Isolated from Brazilian Kefir Grains. *J. Dairy Sci.*, **98**: 3622-3632.
 26. Mahmoudi, I., Ben Moussa, O., MouloukKhaldi, T. E., Kebouchib, M., Soligot, C., Le Roux, Y. and Hassouna, M. 2016. Functional *In Vitro* Screening of *Lactobacillus* Strains Isolated from Tunisian Camel Raw Milk toward Their Selection as Probiotic. *Small Rum. Res.*, **137**: 91-98.
 27. Maragkoudakisa, P. A., Zoumpopoulou, G., Miarisa, C., Kalantzopoulou, G., Potb, B. and Tsakalidoua, E. 2006. Probiotic Potential of *Lactobacillus* Strains Isolated from Dairy Products. *Int. Dairy J.*, **16**: 189-199.
 28. Nami, Y., Abdullah, N., Haghshenas, B., Radiah, D., Rosli, R., YariKhosroushahi, A. 2014a. Probiotic Potential and Biotherapeutic Effects of Newly Isolated Vaginal *Lactobacillus acidophilus* 36YL Strain on Cancer Cells. *Anaerobe*, **28**: 29-36.
 29. Nami, Y., Abdullah, N., Haghshenas, B., Radiah, D., Rosli, R., YariKhosroushahi, A. 2014b. Probiotic Assessment of *Enterococcus durans* 6HL and *Lactococcus lactis* 2HL Isolated from Vaginal Microflora. *J. Med. Microbiol.*, **63**: 1044-1051.
 30. Peres, C. M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M. R., Vilas-Boas, L., Peres, C. and Malcata, F. X. 2014. Novel Isolates of Lactobacilli from Fermented Portuguese Olive as Potential Probiotics. *LWT - Food Sci. Technol.*, **59**: 234-246.
 31. Seifu, E., Abraham, A., Kurtu, M. Y. and Yilma, Z. 2012. Isolation and Characterization of LAB from *Ititu*: Ethiopian Traditional FCM. *J. Camelid Sci.*, **5**: 82-98.
 32. Shamsia, S. M. 2009. Nutritional and Therapeutic Properties of Camel and Human Milks. *Int. J. Genet. Mol. Biol.*, **2**: 52-58.
 33. Soleymanzadeh, N., Mirdamadi, S. and Kianirad, M. 2016. Antioxidant Activity of Camel and Bovine Milk Fermented by LAB Isolated from Traditional FCM (Chal). *Dairy Sci. Technol.*, **96**: 443-457.
 34. Tejero-Sariñena, S., Barlowb, J., Costabile, A., Gibson, G. R. and Rowland, I. 2012. *In Vitro* Evaluation of the Antimicrobial Activity of a Range of Probiotics against Pathogens: Evidence for the Effects of Organic Acids. *Anaerobe*, **18**: 530-538.
 35. Todorov, S. D., Botes, M., Guigas, C., Schillinger, U., Wiid, I. and Wachsmann, M. B. 2008. Boza, a Natural Source of Probiotic LAB. *J. Appl. Microbiol.*, **104**: 465-477.



36. Vera-Pingitore, E., Jimenez, M.E., Dallagnol, A., Belfiore, C., Fontana, C., Fontana, P., Von Wright, A., Vignolo, G. and Plumed-Ferrer, C. 2016. Screening and Characterization of Potential Probiotic and Starter Bacteria for Plant Fermentations. *LWT - Food Sci. Technol.*, **71**: 288-294.
37. Vijayakumar, M., Ilavenil, S., Kim, D. H., Arasu, M. V., Priya, K., Choi, K. C. 2015. *In-Vitro* Assessment of the Probiotic Potential of *Lactobacillus plantarum* KCC-24 Isolated from Italian Rye-Grass (*Lolium multiflorum*) Forage. *Anaerobe*, **32**: 90-97.
38. Zhoua, J. S., Pillidgec, C. J., Gopalc, P. K. and Gill, H. S. 2005. Antibiotic Susceptibility Profiles of New Probiotic *Lactobacillus* and *Bifidobacterium* Strains. *Int. J. Food Microbiol.*, **98**: 211 – 217.

بررسی ویژگی‌های پروبیوتیکی بالقوه باکتری‌های لاکتیک اسید جدا شده از شیر خام و شیر شتر تخمیری سستی

م. محمودی، م. خمیری، م. سعیدی، م. کاشانی نژاد و ه. داوودی

چکیده

در پژوهش حاضر، ویژگی‌های پروبیوتیکی بالقوه *Lactococcus lactis* و *Lactobacillus helveticus* KMCH1 و KMCM3 به ترتیب جدا شده از شیر شتر خام و شیر شتر تخمیری سستی (چال) بررسی شدند. ویژگی‌های پروبیوتیکی جدایه‌ها شامل همولیز، مقاومت به آنتی‌بیوتیک، خاصیت ضد باکتریایی، مقاومت به pH پایین و نمک‌های صفرای، بقاء تحت شرایط شبیه‌سازی شده دستگاه گوارش (GIT)، توانایی چسبندگی به هیدروکربن و در نهایت میزان خود اتصالی و قابلیت تجمع آنها تحقیق شدند. نتایج آشکار کرد که هیچ کدام از جدایه‌ها فعالیت همولیتیکی نشان ندادند و در مقابل آنتی‌بیوتیک‌های تتراسایکلین، پنی‌سیلین، آمپی‌سیلین، کلروآمفنیکل، اریترومایسین و ونکومایسین حساس بودند. *L. helveticus* KMCH1 و *Lac. lactis* KMCM3 قابلیت زنده‌مانی‌شان را در pH=3 (۶۸/۸ و ۶/۶ لگاریتم)، ۰/۳ درصد نمک‌های صفرای (۲۳/۱ و ۵۸/۸ لگاریتم) و تحت شرایط شبیه‌سازی شده دستگاه گوارش (۳۱/۸ و ۴۶/۸ لگاریتم) حفظ کردند. هر دو جدایه قادر بودند رشد همه باکتری‌های بیماری‌زای *S. aureus*، *E. coli*، *S. enterica*، *L. monocytogenes* و *B. cereus* زیر گونه *enterica* سرووارته Typhimurium را با مقادیر MIC از ۲۵-۶/۲۵ mg/mL مهار کنند. به علاوه، آنها توانایی برای چسبیدن به هیدروکربن (زایلن) را نشان دادند و از میزان خود اتصالی و قابلیت تجمع بالایی (بیش از ۴۰ درصد) برخوردار بودند.