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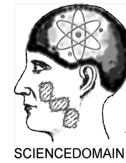

Functional Characteristics of *Lactobacillus* Strains Isolated from Camel's Milk

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMM designed the study, wrote the protocol, and wrote the first draft of the manuscript and managed the literature searches. Author MMA managed the experimental process and typed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed at isolation, identification and evaluation of probiotic potential of *Lactobacillus* isolates from camel's milk. Thirty four *Lactobacillus* isolates coded M 1 to M 34 were Gram positive, rods, catalase and oxidase negative and nonspore-forming bacteria. These isolates were identified by biochemical tests and API 50 CH kits. From these, 14 different *Lactobacillus* isolates (M 1, M 2, M 4, M 5, M 9, M 10, M 12, M 14, M 15, M 18, M 20, M 27, M 29 and M 31) which were tolerant to gastric and intestinal juices in a previous study were now tested for antipathogenic activity which varied according to the *Lactobacillus* species and the challenged pathogen. All 14 isolates demonstrated significant inhibitory effect against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus* and moderate to low activity against *Salmonella typhimurium* and *Escherichia coli*. When tested for bile tolerance at the concentration of 0.3 to 2.0%, the growth rate of 8 isolates M 2, M 5, M 9, M 10, M 12, M 14, M 18 and M 20 exceeded 60% in 0.3 and 0.5% bile.

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M 2 (*L. fermentum*) and M 12 (*L. plantarum*) and M 20 (*L. paracasei* ssp. *paracasei*) exhibited the highest growth rates of 82, 79.4 and 78.8% respectively. At higher levels of 1 and 2% bile, significant reduction ($p < 0.05$) was observed for all tested isolates except M 9 (*L. plantarum*) with growth rate of 66.5% at 2% bile. As for cholesterol reduction, M 10 (*L. plantarum*) and M 15 (*L. paracasei* ssp. *paracasei*) had the highest reduction rate of 58.0 and 53.2% respectively, which is comparable to the reference strain *L. reuteri* DSMZ 20056. Testing adhesion to intestinal epithelial cells and ileal tissues of BALB/c mouse; M 20 (*L. paracasei* ssp. *paracasei*) and M 2 (*L. fermentum*) exhibited highest attachment rate of more than 15 bacterial cells/epithelial cell. SEM images showed variable degrees of bacterial attachment to ileal tissues. These results suggest that camel milk is a rich source for potential probiotic lactobacilli which may be suitable for food and nutraceuticals industries; however, further *in vivo* investigations are needed.

Keywords: Probiotics; antipathogenic; cholesterol reduction; attachment; camel milk.

1. INTRODUCTION

Probiotics refer to live, non-pathogenic microbial preparations that beneficially exert health benefits on the host when administered in adequate amounts [1,2]. Estimates claim that 500-1000 different bacterial species are present in the human intestinal tract [3]. This involves both pathogenic and non-pathogenic microorganisms which are present in a varying complex symbiosis over human life-span [4]. Lactobacilli group are the first residents in the gastrointestinal tract after birth [5]. In healthy persons, they are present in the oral cavity, ileum, colon and in the vagina [6]. Lactobacilli is a group of Gram-positive, non-spore forming, catalase negative rods producing lactic acid by homo- or heterofermentative activity of carbohydrates [7-10]. Lactobacilli have been long known as the main microbiota in dairy industries [11]. Fermentation and other products of lactobacilli are extremely beneficial in controlling pathogens thus preventing spoilage of fermented foods among other functions [12].

To exert health benefits, live probiotic bacterial counts should be at the level of 10^7 cfu/ml of the product at the time of consumption [12]. Previous studies indicated healthy functions of lactobacilli properties pertaining to modulation of gut flora, reduction of gastrointestinal syndromes, diarrhea reduction, immune system enhancement, antimutagenic and anticarcinogenic activity and cholesterol lowering effects [7,13,14]. To achieve some or all of these suggested activities, probiotic microorganism should exhibit characteristics to overcome extreme low pH and bile toxicity, adherence to human intestinal mucosa, inhibitory activity against pathogens and production of antibacterial substances [15-17]. The continued search for new and novel probiotic bacterial strains is indispensable to obtain new functional products and also important to reach a state of producing more active probiotic cultures and hopefully producing designer selective probiotics for specific purposes. The quest in this direction, lead researchers to mine natural resources specially traditionally fermented foods for unique lactobacilli [18-21].

In this study, camel's milk both fresh and spontaneously fermented products which rarely been studied were used to isolate and identify potential probiotic strains of *Lactobacillus* and to study their bile tolerance, adhesion to intestinal tissues, cholesterol reducing level and antimicrobial activity against selected human pathogenic bacteria. These characteristics are among criteria of potential probiotics, hence are tested in this study. Results obtained may also help in clarifying the common belief in this region, of the curing ability of camel's milk products for several ailments [22,23] which may be linked to the presence of such probiotic bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Milk Samples and Enrichment for Indigenous Bacteria Growth

Ten samples (500 ml each) of raw camel's milk were collected from dromedary camel herds from different sites of Jordan during the period extending from April 2009 - May 2010. The samples were collected by manual milking in sterile plastic bottles, kept on ice and transported to the microbiology laboratory of the University of Jordan within 2 h of collection. Aliquots of the samples were used directly and

the remainder was allowed to ferment spontaneously through the raw milk indigenous microorganisms at room temperature, without any additives. The enrichment process of the collected samples was achieved by adding 10 ml of raw camel milk to 80 ml MRS broth medium (Oxoid, UK). The enriched samples were incubated at 30ºC and/or 37ºC for 1 week under static and/or shaking conditions.

2.2 Isolation of Bacterial Strains and Culture Conditions

Lactobacillus species were isolated from camel's milk by aseptic microbiological procedures. Briefly, selective medium for *Lactobacillus* de Man Rogosa and Sharpe (MRS) agar plates were used. One hundred microliters of tenfold dilution of milk samples in sterile normal saline were spread on the surface of MRS agar plates. Incubation was carried out by incubating lots at 30ºC and others at 37ºC. After 2-5 days incubation at anaerobic conditions using anaerogen sachet (AnaeroGen, UK) in anaerobic jar (Oxoid, UK), suspected *Lactobacillus* colonies were picked off and subcultured onto the same medium. In some cases, MRS agar supplemented with bromocresol purple (0.01% w/v) to obviate colonies of *Lactobacillus* species was used. To enhance isolation, MRS medium was supplemented with 0.5 g / L cysteine-HCl.

2.3 Maintenance of Isolated Strains

Isolated strains were stocked as frozen cultures in MRS broth with 20% glycerol at both -80ºC and -20ºC. Working cultures were kept on MRS agar slants and MRS agar plates at 4ºC and were routinely subcultured every 4 weeks.

2.4 Biochemical Identification of Bacterial Strains

All isolates were tested for catalase and oxidase activity, Gram reaction, cell morphology and spore formation. All Gram positive and catalase negative rods were tested for growth in MRS broths at 10, 37 and 45ºC and for growth at pH 3.9 and 9.6. The strains were tested for production of acids from carbohydrates and related compounds by using API 50 CH kits and CHL media ((BioMérieux, France). The API test strips were prepared according to manufacturer's instructions. Results were scored after incubation for 24 and 48 h at 37ºC. These results were joined to the apiweb™ identification software with database (V5.1), which uses the phenotypic data to predict a species identity. Interpretations of the fermentation profiles were facilitated by analytically comparing all results obtained for the isolates studied with information from the computer-aided database.

2.5 Bile Tolerance Test

The tolerance of the bacterial isolates to bile was tested using MRS broth prepared with and /or without 0.3, 0.5, 1 and 2% (w/v) oxgall (Oxoid, UK). Ten milliliter aliquots of bile solutions were transferred into standard glass tubes and sterilized by autoclaving at 121ºC for 15 min. For each new bacterial culture to be tested, three tubes of each concentration were inoculated with 0.2 ml of freshly prepared MRS broth culture of 0.5 McFarland. Inoculated tubes were incubated at 37ºC for 24 h. Bacterial growth was recorded by measuring optical density at 600 nm using a spectrophotometer (Biotech, UK). Growth ability (bile tolerance) was expressed as a percentage of that of the control (inoculated tubes of MRS broth without oxgall), which was assigned a value of 100%.

2.6 Antipathogen Activity of *Lactobacillus* Isolates

2.6.1 Agar spot method

The antibacterial activity of the selected *Lactobacillus* isolates was determined by the agar spot test described by Schillinger and Lücke (1989) [24] with some modifications as follows: five microliters of each overnight culture of *Lactobacillus* isolate were spotted onto the surface of MRS agar plates (containing 0.2% glucose) and were then incubated under anaerobic conditions at 37ºC for 48 h. An overnight culture of four indicator strains *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *B. cereus* (Toxigenic strain, TS), and methicillin resistant *S. aureus* (MRSA clinical isolate) were grown in nutrient broth and were adjusted to 0.5 McFarland and then were diluted 1:10 using nutrient broth to reach 10^7 CFU/ml. Aliquots of 0.25 ml were inoculated into 7 ml of soft nutrient agar (containing 0.2% glucose and 0.7% agar). Inoculated soft agar was immediately poured in duplicates over the MRS plate on which the tested *Lactobacillus* isolate was grown. The plates were incubated aerobically at 37ºC for 24 h.

The antibacterial activity was detected by measuring the diameter of inhibition zones around the *Lactobacillus* bacterial spots. Inhibition was recorded as positive if the diameter of the zone around the colonies of the producer was 2 mm or larger [25].

2.7 Cholesterol-lowering Effect

To test *Lactobacillus* for cholesterol-lowering effect, one fresh colony from each *Lactobacillus* isolate was inoculated into 5 ml MRS broth separately and incubated anaerobically for 24 h at 37ºC. Then, they were inoculated (1%) into MRS-THIO broth with 0.1 g/L filter-sterilized water-soluble cholesterol (polyoxyethanyl cholesteryl sebacate) (Sigma-Aldrich, USA) and incubated under anaerobic conditions at 37ºC for 24 h. After the incubation period, cells were centrifuged at 12,000 x g at 4ºC for 10 min, and the remaining cholesterol concentration in the broth was determined using o-phthalaldehyde modified colorimetric method as described by Rudel and Morris (1973) [26]. One milliliter of the supernatant (broth containing the remaining cholesterol) aliquot was added with 1 ml of KOH (50% w/v) and 2 ml of absolute ethanol, vortexed for 1 min, followed by heating at 37ºC for 15 min. After cooling, 2 ml of distilled water and 5 ml of hexane were added and vortexed for 1 min.

The hexane layer of 2.5 ml was transferred into a glass tube. The hexane was evaporated from each tube at 60ºC under the flow of nitrogen gas. The residue was immediately dissolved in 2 ml of o-phthaldehyde reagent. The reagent contained 0.5 mg of o-phthaldehyde per ml of glacial acetic acid. After complete mixing, the tubes were allowed to stand at room temperature for 10 min, and then 0.5 ml of concentrated sulfuric acid was added and the mixture was vortexed for 1 min. After standing at room temperature for an additional 10 min, the absorbance was read at 550 nm against a reagent blank. The removal rate of every strain was computed by the following formula: the cholesterol reducing rate = $[(A_0 - A) / A_0] \times 100\%$. Where, A_0: absorbance of the unfermented broth. A: absorbance of the broth fermented for 24 h.

2.8 Adhesion of *Lactobacillus* Isolates to the Intestinal Epithelial Cells of BALB/c Mouse

2.8.1 Mouse epithelial cells preparation

BALB/c mouse was sacrificed using high dose of ether and segments of the ileum were taken and opened, washed with phosphate buffer saline (PBS) (pH 7.2) and held in 10 ml PBS at 4ºC for 30 min to loosen the surface mucus. The segments were then rinsed thrice with PBS and the epithelial cells were scraped off with the edge of a microscopic slide and were suspended in PBS. The indigenous bacteria were removed and eliminated completely by washing the cells suspension three times with 10 ml of PBS and centrifugating at 100 x g at 2ºC, and then were examined microscopically to ensure that the adherent bacteria were removed.

2.8.2 *Lactobacillus* cells preparation

Selected *Lactobacillus* species were grown in MRS broth overnight at 37 °C. Aliquots of these were centrifuged and resuspended in PBS to give a cell density of 1×10^8 CFU/ml.

2.8.3 Adhesion assay

Five hundred microliters of each bacterial suspension were added to 500 µl of epithelial cell suspension separately and the mixtures were rotated at 35 rev/min at 37ºC for 1 hour. Then the non-adherent bacteria were removed by centrifuging the mixture for 10 min at 100 x g. The supernatants were discarded and the pellets were resuspended in 1 ml PBS and were washed thrice under the same conditions. The bacterial binding to the epithelial cells in the pellet was measured by observing Gram stained preparations with light compound microscope (Novex, Holland). Positive adhesion was recorded if more than 15 bacterial cells adhered to one epithelial cell [27].

2.9 Evaluation of *Lactobacillus* Adhesion to the Intestinal Ileal Tissue of BALB/c Mouse by Scanning Electron Microscopy

2.9.1 Mouse ileal tissue preparation

BALB/c mouse was sacrificed using high dose of ether and segments of the ileum were taken and opened, washed with phosphate buffer saline (PBS) (pH 7.2) and were held in 10 ml PBS at 4 °C for 30 min to loosen the surface mucus. The segments were then rinsed thrice with PBS. The indigenous bacteria were removed and eliminated completely by washing the ileal segments three times with 10 ml of PBS and centrifuging at 100 x g at 2ºC then the samples were examined microscopically to ensure that the adherent bacteria were removed.

2.9.2 *Lactobacillus* cells preparation

Selected *Lactobacillus* species isolates were grown in MRS broth overnight at 37ºC and were then centrifuged and resuspended in PBS to give a cell density of 1 × 10^8 CFU/ml.

2.9.3 Adhesion assay

One milliliter of each bacterial suspension was added to one ileal segment separately and the mixtures were rotated at 35 rev/min at 37ºC for 1 h. Then the non-adherent bacteria were removed by centrifuging the mixture for 10 min at 100 x g. The supernatants were discarded and the pelleted tissues were resuspended in 1 ml PBS and washed thrice under the same conditions. The bacterial binding to the ileal tissues in the pellet was observed by scanning electron microscopy (Inspect F 50, Netherlands).

2.9.4 Specimen preparation for scanning electron microscopy

Pellets of the ileal tissues binding bacteria were fixed with 1.5% glutaraldehyde solution (25% reagent) prepared in 0.1 M PBS (pH 7.2) and incubated at 4ºC overnight. After the fixation step, specimens were rinsed first with 0.1 M PBS for 10 min, and then rinsed further three times for 20 min each at 4ºC in order to remove the excess fixative. The dehydration was performed with a graded series of ethanol. Specimens were immersed in 30% ethanol for 2 min, 50% ethanol for 5 min, 70% ethanol for 10 min, 90% ethanol for 15 min, and finally 100% ethanol twice for 20 min at 4ºC. Ready specimens were then mounted on a holder that can be inserted into the scanning electron microscope. They were mounted on aluminium stubs using a double-sticky tape. Specimens were coated with a thin layer of approximately 20 nm to 30 nm of conductive metal, platinum, using a sputter coater (Bruker, Germany).

2.10 Statistical Analysis

The results are presented as means ± S.D. Statistical differences among bacterial isolates in the *in vitro* study were determined by two way ANOVA except for cholesterol reduction experiment which was determined by one way ANOVA. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 *Lactobacillus* Isolation

Thirty four confirmed *Lactobacillus* isolates coded M 1 to M 34 were isolated and identified using biochemical and API 50 CH kits. These isolates were diverse in terms of identity. However, the majority belonged to *L.* fermentum, *L. plantarum* and *L. paracasei ssp. paracasei*. As scarce as it is the case with studies on micro flora of camel's milk, Yateem et al. [28] reported the isolation of some lactobacilli from camel milk samples from Kuwait. It was also observed that some isolates of the same species varied slightly, an observation reported by Suriasih et al. [29] with *Lactobacillu*s isolates. Recently, Akhmetsadykova et al. [30] studied the flora of camel milk and among others; they were able to isolate *lactobacillus* species. Fourteen *Lactobacillus* isolates coded M 1, M 2, M 4, M 5, M 9, M 10, M 12, M 14, M 15, M 18, M 20, M 27, M 29 and M 31 were selected for further probiotic characterization (Table 1). Siezen et al. [31] reported variations on phenotypic and genotypic levels within the genus *Lactobacillus* specially with isolates of different environments. Experimental results of this study (Table 1) showed that *Lactobacillus species* from camel milk were mostly able to survive acidic pH (3.9) and the extreme alkaline pH of 9.6, unlike results of Ammor et al. [32] where little of their isolates tolerated such pH values. Ashmaig et al. [33] results of *Lactobacillus* isolates from camel milk showed somehow a similar trend in response to different parameters.

3.2 Antipathogen Activity

Table 2 showed varying degrees of the antipathogen activity of *Lactobacillus* species. Highest activity was observed for M 2 (*L. fermentum*) against methicillin resistant *Staphylococcus aureus* followed by M 10 (*L. plantarum*) against *Bacillus cereus*. M 4 (*L. fermentum*) was the least active against *Escherichia coli* and *Salmonella typhimurium*. In general all 14 isolated *Lactobacillus* exhibited significant antipathogenic activity against MRSA and *B. cereus*. Moderate activity against *S. typhimurium* was recorded by M 4 (*L. fermentum*), M 9 and M 10 (*L. plantarum*), M 18 (*L. paracasei* ssp. *paracasei*) and M 29 and M 31 (*L. rhamnosus*). These results were different compared with Coeuret et al. [34] who observed

strongest activity of *L. plantarum* and *L. paracasei* against *Salmonella* species. Soleimani et al. [35] reported antipathogenic activity of *L. plantarum* against different pathogenic *S. aureus* which is comparable with our isolates against MRSA. *Lactobacillus* species antipathogenic activity against Gram positive and Gram negative bacteria is associated with accumulation of primary metabolites [36,37] and production of antimicrobials and other mechanisms [38,12,28].

3.3 Bile Tolerance

Fourteen *Lactobacillus* isolates (M 1, M 2, M 4, M 5, M 9, M 10, M 12, M 14, M 14, M 15, M 18, M 20, M 27, M 29 and M 31) were chosen for further analysis due to their tolerance to simulated gastric and intestinal juices in a previous study. The relative growth rate of these isolates (Table 3) in the presence of different bile concentrations varied according to species. M 2 (*L. fermentum*) exhibited the highest growth rate of 82% at 0.3% bile concentration. M 12 (*L. plantarum*) and M 20 (*L. paracasei* ssp. *paracasei*) where as active at the relative growth rate of 79.4% and 78.8% respectively. At elevated concentrations of bile of 0.5%, M 12 showed excellent survival at 0.5% bile amounting to a relative growth rate of 73%. Both M 9 and M 10 (*L. plantarum*) were almost similar (Table 3). At 1% and 2% bile, M 2 (*L. fermentum*) and M 9, M 10 and M 12 which belong to *L. plantarum* showed relative growth rates above 65% and above the *L. reuteri* DSMZ 20056 which is a reference probiotic strain [39]. Most of the other isolates were significantly lower ($p < 0.05$) than these values and comparable with the reference strain. Bile is fundamental in the defenses of the gut [40]. The range of physiological concentrations of the human bile lies between 0.3 to 0.5% [41]. As presented above considerable species and strain variations to bile resistance are observed in this study and other studies [42], most likely due to expression of bile resistance mediator proteins by bacterial cells [43]. Some of our isolates specially *L. plantarum* isolates M 9, M 10 and M 12 (Table 3) were superior to lactobacilli isolates of Kaboré et al. [44] and comparable to Tambekar and Bhutada [45] isolates.

These variations in bile tolerance of isolates of this study agrees with previous findings of probiotic cultures being species as well as strain specific in response to bile concentrations [46,47].

Bacterial salt tolerance is indirectly related to cholesterol lowering through its incorporation into the cellular membranes of probiotic bacteria from the media during growth [48,49]. Current research findings suggest that probiotic bacterial function in the detoxification of bile salts increases their intestinal survival and persistence of producer strains [50,51]. This in turn improves the efficiency of the probiotic strains [52] and forms an important basic property in screening for novel probiotic strains. However, sensitivity to bile salts may be related to absence of bile salt hydrolase among other factors [12].

3.4 Cholesterol Lowering Effects

All selected *Lactobacillus* isolates (Fig. 1) showed high ability of cholesterol *in vitro* reduction. No significant ($p < 0.05$) variations were recorded. However M 10 (*L. plantarum*) was superior (58% reduction rate) to others as well as to the control strain of *L. reuteri* DSMZ 20056 (53.2%) which was similar to M 15 (*L. paracasei* ssp. *paracasei*) (53.2%). M 31 (*L. rhamnosus*) had the lowest reduction rate of 41%. Zheng et al. [40] isolated probiotic *Lactobacillus* strains including *L. plantarum* B23 which were able to assimilate and precipitate cholesterol with variations between isolates of the same and different species. It is recognized that high cholesterol levels in the human blood is a risk factor for coronary heart diseases [53]. It is also known that *Lactobacillus* strains that were able to assimilate cholesterol *in vitro* were also capable of reducing it *in vivo* [54,55]. In this study, the 14 selected *Lactobacillus* strains showed a well defined potential for cholesterol reduction *in vitro*. Some of these isolates as indicated above were superior even for *L. reuteri* DSMZ 20056 which is known in probiotic understanding to be of great cholesterol lowering effect [56]. These results agree with other findings Wang, et al. [41] both *in vivo* and *in vitro*. It is now known that lactobacilli with lowering cholesterol activities do that in multiple ways [57-59,41]. Lavanya, et al. [60] isolated lactic acid bacteria from fermented milk which assimilated and reduced cholesterol levels at the rate of 28-83%. Isolates of this study needs further testing to substantiate their probiotic characteristics *in vivo* as well as on improving their technological properties.

Table 1. Effect of NaCl, temperature and pH on the growth of *Lactobacillus* isolates and summary of API 50 biochemical identification results

Isolate	NaCl %				Temperature ºC			pH		API 50 CH designated species
	4	**6.5**	**8**	**10**	**10**	**37**	**45**	**3.9**	**9.6**	
M 1	+	-	-	-	-	+	+	+	+	*Lactobacillus fermentum*
M 2	+	+	-	-	+	+	+	+	+	*Lactobacillus fermentum*
M 4	+	+	+	-	-	+	+	+	+	*Lactobacillus brevis*
M 5	+	+	v	-	+	+	+	+	+	*Lactobacillus plantarum* 1
M 9	+	+	+	-	+	+	+	+	+	*Lactobacillus plantarum* 1
M 10	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum* 1
M 12	+	+	+	-	-	+	+	+	+	*Lactobacillus plantarum*
M 14	+	+	+	+	+	+	+	+	+	*Lactobacillus paracasei* ssp. *paracasei* 1
M 15	+	+	+	+	+	+	+	+	+	*Lactobacillus paracasei* ssp. *paracasei*
M 18	+	+	+	+	+	+	+	+	+	*Lactobacillus paracasei* ssp. *paracasei*
M 20	+	+	+	+	+	+	+	+	+	*Lactobacillus paracasei* ssp. *paracasei* 1
M 27	+	+	+	+	+	+	-	+	-	*Lactobacillus rhamnosus*
M 29	+	+	+	+	+	+	+	+	+	*Lactobacillus rhamnosus*
M 31	+	+	+	+	+	+	-	+	-	*Lactobacillus fermentum*

All isolates were Gram positive rods, catalase and oxidase negative and non-spore formers. (+): Positive growth; (-): Negative growth and (v): Variable

Table 2. Antibacterial activity (inhibition zones diameter) of the selected *Lactobacillus* species

Inhibition zone diameter (mm) of indicator strains, mean ± S.D, n=2				
	B. cereus	**MRSA**	***E. coli***	***S. typhimurium***
M1	42.5 ± 3.5	42.0 ± 1.4	34.0 ± 2.8	36.0 ± 2.8
M2	28.5 ± 0.7	54.5 ± 2.1	28.5 ± 0.7	32.5 ± 0.7
M4	40.5 ± 0.7	26.5 ± 4.9	25.5 ± 0.7	29.5 ± 2.1
M5	36.0 ± 1.4	36.5 ± 0.7	34.0 ± 4.2	30.0 ± 2.8
M9	49.0 ± 1.4	46.5 ± 0.7	35.5 ± 2.1	27.5 ± 0.7
M10	54.0 ± 1.4	44.0 ± 1.4	39.0 ± 1.4	28.5 ± 3.5
M12	37.0 ± 2.8	44.5 ± 0.7	38.5 ± 2.1	26.0 ± 1.4
M14	44.5 ± 2.1	43.0 ± 1.4	38.0 ± 1.4	32.5 ± 0.7
M15	42.5 ± 4.9	48.5 ± 3.5	36.5 ± 0.7	32.0 ± 2.8
M18	42.0 ± 2.8	45.5 ± 0.7	41.0 ± 2.8	29.5 ± 0.7
M20	44.5 ± 3.5	49.0 ± 2.8	40.5 ± 0.7	39.5 ± 0.7
M27	39.0 ± 1.4	43.5 ± 2.1	36.5 ± 0.7	39.0 ± 1.4
M29	41.0 ± 0.0	42.0 ± 1.4	36.5 ± 2.1	29.5 ± 6.3
M31	41.5 ± 2.1	47.5 ± 0.7	37.5 ± 2.1	28.0 ± 1.4
L. reuteri (DSMZ 20056)	26.5 ± 2.1	41.0 ± 1.4	47.5 ± 0.7	36.5 ± 4.9

Bacterial species: Bacillus cereus (B. cereus); Methicillin resistant Staphylococcus aureus (MRSA); Escherichia coli (E. coli); and Salmonella typhimurium (S. typhimurium). Low activity < 32 mm, moderate 33-39 mm, and significant > 39 mm. Differences were considered significant at p <0.05

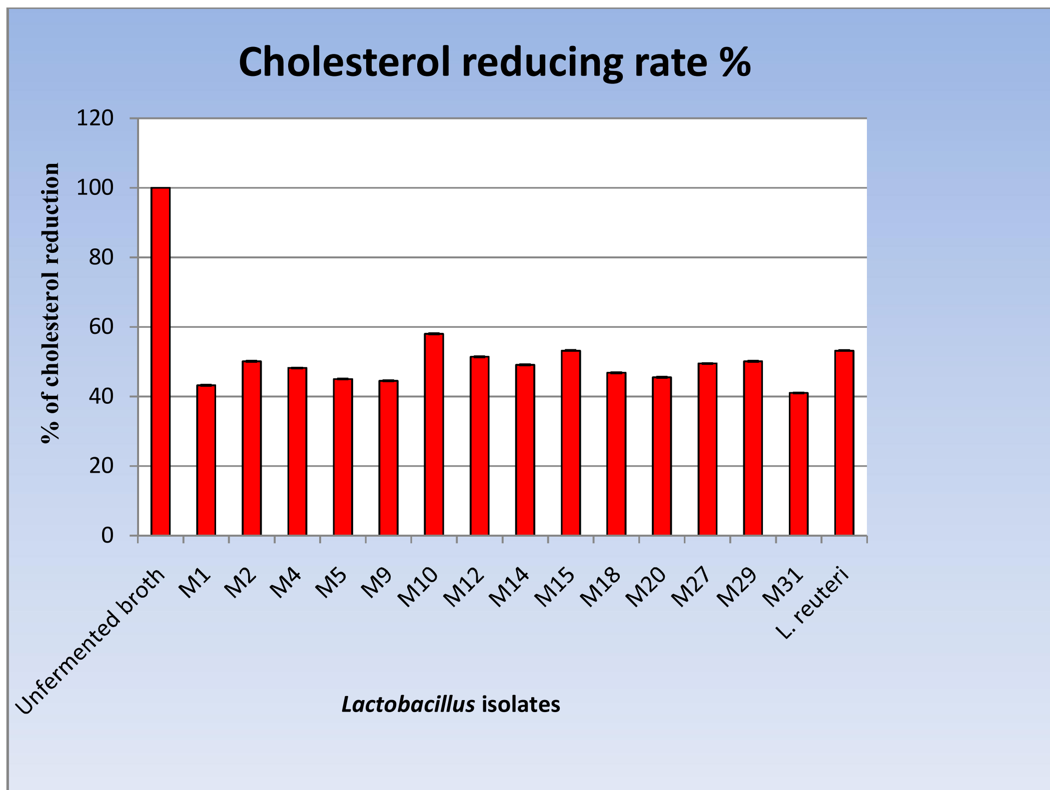


Fig. 1. Cholesterol-reducing rate of *Lactobacillus* isolates in addition to the control *L. reuteri* DSMZ 20056. The first column represents the control (unfermented MRS-THIO broth)

3.5 Adhesion of *Lactobacillus* Isolates to Intestinal Epithelial Cells and Intestinal Ileal Tissue of BALB/c Mouse

The adhesion ability of some selected isolates varied depending upon the species and strains. All were able to adhere to BALB/c mouse epithelial cells and the rate varied between 15 cells/epithelial cell: M 2 (*L. fermentum*) (Fig. 2A) and more than 15 cells/epithelial cell as in the case of M 20 (*L. paracasei* ssp. *paracasei*) (Fig. 2B) which showed significantly better binding. These values are better than the rate of attachment ability of the reference strain *L. reuteri* DSMZ 20056 (Fig. 2C). Other tested isolates exhibited significantly ($p < 0.05$) less adhesion ability. As for adhesion for BALB/c mouse ileal tissue, scanning electron micrographs showed variable degrees of attachment of bacterial cells. M 15 and M 18 (*L. paracasei* ssp. *paracasei*) (Fig. 3A and Fig. 3B) show well elaborate, crowded attachment on the surface area of the tissue. M 5 (*L. brevis*) exhibited strong adhesion affinity exemplified by the dense aggregates (Fig. 3C). Adhesion and colonization of probiotic bacteria is an essential character to express their health benefits [27,61]. It is established now that adhesion is a prerequisite for colonization and antagonistic activity against enteropathogens and immunomodulations [62,63]. Isolates of this study exhibited attachment ability to both epithelial cells and ileum tissue of BALB/c mouse and they were significantly better adherent than the reference probiotic strain of *L. reuteri* DSMZ 20056. Tsai et al. [64] found that *Lactobacillus* strains of animal origin were able to adhere strongly to different types of epithelial cells isolated from BALB/c mouse. Both et al. [65] recorded good adhesion ability of *L. acidophilus* and *L. casei* to epithelial cells. Martín et al. [66] attributed the good adhesion capacity of lactobacilli to mucin mediated by an extracellular form of glyceraldehyde 3-phosphate dehydrogenase. Although the results of *in vitro* testing may not be the same as *in vivo* [67], we can be sure that an association between adhesion ability and health benefits of probiotic bacteria exists [27,66]. It is concluded that the adhesion ability of lactobacilli is relatively dependent on variations among species, strains and their origin. This is also linked to adhesion factors on bacterial cell surfaces which invites for further investigations.

Finally, looking at the above results, it is rather clear that camel's milk is a good and unique source of interesting probiotic *Lactobacillus* strains. Most isolates exhibited significant activities pertaining to criteria needed for any bacterial isolate to be of potential use as a probitic in foods industries. Additionally, this study on camel milk shed some light on the traditional belief in Middle-Eastern countries that camel milk and products have a curative abilities as a nutraceutical against several ailments [68,23], a claim which needs further *in vivo* studies.

Table 3. Effect of bile concentration on the relative growth rates of *Lactobacillus* species

		Relative growth rate* (%)			
Isolates	**Control (without bile)**	**0.3**	**0.5**	**1**	**2**
M1	100	2.20±0.003	2.30±0.003	3.40±0.01	6.18±0.003
M2	100	82.0±0.009	72.1±0.02	73.8±0.02	33.5±0.003
M4	100	2.62±0.002	3.15±0.003	4.05±0.003	6.9±0.009
M5	100	76.2±0.05	52.1±0.05	24.5±0.004	22.7±0.11
M9	100	75.3±0.007	69.4±0.011	65.9±0.031	66.5±0.002
M10	100	77.9±0.005	72.1±0.03	69.2±0.04	37.2±0.03
M12	100	79.4±0.01	73.0±0.02	67.6±0.01	34.5±0.006
M14	100	76.7±0.03	71.5±0.009	62.4±0.004	32.8±0.003
M15	100	33.2±0.03	30.4±0.03	10.6±0.03	9.87±0.03
M18	100	60.5±0.02	46.8±0.003	31.7±0.02	27.2±0.009
M20	100	78.8±0.004	66.1±0.007	27.0±0.009	19.9±0.004
M27	100	2.92±0.007	2.73±0.004	3.35±0.0006	6.27±0.006
M29	100	3.26±0.004	2.67±0.004	3.73±0.006	6.82±0.005
M31	100	2.66±0.003	2.68±0.005	3.85±0.008	6.60±0.007
L. reuteri	100	3.42±0.007	2.42±0.004	4.60±0.01	6.11±0.008

** Data are expressed as percentage of the growth rate (h^{-1}) obtained in absence of bile, which was assigned a value of 100 %. Means ± SDs of three independent experiments are given. Differences were significant at ($p < 0.05$)*

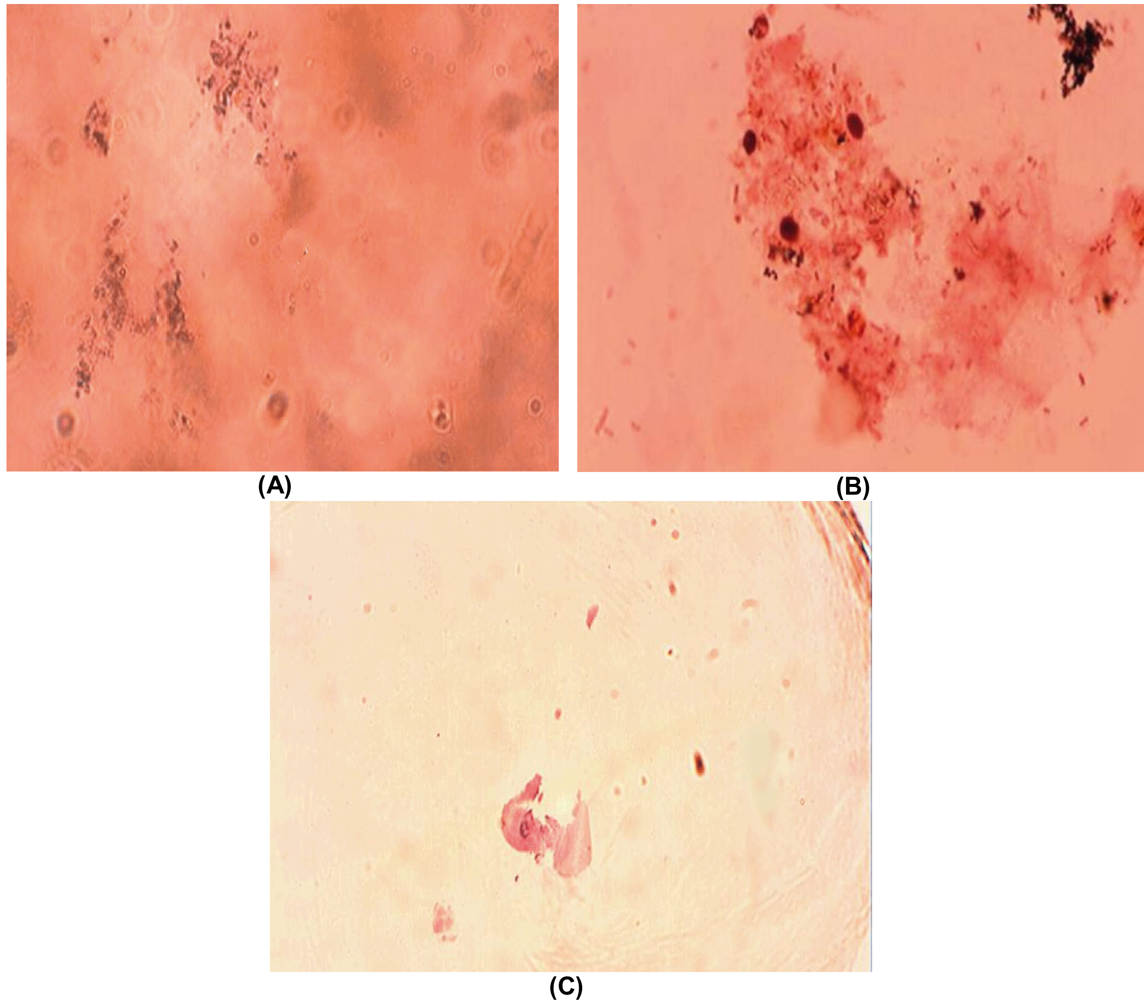
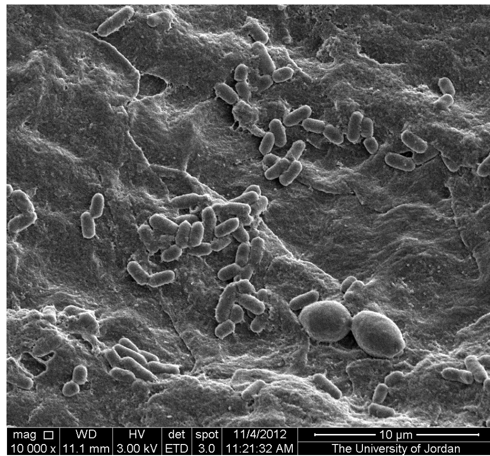
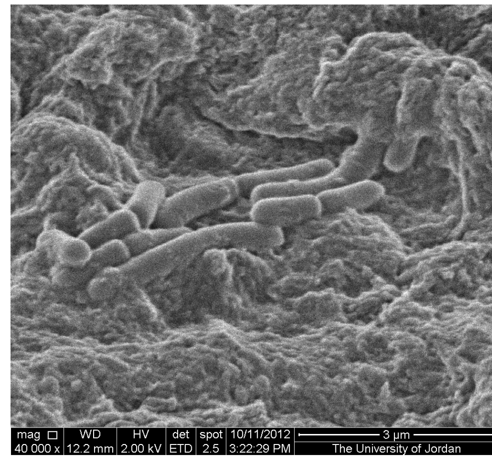


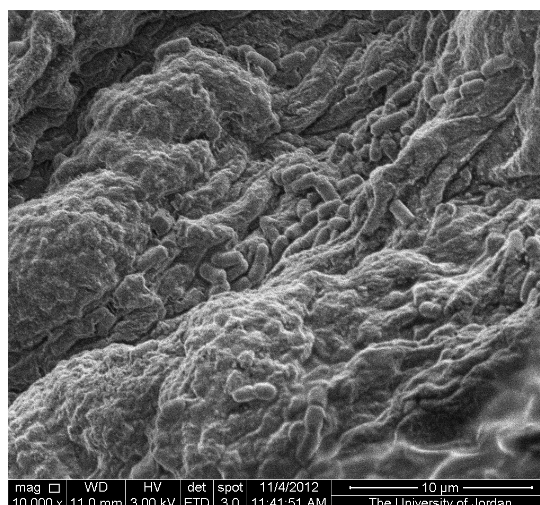
Fig. 2. Adhesion of *Lactobacillus* species to mouse intestinal epithelial cells with rate of more than 15 bacterial cells/epithelial cell. (A). M 2 (*L. fermentum)* (460 x), (B). M 20 *(L. paracasei ssp. paracasei*) (565 x), (C). *L. reuteri* DSMZ 20056(733 x)



(A)



(B)



(C)

Fig. 3. SEM images of adhesion of *Lactobacillus* isolates to the surface of epithelial cells mainly to the ileal microvilli. (A). M 18 (*L. paracasei ssp. paracasei*), (B). M 5 (*L. brevis*), (C). Crowded embedded aggregates of M 15 (*L. paracasei ssp. paracasei*)

4. CONCLUSION

This study proved that camel milk may be used to isolate unique probiotic lactobacilli isolates. These isolates met most criteria needed for a potential probiotic bacterial isolate. Results also may substantiate the belief in the curative abilities and probable use of camel milk as a nutraceutical food product.

CONSENT

Not applicable.

ETHICAL APPROVAL

We hereby declare that principles of laboratory animal care (NHI publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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