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Isolation of *Lactobacillus* strains with probiotic potential from camel's milk

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The study aimed to isolate and evaluate the probiotic potential of *Lactobacillus* species from fresh and fermented camel's milk samples. Isolates were identified morphologically and biochemically. Biochemical features investigated included temperatures, pH and NaCl concentrations effects on growth, survival in simulated gastrointestinal tract conditions, bacteriocin like activity and antibiotic resistance of the tested strains. Thirty four (34) isolates coded M1 to M 34 belonging to different *Lactobacillus* species (41% *Lactobacillus paracasei* ssp. *paracasei*, 23% *Lactobacills plantarum*, 18% *Lactobacillus rhamnosus*, 12% *Lactobacillus fermentum*, 6% *Lactobacillus brevis*) were subjected to the above criteria. All isolates grew well at 37°C and pH 3.9 and 9.6, while they varied in growth at 10 and 45°C. However, all failed to grow after 3 h exposure to gastric juice at pH 2.0 but growth variations were obsereved after 3 h exposure at pH 3.0 followed by 4 h exposure to simulated intestinal juice of pH 8.0. *Lactobacillus fermentum* isolates M 1, M 2, M 4 recorded best survival rates. NaCl was tolerated by all isolates whereas elevated concentrations affected growth differently to the point of inhibition at 10%. Bacteriocin like activity was highest by *Lactobacillus paracasei* ssp. *paracasei* M 27 against MRSA and lowest by *Lactobacillus fermentum* M 1 against *Bacillus* cereus and *Salmonella typhimurium*. Testing for antibiotic susceptibility showed 6 out of 14 strains to be resistant to all antibiotics under study. However, *Lactobacillus paracasei* ssp. *paracasei* M 15 was sensitive to all except tetracycline. Other isolates varied, being susceptible to between 1 and 4 antibiotics. These results show the *in vitro* probiotic potential of *Lactobacillus* isolates from camel milk and further *in vivo* investigations are needed.

Key words: Camel milk, probiotics, *Lactobacillus*, functional characteristics.

INTRODUCTION

Lilly and Stillwell (1965) were the first to address meaning "for life", thus conferring beneficial health effects to humans and animals. Recent literature refers to probiotics as viable, non-pathogenic microorganisms that when administered in adequate amounts confer a health benefit on the host (Argyri et al., 2013; Lavilla-Lerma et

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al., 2013). Among the commonly used probiotic bacteria are different species of the genera *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Enterococcus* and very few probiotic yeasts (Morrow et al., 2012; Zheng et al., 2013). Although ample evidence exists supporting functionality of some probiotic strains (Kotzamanidis et al., 2010), this cannot be extrapolated to others without experimentation (Bao et al., 2010; Huang et al., 2013). However, it is well established that intake of probiotic formulations stimulate growth of beneficial bacteria and reduce pathogen activity thus improving the intestinal microbial balance of the host (Chiang and Pan, 2012). Prado et al. (2008) reported certain probiotics to relieve symptoms of inflammatory bowel diseases, colitis, constipation and reduce liver, breast and colon cancers (Zhu et al., 2011; Lee et al., 2011).

Foods fortified with probiotics received expanding market interest as health promoting functional foods (Argyri et al., 2013). To achieve the expected health benefits, such probiotic foods need to contain adequate amount of live bacteria no less than 10^7 CFU/g (Pundir et al., 2013). However, there are some literatures on beneficial immunological effects derived from non viable bacterial probiotic cells (Morrow et al., 2012; Tulini et al., 2013).

An ample amount of literature reported that the most suitable matrices to deliver probiotics are dairy products both fresh and fermented including milk, yoghurt and cheese (Granato et al., 2010; Mahasneh and Abbas, 2010).

Lactic acid bacteria forms the corner stone for probiotics use and no doubt lactobacilli represent the fundamental group (Rivera-Espinoza and Gallardo-Navarro, 2010). Although, there is a drive towards non-dairy and novel probiotics where traditional fermented foods would form an area of search for new probiotic-type functional foods (Sánchez et al., 2012), it is thought that some unusual traditional dairy foods are prospective mining areas for unique probiotics. Among such traditional foods, which did not receive the necessary attention are camel's milk and its fermented products (Mahasneh and Abbas, 2010). In dairy industries, well-adapted commercial starters fail to compete metabolically with wild strains that out-grow others in traditional fermentations (Argyri, et al., 2013). It is recognized now that results obtained from understanding traditional fermented foods would help in securing new probiotics for a wide array of applications (Lavilla-Lermaet al., 2013)

In the last few years, great deal of research on probiotic lactobacilli isolated from novel fermented foods including exotic dairy products was successfully carried out (Bao et al., 2010; Espeche et al., 2012; Monteagudo-Mera et al., 2012). The objective of this study was to isolate and identify selected *Lactobacillus* strains originating from fresh and fermented camel's milk and to study some of their functional properties, antimicrobial abilities against pathogens and survival in certain gut related conditions. This was carried out in an effort to establish their prospective probiotic potential.

MATERIALS AND METHODS

Collection of milk samples and enrichment for indigenous bacteria growth

Ten samples of raw camel's milk were collected directly from camel herds in Jordan. Aliquots of each fresh sample were used and the remainder was allowed to ferment spontaneously at room temperature (25 - 30°C)

The enrichment process of the collected samples was carried out by inoculating 80 ml of MRS broth with 10 ml of the fresh milk samples and incubated anaerobically at 37°C for 5-7 days. All samples were collected into sterile plastic bottles, kept on ice and were transported to the microbiology laboratory within 2 h of collection.

Isolation of *Lactobacillus* strains

Fresh or fermented camel's milk samples were serially diluted in sterile saline and 100 µm were then plated onto de Man Rogosa and Sharpe agar (MRS, Oxoid, UK). Plates were incubated anaerobically using anaerogen bags (AnaeroGen, UK) at 37°C for 2-5 days. Presumptive *Lactobacillus* colonies were randomly picked off the MRS plates and were subcultured onto fresh plates of the same medium to ensure purity. To enhance the chances of isolating *Lactobacillus* strains, MRS medium was supplemented with 0.5 g/L cysteine-HCl (Gomes et al., 1998).

Identification of bacterial strains

All isolates were tested for catalase and oxidase activity, Gram reaction, cell morphology and spore formation (Guessas and Kihal, 2004; Ashmaig et al., 2009). All Gram positive and catalase negative rods were tested for growth in MRS broths at 10, 37 and 45°C (Togo et al., 2002) and for growth at pH 3.9 and 9.6 (Ammor et al., 2005).

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 37°C. 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 comparing all results obtained for the tested isolates with information from the computer-aided database.

Maintenance of bacteria

Bacterial cultures were maintained in MRS broth with 20% glycerol and kept stored at -80°C. Working cultures were kept on MRS agar plates or slants stored at 4°C and were routinely sub- cultured every four weeks. For comparative purposes, *Lactobacillus reuteri* DSMZ 20056 purchased from the German Microbiological Collection and known as a probiotic strain was included in some tests.

Preparation of simulated gastric and intestinal juices

Simulated fresh gastric and intestinal juices were prepared daily by

suspending pepsin (P 7000-25G) (Sigma-Aldrich, USA) (0.3% w/v) and pancreatin USP (P-1500) (Sigma-Aldrich, USA) (0.1% w/v), respectively, in sterile NaCl (0.5% w/v) and adjusting the pH to 2.0 and 3.0 for gastric juices using HCl, and 8.0 for intestinal juice with 0.1 mol/L NaOH using pH meter (Eutech 510, Singapore).

Bacterial tolerance to simulated gastric and intestinal juices

Overnight bacterial cultures (30 ml) grown in MRS broth were adjusted to 0.5 McFarland and were centrifuged (2500 × g, for 20 min, at 5°C). The pellets were then washed twice in 50 mM K_2HPO_4 (pH 6.5) and finally were resuspended in 3 ml of the same buffer. One milliliter aliquots of this cell suspension were harvested by centrifugation (12,000 × *g*, for 20 min, at 5°C) and resuspended in 9 ml of gastric solution pH 2 and 3. Total viable counts on MRS plates were recorded, both before and after incubation period of 3 h at 37°C. Then, one milliliter of gastric juices pH 2 and 3 were taken and added separately to 9 ml each of intestinal solution pH 8. Total viable counts on MRS plates were also recorded, after an incubation period of 4 h at 37°C. The results were expressed as colony counts (log_{10} orders CFU/ml).

Determination of total viable counts

Total viable counts of *Lactobacillus* species were determined by spread plate method using MRS agar. Serial tenfold dilutions were prepared in sterile normal saline. Triplicate plates of each suitable dilution were inoculated with 100 µl each and incubated anaerobically (AnaeroGen, UK) at 37°C for 48 h after which numbers of CFU/ml were determined.

Detection of bacteriocin like activity of the bacterial isolates

Preparation of cell-free supernatant of the bacterial isolates

The antibacterial activity of neutralized cell-free supernatants was determined using the agar well diffusion assay. Filter sterilized (0.22 µm syringe filter (Macherey-Nagel, Germany)) cell-free supernatant was obtained from 36 h culture of the selected *Lactobacillus* isolates grown in MRS broth at 37°C under anaerobic conditions. This sterile supernatant was used for the agar well diffusion assay.

Agar well diffusion assay

Bacteriocin-like antibacterial activity was assayed by the agar-well diffusion. One hundred microliters of culture filtrates of selected *Lactobacillus* isolates prepared as above were introduced in triplicates into 8 mm diameter wells of a plate of Müeller-Hinton agar. These plates were previously inoculated with 100 µl of approximately 10^7 CFU/ml of an overnight culture of indicator strains. These strains included (*E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), *B. cereus* (Toxigenic strain, TS), and MRSA (clinical isolate). Müeller-Hinton broth cultres of these were adjusted to 0.5 McFarland and were then diluted 1:10 using the same broth. The plates were placed initially at 4°C for 1 h to allow the diffusion of the cell free supernatant and were then incubated aerobically at 37°C for 24 h. Inhibition zones diameter were recorded as positive if the diameter of the zone was 1 mm or larger.

Antibiotic susceptibility testing

The antibiotic susceptibility test was done according to the agar diffusion method published by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The determination of minimum inhibitory concentration (MIC) to certain antimicrobial agents recommended by Scientific Committee on Animal Nutrition (SCAN, 2002) included ampicillin, ciprofloxacin, erythromycin, gentamycin, kanamycin, streptomycin, tetracycline and trimethoprim. Müeller-Hinton agar (Merck, Darmstadt, Germany) plates were used and incubated under anaerobic conditions. Serial dilutions of antibiotics were prepared using distilled water and were sterilized using 0.22 µm syringe filters (Macherey-Nagel, Germany). One milliliter of each suitable antibiotic concentration was added to 9 ml of molten agar, mixed thoroughly, and poured into sterile petri dishes. The agar plates were allowed to set at room temperature. Bacterial inoculum was prepared by suspending several bacterial colonies from a fresh agar plate in normal saline to a McFarland 0.5 turbidity standard. The 0.5 McFarland suspensions were diluted 1:10 in sterile normal saline to obtain a concentration of 10^7 CFU/ml. A spot of 1 µl of the inocula was placed on the agar surface yielding approximately 10^4 CFU/spot. The inoculated plates were allowed to stand at room temperature for about 30 min. The triplicate plates were transferred into anaerobic jars and were then incubated at 37°C for 24 h. The MIC (Minimum Inhibitory Concentration) was recorded as the lowest concentration of antimicrobial agent that completely inhibited growth.

Statistical analysis

The results are presented as means ± S.D. Statistical differences among bacterial isolates were determined by two way ANOVA except for tolerance to simulated gastric and simulated intestinal juices which were determined by three way ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

Isolation and identification of *Lactobacillus* potential probiotic strains

A total of 400 isolates from fresh and fermented camel milk samples were cultured using MRS medium. Of these isolates only 34 were Gram positive rods, catalase and oxidase negative and non-spore formers and tentatively presumed to be *Lactobacillus* species. These isolates were further characterized using API 50 CH strips. Results of the API 50 tests confirmed the identity of the 34 *Lactobacillus* isolates (Table 1) which were coded M 1 to M 34. As Table 1 shows the substrate utilization results of API 50 test showed that camel's milk originated *Lactobacillus* isolates were mostly identical in their biochemical and carbohydrate fermentation profiles and they were unable to utilize L-xylose, adonitol and L-rhamnose except M 6, M 7, M 21, M 29, M 31 and M 32. These profiles differed slightly for some isolates compared with the reference strain *Lactobacillus reuteri* (DSMZ 20056) which was purchased from the german microbiological collection (DSMZ) and known for its probiotic properties (Forsberg et al., 2013).

Temperature, NaCl and pH effect on the growth of potential probiotic isolates

The majority of the selected *Lactobacillus* isolates were

Table 1. Summary of API 50 identification results. All isolates were Gram positive rods, catalase and oxidase negative and non-spore formers.

Isolate	API 50 CH Profile																Designated species
	LARA	RIB	LXYL	ADO	GAL	MNE	RHA	MAN	SOR	ARB	ESC	CEL	LAC	MEL	RAF	TUR	
M 1	-	+	-	-	+	v	-	-	-	-	w	-	+	+	+	-	*Lactobacillus fermentum*
M 2	-	+	-	-	+	v	-	-	-	-	w	-	+	+	+	-	*Lactobacillus fermentum*
M 3	-	+	-	-	+	v	-	-	-	-	v	-	+	+	+	-	*Lactobacillus fermentum*
M 4	-	+	-	-	+	v	-	-	-	-	v	-	+	+	+	-	*Lactobacillus fermentum*
M 5	+	+	-	-	-	-	-	-	-	-	+	-	-	v	-	-	*Lactobacillus brevis*
M 6	-	+	-	-	+	+	+	+	v	+	+	+	+	+	+	-	*Lactobacillus rhamnosus*
M 7	+	+	-	-	+	+	v	+	-	+	+	+	+	-	-	+	*Lactobacillus plantarum*
M 8	-	+	-	-	+	+	-	+	-	+	+	+	+	-	-	+	*Lactobacillus plantarum* 1
M 9	+	+	-	-	+	+	-	+	-	+	+	+	+	+	+	+	*Lactobacillus plantarum* 1
M 10	-	+	-	-	+	+	-	+	+	+	+	+	+	+	-	+	*Lactobacillus plantarum* 1
M 11	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	*Lactobacillus plantarum* 1
M 12	+	+	-	-	+	+	-	+	+	+	+	+	+	+	w	+	*Lactobacillus plantarum* 1
M 13	-	+	-	-	+	+	-	+	+	+	+	+	+	v	-	+	*Lactobacillus plantarum*
M 14	-	+	-	-	+	+	-	+	+	+	+	+	+	+	-	+	*Lactobacillus plantarum*
M 15	-	+	-	-	-	+	-	+	+	+	+	+	+	-	-	v	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 16	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	*Lactobacillus paracasei* ssp. *Paracasei* 3
M 17	-	+	-	-	+	+	-	+	+	+	+	+	v	-	-	v	*Lactobacillus paracasei* ssp. *Paracasei* 2
M 18	-	+	-	-	-	+	-	+	+	+	+	+	+	-	-	-	*Lactobacillus paracasei* ssp. *paracasei*
M 19	-	+	-	-	+	+	-	+	+	+	+	+	v	-	-	v	*Lactobacillus rhamnosus*
M 20	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	*Lactobacillus paracasei* ssp. *paracasei*
M 21	-	+	-	-	+	+	+	+	v	+	+	+	+	-	-	-	*Lactobacillus rhamnosus*
M 22	-	+	-	-	-	+	-	+	+	+	+	+	+	-	-	-	*Lactobacillus paracasei* ssp. *paracasei*
M 23	-	+	-	-	v	+	-	+	+	+	+	+	+	-	-	-	*Lactobacillus paracasei* ssp. *Paracasei* 3
M 24	-	+	-	-	+	+	-	+	+	+	+	+	v	-	-	v	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 25	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 26	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	v	*Lactobacillus paracasei* ssp. *paracasei*
M 27	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 28	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 29	-	+	-	-	+	+	v	+	v	+	+	+	+	-	-	-	*Lactobacillus rhamnosus*
M 30	-	+	-	-	+	+	-	+	+	+	+	+	+	-	-	+	*Lactobacillus paracasei* ssp. *Paracasei* 1
M 31	-	+	-	-	+	+	+	+	v	+	+	+	+	-	-	-	*Lactobacillus rhamnosus*
M 32	-	+	-	-	+	+	+	+	v	+	+	+	+	-	-	-	*Lactobacillus rhamnosus*
M 33	-	+	-	-	+	+	-	-	-	-	+	+	-	+	+	+	*Lactobacillus brevis*
M 34	-	+	-	-	+	+	-	+	+	+	+	+	v	-	-	v	*Lactobacillus paracasei* ssp. *Paracasei* 2

(+): Positive reaction; (-): Negative reaction; (w): Weak reaction and (v): Variable reaction.

able to grow very well at 10 and 37°C, however, M 1, M 3, M 4, M 12 and M 28 failed to grow at 10°C (Table 2). At elevated temperatures of 45°C, most isolates grow satisfactorily except for M 25, M 26, M 27, M 30 and M 31 which failed to show any degree of growth (Table 2). As for NaCl tolerance, bacterial isolates were able to tolerate 4-6.5% NaCl except M 1 and M 33 which were unable to grow at 6.5% (Table 2). At 8-10% NaCl most isolates were able to show good growth, however, some variations were recorded. M 1, M 2, M 3 and M 33 failed to grow at both 8 and 10% NaCl concentration. As for 10% NaCl, another five isolates were unable to grow, these included M 4, M 5, M 9, M 11, M 12. The pH effect on growth showed the ability of the majority of the isolates to grow well at pH 3.9 and 9.6 except M 33 which was unable to grow at pH 3.6 while it grew well at pH 9.6.

Table 2. Effect of NaCl, temperature and pH on the growth of *Lactobacillus* isolates.

Isolate	NaCl (%)				Temperature (°C)			pH	
	4%	6.5%	8%	10%	10	37	45	3.9	9.6
M 1	+	-	-	-	-	+	+	+	+
M 2	+	+	-	-	+	+	+	+	+
M 3	+	+	-	-	-	+	+	+	+
M 4	+	+	+	-	-	+	+	+	+
M 5	+	+	V	-	+	+	+	+	+
M 6	+	+	+	+	+	+	+	+	+
M 7	+	+	+	+	+	+	+	+	+
M 8	+	+	+	+	+	+	+	+	+
M 9	+	+	+	-	+	+	+	+	+
M 10	+	+	+	+	+	+	+	+	+
M 11	+	+	+	-	+	+	+	+	+
M 12	+	+	+	-	-	+	+	+	+
M 13	+	+	+	+	+	+	+	+	+
M 14	+	+	+	+	+	+	+	+	+
M 15	+	+	+	+	+	+	+	+	+
M 16	+	+	+	+	+	+	+	+	+
M 17	+	+	+	+	+	+	+	+	+
M 18	+	+	+	+	+	+	+	+	+
M 19	+	+	+	+	+	+	+	+	+
M 20	+	+	+	+	+	+	+	+	+
M 21	+	+	+	+	+	+	+	+	+
M 22	+	+	+	+	+	+	+	+	+
M 23	+	+	+	+	+	+	+	+	+
M 24	+	+	+	+	+	+	+	+	+
M 25	+	+	+	+	+	+	-	+	+
M 26	+	+	+	+	+	+	-	V	+
M 27	+	+	+	+	+	+	-	+	-
M 28	+	+	+	+	-	+	V	+	-
M 29	+	+	+	+	+	+	+	+	+
M 30	+	+	+	+	+	+	-	+	-
M 31	+	+	+	+	+	+	-	+	-
M 32	+	+	+	+	+	+	+	+	+
M 33	+	-	-	-	+	+	+	-	+
M 34	+	+	+	+	+	+	+	+	+

+, Growth; -, no growth; V, variable result. No growth was recorded at pH 2.

On the contrary isolates M 27, M 28, M 30 and M 31 failed to grow at pH 9.6 but grew well at pH 3.6.

Effect of simulated gastric juice and small intestine transit on the viability of the *Lactobacillus* probiotic isolates

The simulated tolerance test results indicated the inability of all isolates to survive 3 h treatment at pH 2 (Table 3). However, at pH 3 and after 3 h exposure, the viable counts of some isolates (M 1, M 2, M 4 and M 5) increased and were highly tolerant where after 7 h at this pH the numbers of these isolates were not greatly affected (Table 3). At the same time, isolates M 9, M 10, M 12, M 14, M 15, M 18, M 20, M 27, M28, M 29 and M31 were tolerant in the sense of being a good probiotic candidate.

These significant results ($p < 0.05$) indicate real probiotic potential of some of these isolates compared to the reference strain *Lactobacillus reuteri* (DSMZ 20056).

Bacteriocin like activity

The 14 *Lactobacillus* isolates were examined for the

Table 3. Effect of simulated gastric juice and intestinal juice on viability of *Lactobacillus* isolates.

Isolate	Viable count (log CFU/ml ± S.D)		
	Gastric juice (pH 3)		Intestinal juice (pH 8)
	0 h	3 h	4 h
M 1	7.65 ± 0.02	8.97 ± 0.03	7.01 ± 0.09
M 2	8.17 ± 0.1	9.8 ± 0.07	7.9 ± 0.15
M 4	7.63 ± 0.12	7.90 ± 0.04	6.91 ± 0.18
M 5	10.63 ± 0.05	11.0 ± 0.03	7.36 ± 0.04
M 6	10.20 ± 0.07	-	-
M 7	9.93 ± 0.08	-	-
M 8	8.89 ± 0.03	8.50 ± 0.03	-
M 9	10.84 ± 0.05	10.61 ± 0.03	6.59 ± 0.05
M 10	9.15 ± 0.07	8.69 ± 0.06	6.98 ± 0.02
M 11	11.67 ± 0.08	-	-
M 12	8.15 ± 0.05	7.67 ± 0.09	6.15 ± 0.02
M 13	8.10 ± 0.05	7.38 ± 0.02	ND
M 14	7.96 ± 0.06	7.69 ± 0.08	7.45 ± 0.04
M 15	10.18 ± 0.03	10.15 ± 0.06	6.47 ± 0.2
M 16	10.97 ± 0.1	ND	-
M 17	8.74 ± 0.06	-	-
M 18	9.08 ± 0.07	8.99 ± 0.02	7.66 ± 0.05
M 19	10.9 ± 0.04	-	-
M 20	9.31 ± 0.03	9.16±0.05	7.25±0.15
M 21	8.32 ± 0.1	-	-
M 22	9.52 ± 0.02	-	-
M 23	12.16 ± 0.05	7.90 ± 0.05	7.72 ± 0.3
M 24	8.33 ± 0.04	-	-
M 26	9.09 ± 0.13	-	-
M 27	11.16 ± 0.07	7.75 ± 0.02	6.83 ± 0.06
M 28	7.98 ± 0.08	7.74 ± 0.16	6.71 ± 0.2
M 29	8.84 ± 0.13	8.23 ± 0.21	-
M 31	9.60 ± 0.1	8.86 ± 0.2	-
M 32	8.85 ± 0.07	7.02 ± 0.04	-
L. reuteri DSMZ 20056	9.61 ± 0.06	9.38 ± 0.03	ND

Results are shown as (mean of log CFU/ml ± S.D, n=3). ND: Not Determined. Differences between all isolates in resistance pattern were significant at ($p < 0.05$). None of the isolates was able to grow at p H 2 .

bacteriocin like inhibitory activity against *B. cereus*, MRSA, *E. coli* ATCC 25922 and *S. typhmurium* ATCC 14028. Table 4 indicates that the supernatant of M 27 (*L. paracasei* ssp. *paracasei*) possessed the highest inhibitory activity (17.7 mm inhibition zone diameter) against MRSA. The lowest activity of the supernatant was observed with M 1 (*L. fermentum*) isolate against *B. cereus* (9.7 mm) and *S. typhimurium* (10.7 mm). Both M 1 and M 2 were active against *E. coli*. On the otherhand, no activity was recorded for M 4 (*L. fermentum*), M 5 (*L. brevis*) and the probiotic control *L. reuteri* DSMZ 20056 against *E. coli*, *S. typhimurium* or *B. cereus*, however they were active against MRSA (Table 4). These results lead to the assumption that these fourteen isolates are capable of producing different levels of bacteriocin like compounds; however, this needs further substantiation.

Antibiotic resistance

Table 5 shows minimum inhibitory concentrations (MICs) of the 14 *Lactobacillus* isolates tested with different antibiotics of different modes of action. Isolates showing MIC values higher than the MIC breakpoint established by the European Safety Authority (EFSA, 2005) were resistant to Ampicillin (cell wall inhibitor) except for strain M 15 (*L. paracasei* ssp. *paracasei* 1) which was susceptible as it is the case with the control *L. reuteri* DSMZ 20056. When protein synthesis inhibitors (erythromycin, gentamycin, streptomycin and tetracycline) were tested,

Table 4. Antibacterial activity of cell free supernatant (inhibition zones diameter) of the selected *Lactobacillus* species. Bacterial species: *Bacillus cereus* (*B*. *cereus*); Methicillin resistant *Staphylococcus aureus* (MRSA); *Escherichia coli* (*E*. *coli*) and *Salmonella typhimurium* (*S*. *typhimurium*).

Isolate	*B. cereus*	MRSA	*E. coli*	*S. typhimurium*
M 1	9.7 ± 0.58	13.7±1.7	11.3 ± 0.58	10.7 ± 0.58
M 2	12.3 ± 0.58	14.7±0.58	10.7 ± 0.58	13.3 ± 1.2
M 4	0.0 ± 0.0	14.7±0.58	0.0 ± 0.0	0.0 ± 0.0
M 5	0.0 ± 0.0	14.3±1.2	0.0 ± 0.0	0.0 ± 0.0
M9	14.0 ± 1.7	14.3±0.58	12.3 ± 1.5	13.7 ±0.58
M10	14.2 ± 2.0	16.8±0.76	12.3 ± 0.58	14.3 ± 1.5
M12	14.0 ± 1.7	15.8±0.76	13.0 ± 1.0	15.0 ± 1.0
M14	14.8 ± 1.4	16.3±0.58	11.3 ± 0.58	14.7 ±0.58
M15	13.3 ± 0.58	14.3±0.58	11.7 ± 0.58	15.7 ± 1.5
M18	13.0 ±0.00	14.0±1.0	11.3 ± 0.58	13.0 ± 1.0
M20	12.3 ± 2.1	14.7±1.5	13.3 ± 1.2	15.0 ± 2.0
M27	14.3 ± 1.5	17.7±0.58	12.3 ± 0.58	14.0 ± 2.0
M29	12.8 ± 1.0	15.0±1.7	12.0 ± 1.0	13.7 ± 1.2
M31	13.3 ± 0.58	15.7±0.58	12.2 ± 0.76	13.8 ± 1.0
L. *reuteri DSMZ 20056*	0.0 ± 0.0	12.3± 0.58	0.0 ± 0.0	0.0 ± 0.0

Inhibition zone diameter (mm) of indicator strains, mean ± S.D, n=3.

Table 5. Antibiotic susceptibility of the selected *Lactobacillus* species. [a]The breakpoints for *Lactobacillus* sp. by SCAN category. Minimum Inhibitory Concentration (MIC) equal to or higher than the breakpoint is considered as resistant.(R): Resistant; (S): Susceptible; (A): Ampicillin; (C): Ciprofloxacin; (E): Erythromycin; (G): Gentamycin; (K): Kanamycin; (S): Streptomycin; (Te): Tetracycline and (Tr): Trimethoprim.

Isolate	Antibiotic breakpoint[a] (µg/ml)							
	A (2)	C (4)	E (4)	G (1)	K (32)	S (16)	Te (16)	Tr (32)
M 1	R	R	R	R	R	R	S	R
M 2	R	R	R	R	R	R	R	R
M 4	R	S	R	R	R	S	S	S
M 5	R	R	R	R	S	R	R	R
M 9	R	R	R	R	R	R	R	R
M 10	R	R	R	R	R	R	R	R
M 12	R	R	R	R	R	R	R	R
M 14	R	R	R	R	R	R	R	R
M 15	S	S	S	S	S	S	R	S
M 18	R	R	R	R	R	R	R	S
M 20	R	R	R	R	R	R	R	R
M 27	R	R	R	R	R	R	S	R
M 29	R	S	R	S	R	R	S	R
M 31	R	R	R	R	R	R	S	R
L. *reuteri DSMZ 20056*	S	R	R	R	S	S	S	S

the same profile of resistance was recorded for all 14 isolates. Variable results were observed, for example M 15 (*L*. *paracasei* ssp. *paracasei* 1) and M 29 (*L*. *rhamnosus*) were sensitive to erythromycin, gentamycin, and streptomycin. However, strain M 15 was resistant to tetracycline while M 29 (*L*. *rhamnosus*) and M 31 (*L*.

rhamnosus) were tetracycline sensitive. Regarding the DNA interferring antibiotics (ciprofloxacin and trimethoprim), 11 out of 14 of isolates were resistant to ciprofloxacin and trimethoprim and only strain M 4 (*L*. *fermentum*) and strain M 15 were sensitive to both. Strains M 4, M 15 and M 29 were sensitive to ciprofloxacin. *L*. *reuteri* DSMZ 20056 control strain showed a rather different profile where it was sensitive to all antibiotics tested except ciprofloxacin, erythromycin and gentamycin. It is clear from these results that it is difficult to judge whether resistance of probiotics to specific antibiotics is desirable or not depending upon what these probiotic formulations are used for.

DISCUSSION

The eternal connection between health, disease and diet always stimulated the quest for novel products of unique functional properties (Meira et al., 2012). As a result and since camel's milk is an exotic food and may form a potential source of probiotic bacteria (Yateem et al., 2008), this study has been carried out. The main objectives were to isolate *Lactobacillus* strains from fresh and fermented camel's milk samples and to evaluate some of the selected isolates for their beneficial probiotic properties. Following laboratory screening of different camel's milk samples, 34 isolates were found to be Gram-positive rods, catalase and oxidase negative and non-spore forming bacteria. These isolates were assigned as members of the genus *Lactobacillus*. With the use of the API 50 CH Kits, 12% of the isolates were identified as *L*. *fermentum* and these included M 1, M 2, M 3 and M 4 (Table 1). Twenty three percent of the isolates belonged to *L*. *plantarum* (M 7, M 8, M 9, M 10, M 11, M 12, M 13 and M 14). Forty one percent were identified as *L*. *paracasei* ssp. *paracasei* (M 15, M 16, M 17, M 18, M 20, M 22, M 23, M 24, M 25, M 26, M 27, M 28, M 30 and M 34). The API fermentation profile varied with the different species and this is not unusual where similar results are reported (Ashmaig et al., 2009; Suriasih et al., 2012; Diaz et al., 2013; Tulini et al., 2013).

Six isolates (18%), which also showed variations in sugars fermentations, were identified as *L*. *rhamnosus* and these were M 6, M 19, M 21, M 29, M 31 and M 32. The remainder (6%) of isolates (M 5 and M 33) belonged to *L. brevis,* where unexpectedly, these two isolates showed variations in their fermentation profiles. *Lactobacilli* are considered part of the indigenous microflora of the mammalian gastrointestinal tract and of many other niches and fermented foods (Jara et al., 2011; Neville and O'Toole, 2010; Messaoudi et al., 2013) which may explain some limited variations in these profiles.

Temperature, NaCl and pH tolerance

In this study, the 34 selected isolates were able to grow optimally at 37°C (Table 2). However, variations of growth at 10 and 45°C were observed. M 1, M 3 and M 4 were able to grow at 45°C but not at 10°C and they belonged to *L*. *fermentum*. Pancheniak and Soccol (2005) isolated *L*. *fermentum* which grow well at 45°C but poorly at 10°C. Isolates M 25, M 26, M 27 and M 30 all grew at 10°C but not at 45°C and they were identified as *L*. *paracasei* ssp. *paracasei*. Suriasih et al. (2012) observed similar variations with their *Lactobacillus* isolates and this was related, according to Siezen et al. (2010) and Neville and O'Toole (2010), to different isolation environments. Pundir et al. (2013) isolated lactic acid bacteria from fermented foods which were able to grow at 25, 37 and 40°C.

As for NaCl effect, all isolates were able to grow at 4 and 6.5 % concentrations except M 1 and M 33. At elevated concentrations of 8 and 10%, great variations were recorded. For example, M 1, M 2, M 3, M 4, M 5, M 9, M 11, M 12 and M 33 failed to grow at both concentrations of 8 and 10% NaCl. Hoque et al. (2010) isolated *Lactobacillus*—strains from different regional yoghurts, which tolerated 4-8% NaCl. Pundir et al. (2013) isolated lactobacilli from different foods, which were able to tolerate 1-6.5 % only. Experimental results showed that *Lactobacillus* species isolated from camel's milk were able to grow in acidic pH 3.9 except for M 33 and also to survive at pH 9.6 except for M 27, M 28, M 30 and M 31 (Table 2). Ammor et al. (2005) isolated 36 lactobacilli from dried sausage and found only 4 isolates capable of growing at 3.9 but all grew at pH 9.6. Pundir et al. (2013) reported the ability of *Lactobacillus* isolates to grow at 3.5, 3.7 and 4 pH values but did not mention the alkaline side.

Gastric and intestinal juice tolerance

Considering the fact that most microorganisms are destroyed by the gastric acid (pH 1.4-2.0) in the stomach (Chang et al., 2010), probiotic strains need to exhibit tolerance to such condition for survival. None of the isolates in this study were able to survive gastric and intestinal juices after 3 and 4 h at pH 2.0 (Table 3). However, great variations were observed after 3 h exposure to gastric juice at pH 3 and further 4 h exposure to intestinal juice at the same pH (Table 3). In this context, isolates were grouped into isolates of high tolerance (M 1, M 2, M 4 M 5, and M14). Members of this group were able to grow after 3 h exposure to gastric juice at pH 3 and then their population is slightly declined after 4 h exposure to the intestinal juice. Strain M 2 (*L. fermentum*) presented an increase in the growth of 4000% after 3 h exposure to the gastric juice. Botes et al. (2008) observed an increase in number of some lactobacilli isolates after exposure to simulated intestinal juices. The other group included M 6 to M 32 isolates were less resistant but survived at the rate of (99%) for

strain M 15 (*L*. *paracasei* ssp. *paracasei*) and lowest (65%) for strain M 23 (*L*. *paracasei* ssp. *paracasei*3). The highest tolerance rate (93%) for the intestinal juice was for strain M 14 (*L*. *plantarum*) and the lowest (61%) was recorded for strain M 27 (*L*. *paracasei* ssp. *paracasei* 1). Horáčková et al. (2011) reported failure of *L*. *rhamnosus* to tolerate simulated stomach conditions. Argyri et al. (2013) isolated several *L*. *plantarum*, *L*. *paracasei* and *L*. *pentosus* strains, which were highly resistant to low pH and comparable to reference strain *L*. *casei* Shirota. These results, agree with earlier reports about the survival rate of probiotic bacteria through the digestive tract which was variable and strain dependent (Karasu et al., 2010; Meira et al., 2012; Argyri et al., 2013).

Pathogens inhibition

It is usually expected that potential probiotic lactobacilli would be capable of inhibiting the growth of pathogens (Mahasneh and Abbas, 2010; Khay et al., 2011; Kazemipoor et al., 2012; Rushdy and Gomaa, 2013). In this study, the inhibitory potential of the selected isolates against Gram-positive bacteria (*B*. *cereus*, methicillin resistant *S*. *aureus* (MRSA)) and Gram-negative bacteria (*E*. *coli* ATCC 25922 and *S*. *typhimurium* ATCC 14028) indicates the significant inhibition showed by the strain M 2 (*L*.*fermentum*) against MRSA and the lowest activity of M 4 (*L*. *fermentum*) against *E*. *coli*. These results were different from these of Coeuret et al. (2004) who observed better results of *L*. *plantarum* against *Salmonella* species and *E*. *coli*. The same trend of significant activity of *L*. *plantarum* isolates against Gram-negative pathogen was reported by Yateem et al. (2008). Soleimani et al. (2010) found that probiotic *L*. *plantarum* ATCC 8014 was very active against bovine mastitis *S*. *aureus* and *S*. *aureus* ATCC 25923. Rushdy and Gomaa (2013) reported substantial activity of *L*. *brevis* isolate against an array of Gram-positive and Gram-negative bacteria. These results are comparable with our isolates M 9, M 10, M 12 and M 14 (all *L*. *plantarum*) in being active against MRSA.

Testing the supernatant bacteriocin-like substances of the isolates, strain M 27 (*L*. *paracasei* ssp. *paracasei*) yielded the highest inhibitory activity against MRSA, while strain M 1 was the lowest against *B*. *cereus*. Khay et al. (2011) reported the bacteriocin-like activity of lactobacilli from Moroccan camel's milk against Gram-positive pathogens only. Conversely, the probiotic bacteria, despite their origin are capable of inhibiting bacteria by several mechanisms (Rodríguez et al., 2012). Hence, further investigations to define the type of the probable bacteriocin present are an idea we share with Kazemipoor et al. (2012). In this context, probiotic bacteria, despite their origin, are capable of inhibiting pathogens by several mechanisms among which are nutrient competition, antimicrobial production, competitive exclusion, immune modulation and modifications pertaining to toxins and their receptors (Rodríguez et al., 2012).

Antibiotic susceptibility

The antibiotic susceptibility of isolates was studied by using eight antibiotics. Results indicate–resistance to ampicillin except for strain M 15 (*L.paracasei ssp. paracasei1)*. Ciprofloxacin resistance was also common except for isolates M4 (*L.fermentum*), M 15 (*L.paracasei* ssp. *paracasei*1) and M 29 (*L. rhamnosus*). Resistance to ampicillin and ciprofloxacin is commonly observed in members of genus *Lactobacillus* (Klayraung et al., 2008). Rojo-Bezares et al. (2006) reported resistance of lactobacilli to ciprofloxacin, gentamycin and other aminoglycosides antibiotics. This inherent resistance is probably due to cell wall structure, membrane permeability and potential efflux mechanisms (SCAN, 2002; EFSA, 2005). Variations in resistance to tetracycline were reported for lactobacilli (Temmerman et al., 2003) and most of our isolates were in this trend, however strain M 15 was sensitive to all eight tested antibiotics except tetracycline. Considering the intrinsic resistance of lactobacilli strains to several antibiotics, one would think that lactobacilli isolated in this study from camel milk would lie in the same category of many *Lactobacillus* probiotic strains.

Conclusively, the results of this study showed that camel's milk is an exotic source for probiotic lactobacilli isolation. These isolates were found to possess functional properties *in vitro* comparable to reference strains and form a suitable material for further studies for their technological characteristics, among which is the claimed protective effect of camel's milk to bacteria during gastric transit. Furthermore, food is the common delivery system for probiotic bacteria and can protect such bacteria during passage through the digestive tract. The probiotic potential of lactobacilli is expected to enhance the nutritional value of foods especially if we know that it is increasingly clear that probiotics in general would provide beneficial impacts even without full colonization of the digestive tract (Ohland and MacNaughton, 2010; Rijkers et al., 2011; Seale and Millar, 2013).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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