JJBS

Volume 8, Number 2, June .2015
ISSN 1995-6673
Pages 81 - 87

Jordan Journal of Biological Sciences

Probiotic Properties of *Lactobacillus* Species Isolated from Local Traditional Fermented Products

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Received: December 28, 2014 Revised: January 25 , 2015 Accepted: February 2, 2015

Abstract

The present study aims to isolate *Lactobacillus* species from locally fermented vegetables and to characterize selected isolates for their probiotic potential. Seventeen *Lactobacillus* strains (9 *Lactobacillus plantarum* 1, 3 *Lactobacillus pentosus*, 2 *Lactobacillus brevis* 1, 2 *Lactobacillus brevis* 3 and 1 *Lactobacillus salivarius*) were isolated and tested for their probiotic potential. This included survival in gastrointestinal simulated juice, antagonistic activity against *Salmonella typhimurium*, *Escherichia coli*, *Bacillus cereus* and a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate, bile tolerance and antibiotic resistance to 8 antibiotics. Most isolates, especially *Lactobacillus plantarum* 1, were tolerant to the acidity and intestinal conditions after exposure for three and four hours, respectively, with reduction less than one log cycle of the starting CFU/ml. The same trend was observed in respect to bile tolerance with slight variations. All isolates inhibited the growth of the tested pathogens and were the most effective against MRSA isolate. As for antibiotic resistance, it was pronounced against kanamycin, ampicillin, erythromycin and tetracycline. Some isolates (M3, M5, M6, M12, B14) showed a resistance to 6 or more antibiotics of those tested. These results prove that the traditionally fermented vegetables are a good source for probiotic *Lactobacillus*. However, further *in vivo* studies are needed to substantiate the potential of these isolates.

Keywords: *Lactobacillus*, Probiotics, Fermented vegetables, Characteristics..

1. Introduction

The increased demand for complimentary and health foods encourages innovation as well as new and novel product development in the food industries (Guo *et al.*, 2010; Tham *et al.*, 2012; Abbas and Mahasneh, 2014). It is well established that the consumption of probiotic bacteria as formulation or fermented food ingredients stimulates growth of beneficial bacteria and reduces pathogen activity (Chiang and Pan, 2012). To attain the health benefits of probiotic foods, it should contain no less than 10^7 CFU of viable bacteria per gram (Pundir *et al.*, 2013). As a result, probiotic fermented or fortified foods have received a wide interest in an expanding market (Argyri *et al*., 2013). For *Lactobacillus* strains to exert the expected benefits as probiotics, they should fulfill these criteria: the ability to survive in the gastrointestinal tract of high acidity, tolerate bile salts as well as adhesion and persistence to resist pathogens through production of antimicrobial substances (Giraffa *et al.*, 2010) and other means (Lee *et al.*, 2011; Tulini *et al.*, 2013).

Lactic acid bacteria mainly *Lactobacillus* and *Bifidobacterium* species are considered very basic in probiotic development; however, lactobacilli are the fundamental group (Rivera-Espinosa and Gallardo-Navaro 2010). They have been used in naturally fermented or fortified dairy products (Granato *et al*., 2010). Recently, a drive towards non-dairy novel probiotics has been observed to span traditionally fermented foods of vegetable origin (Sanchez *et al.*, 2012). No doubt such traditionally fermented foods would be a unique mining area for new and novel probiotic *Lactobacillus* isolates. It is recognized that wild probiotic strains would compete better in food traditional fermentation settings (Lavilla-Lerma *et al.*, 2013). Accordingly, new and novel specific probiotic candidate bacterial strains are being sought. The efficacy of such strains is mandatory and should be carefully evaluated for safety.

In Jordan, pickled and traditionally fermented vegetables form a reasonable part of the homemade stored foods; for instance, Makdoos, in its different forms, whether it is made of traditionally fermented aubergine stuffed with ground walnuts, garlic, hot dried pepper and left to ferment in vegetable oil, mainly olive oil (Hamady 2003). The other type of Makdoos is the big green pepper Makdoos which is stuffed with cut tomato, parsley, garlic and hot dried pepper and traditionally soaked and

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fermented in vegetable oil preferably olive oil. The fermentation process starts with a wide variety of indigenous microorganisms present in the vegetables and the stuffing material used. However, the bacteria responsible for fermentation in this case are lactic acid bacteria mainly *Lactobacillus* spp.

Due to the increasing concern of the interrelationship between diet and health, a great attention is given to the functional properties of indigenous lactobacilli involved in traditional fermented foods (Pisano *et al.,* 2014). It is assumed that these foods could provide an alternative source of new novel probiotics with unique properties. This study aims at isolating and identifying selected *Lactobacillus* strains from Makdoos and at studying some of their probiotic characteristics such as acid tolerance, tolerance to gastrointestinal juice and bile, and their antagonistic activity against some pathogens.

2. Materials and Methods

2.1. Collection of Makdoos Samples and Bacterial Growth Enrichment

Homemade and commercial samples of fermented Makdoos were collected and included in this investigation. The enrichment process was carried out by inoculating approximately 1 ml of a mix of the liquor and Makdoos homogenate into 50 ml sterile MRS broth (Oxoid, UK) and incubated anaerobically at 37˚C for (2-5) days (Abbas and Mahasneh, 2014). All samples were collected into sterile glass bottles and were kept in the laboratory at room temperature for further analysis.

2.2. Isolation of Lactobacillus Strains

Enriched Makdoos samples were serially diluted in sterile normal saline. Aliquots of 100 µl from each dilution were then plated onto de Man, Rogosa and Sharpe agar (MRS, Oxoid, UK) supplemented with 0.01% bromocresol purple as a pH indicator. Plates were incubated anaerobically using anaerogen bags (AnaeroGen, UK) at 37˚C for 24 hours. Presumptive Lactobacillus colonies with yellow halos were randomly picked off the MRS plate and were further subcultured onto fresh plates of the same medium to ensure purity.

2.3. Identification of Bacterial Strains

All isolates were tested for catalase and oxidase activity, Gram reaction, cell morphology as well as spore formation (Guessas and Khal, 2004; Ashmaig *et al.,* 2009). The strains were tested for the production of acids from carbohydrates and related compounds using API 50 CH kits and CHL media (BioMèrieux, France) according to the manufacturer's instructions. Results were scored after incubation at 37˚C for 24 and 48 hours. 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 fermentations profiles were facilitated by comparing all results obtained for the tested isolates with information from the computer aided database. Isolates were also tested for their hemolytic patterns, gelatinase and DNase activity according to Gupta and Malik (2007).

2.4. 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 at 4˚C and were routinely sub cultured every 2-4 weeks. For comparative purposes, *Lactobacillus reuteri* DSMZ 20056, a probiotic strain, was included in some tests.

2.5. Preparation of Simulated Gastric and Intestinal Juices

Fresh simulated gastric and intestinal juices were prepared daily by suspending pepsin (P7000-25G Sigma-Aldrich, USA) at 0.3% w/v and pancreatin USP (P-1500, Sigma-Aldrich, USA) at 0.1% w/v in sterile 0.5% w/v NaCl. The pH was adjusted to 3.0 for gastric juices using HCl and pH 8.0 for intestinal juices with 0.1M NaOH using pH meter (Eutech 510, Singapore).

2.5.1. Bacterial Tolerance to Simulated Gastric and Intestinal Juices

Overnight bacterial cultures grown in MRS broth of each test isolate were adjusted to 0.5 McFarland and 30 ml aliquot of that suspension was centrifuged (2500 x g, for 20 minutes, at 5˚C), washed twice in 50 mM K2HPO4 (pH 6.5) and resuspended in 3 ml of the same buffer. One milliliter of each isolate suspension was harvested by centrifugation (12,000 x g, for 20 minutes, at 5˚C) and resuspended in 9 ml of gastric solution. Total viable counts on MRS plates were recorded, both before and after incubation period of 3 hours at 37˚C. Then, one milliliter of gastric juice was taken and added to 9 ml of intestinal juice solution. Total viable counts on MRS plates were also recorded, after an incubation period of 4 hours at 37˚C. The results were expressed as colony counts (log10 orders CFU/ml).

2.5.2. Determination of Total Viable Counts

The total viable counts of *Lactobacillus* species were determined by spread plate method using MRS agar. Serial ten-fold dilutions were prepared using 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 hours after which numbers of CFU/ml were determined.

2.6. Detection of Antibacterial Activity of the Bacterial Isolates

For the detection of antagonistic activities of the isolates, an agar spot procedure was used. The antibacterial activity of the selected *Lactobacillus* isolates was determined by the test described by Schillinger and Lucke (1989) with some modifications as follows: Five microliters of each overnight culture of *Lactobacillus* isolate grown in MRS broth were spotted onto the surface of MRS agar plates (containing 0.2% glucose) and were then incubated under anaerobic conditions at 37˚C for 48 hours. An overnight culture of four indicator strains (*E. coli* ATCC 25922), (*S. typhimurium* ATCC 14028), (*B. cereus* toxigenic strain TS) and (MRSA clinical isolate) were grown in nutrient broth and were adjusted to 0.5 McFarland solution standard which is equivalent to about 10^8 CFU/ml. Aliquots of 0.25 ml were inoculated into 7 ml of soft/semi-solid nutrient agar (containing 0.2% glucose and 0.7% agar). Inoculated semi-solid agar was

immediately poured in duplicates over the MRS plate on which the tested Lactobacillus isolate was grown. The plates were incubated aerobically at 37˚C for 24 hours. The antibacterial activity was detected by measuring the 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 more (Mami *et. al.* 2008).

2.7. Bile Tolerance Test

The tolerance of the bacterial isolates to bile was tested using MRS broth prepared with 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 121˚C for 15 min. Bacterial cultures were inoculated into sterile MRS broth, incubated overnight and adjusted to 0.5 McFarland at the time of use. Two hundred microliters of the adjusted bacterial cultures were inoculated into different bile concentrations for each isolate. One milliliter aliquots were taken from each inoculated bile tube at zero hours of incubation and after 24 hours, serially diluted with sterile normal saline and inoculated in triplicates onto MRS agar to determine total viable counts.

2.8. 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, Gentamicin, Kanamycin, Streptomycin, Tetracycline and Trimethoprim. Müller-Hinton agar (Merck, Darmstadt, Germany) plates were used and incubated under anaerobic conditions. Serial dilutions of antibiotics were prepared using sterile distilled water, DMSO and/or ethanol and were sterilized using 0.22 µm syringe filters (Macherey-Nagel, Germany). One ml 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 inocula were prepared by suspending several bacterial colonies from fresh agar plates in normal saline to a 0.5 McFarland turbidity standard. A spot of 4 µl of the inocula was placed on the agar surface. The inoculated plates were allowed to stand at room temperature for about 30 minutes. The triplicate plates were then transferred into anaerobic jars and were then incubated at 37˚C for 24 hours. The MIC (Minimum Inhibitory Concentration) was recorded as the lowest concentration of the antimicrobial agent that completely inhibited growth.

2.9. Statistical Analysis

The results are presented as means $\pm$ SD. 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 $P<0.05$.

3. Results and Discussion

3.1. Isolation and Identification of Lactobacillus Potential Probiotic Strains

A total of seventeen isolates from both types of Makdoos were chosen to be used in the present study. All isolates were Gram positive rods, catalase and oxidase negative, non-spore forming, non- hemolytic, and DNase as well as gelatinase negative (Table 1). Absence of hemolytic activity of these isolates is a positive sign in favor of being suitable probiotic isolates irrespect of being *L. plantarum*, *L. pentosus*, *L. brevis* or *L. salivarius*. Similar observations were recorded for *Lactobacillus* isolates from dairy and other sources (Maragkoudakis *et al*., 2006). These isolates were further characterized using API 50 CH strips. Results of the API 50 test confirmed the identity of the 17 *Lactobacillus* isolates (Table 2). Identification of the isolates (Table 2) indicated the dominant presence of *L. plantarum* where 8 out of the 17 isolates belonged to this species, followed by 3 *L. pentosus* isolates and 2 *L. brevis*. Four isolates were not designated to any species.

Probiotic lactobacilli were isolated from foods of plants origin (Husmaini *et al*., 2011) and cereals (Rivera-Espinosa and Gallardo-Navarro 2010). It is recognized that wild type strains that dominate naturally fermented products tend to have higher metabolic capabilities, thus affecting the final quality of the traditionally fermented product (Ayed *et al*., 2002).

3.2. Resistance to Simulated Gastrointestinal Juices

In order for probiotic candidates to exert their beneficial activity, they should survive exposure to gastrointestinal environment of low pH and others (Begley *et al*., 2005). The viable counts of all isolates of the different strains of *Lactobacillus* species were less than or equal to 1 log CFU/ml as compared with the zero time count (7-8 log CFU/ml). This was noted at both pH 3 and 8 after 3 h and 4 h exposure, respectively. This high resistance was observed among *L. plantarum*, *L. pentosus* and *L. brevis* isolates (Table 3). Maragkoudakis *et al*. (2006) and Dunne *et al*. (2001) tested the acid resistance of probiotic bacteria isolated from different sources and reported results in agreement with those reported herein. Abbas and Mahasneh (2014) isolated *Lactobacillus* isolates from camel milk and they had a similar trend in tolerance to gastrointestinal juices as those observed in this investigation.

Table 1. Some primary characteristic of bacterial isolates; all were non-sporeforming rods

Isolate	Gram reaction	Catalase	Oxidase	Hemolysis	DNase	Gelatinase
M3	+	-	-	-	-	-
M4	+	-	-	-	-	-
M5	+	-	-	-	-	-
M6	+	-	-	-	-	-
M7	+	-	-	-	-	-
M8	+	-	-	-	-	-
M9	+	-	-	-	-	-
M10	+	-	-	-	-	-
M11	+	-	-	-	-	-
M12	+	-	-	-	-	-
B13	+	-	-	-	-	-
B14	+	-	-	-	-	-
B16	+	-	-	-	-	-
B17	+	-	-	-	-	-
B18	+	-	-	-	-	-
B19	+	-	-	-	-	-
B20	+	-	-	-	-	-

Table 2. Biochemical identification of bacterial isolates according to API CH 50 strips.

Isolate code number	Identity of the bacterial isolate
M3	*Lactobacillus plantarum* 1
M4	*Lactobacillus plantarum* 1
M5	*Lactobacillus pentosus*
M6	*Lactobacillus plantarum* 1
M7	*Lactobacillus plantarum* 1
M8	*Lactobacillus plantarum* 1
M9	*Lactobacillus pentosus*
M10	*Lactobacillus plantarum* 1
M11	*Lactobacillus brevis* 3
M12	*Lactobacillus plantarum* 1
B13	*Lactobacillus brevis* 3
B14	*Lactobacillus plantarum* 1
B16	*Lactobacillus plantarum* 1
B17	*Lactobacillus brevis* 1
B18	*Lactobacillus salivarius*
B19	*Lactobacillus brevis* 1
B20	*Lactobacillus pentosus*

Table 3. Effect of simulated gastric juice and intestinal juice on viability of *Lactobacillus* isolates. Results are presented as mean log CFU/ml ± S.D. after 3 h exposure at pH 3 and 4 h exposure at pH 8.

Viable count (log CFU/ml ± S.D.)			
Isolate	**Gastric juice (pH 3)**		**Intestinal juice (pH 8)**
	0 h	**3 h**	**4 h**
M3	8.82 ± 0.13	7.68 ± 0.14	6.70 ± 0.20
M4	8.94 ± 0.05	7.78 ± 0.02	6.87 ± 0.38
M5	7.91 ± 0.11	7.35 ± 0.12	6.73 ± 0.15
M6	8.61 ± 0.16	8.04 ± 0.09	6.97 ± 0.14
M7	8.01 ± 0.15	7.83 ± 0.09	7.02 ± 0.06
M8	8.58 ± 0.02	8.20 ± 0.04	7.26 ± 0.07
M9	8.15 ± 0.12	8.15 ± 0.05	6.96 ± 0.12
M10	7.91 ± 0.12	7.95 ± 0.05	6.92 ± 0.08
M11	8.62 ± 0.04	8.19 ± 0.03	7.11 ± 0.16
M12	7.52 ± 0.54	0.00 ± 0.00	0.00 ± 0.00
B13	8.96 ± 0.05	8.30 ± 0.05	7.31 ± 0.08
B14	8.78 ± 0.08	8.07 ± 0.02	6.96 ± 0.08
B16	8.57 ± 0.03	8.26 ± 0.02	7.29 ± 0.09
B17	8.51 ± 0.03	8.59 ± 0.07	7.50 ± 0.08
B18	8.61 ± 0.63	8.43 ± 0.09	7.16 ± 0.06
B19	8.02 ± 0.03	8.05 ± 0.10	7.09 ± 0.14
B20	8.32 ± 0.17	8.12 ± 0.06	7.16 ± 0.27
L. reuteri	7.10 ± 0.17	7.52 ± 0.09	7.06 ± 0.25

3.3. Antagonistic Activity Against Pathogens

All isolates inhibited the pathogenic target bacteria with varying degrees (Table 4). This leads to the assumption that some bacteriocins were being produced by these isolates which need further testing. Antibacterial substance production is a functional property to characterize probiotics (Shah, 2007). The ability to produce such compounds is very basic for competitive exclusion of pathogens and is a critical characteristic for probiotic bacterial candidates (Begley *et al.*, 2005). Of interest is the superior antagonistic activity of *L. plantarum* 1 M6, *L. plantarum* 1 M8 and *L. brevis* 1 B17 against multiresistant *S. aureus*.

Table 4. Antagonistic activity of *Lactobacillus* isolates against pathogenic bacteria. Inhibition zone diameters (mm) are presented as mean ± S.D.; n=2.

Inhibition zone diameters (mm) of indicator strains				
Bacterial isolate	*B. cereus*	*E. coli*	MRSA	*S. typhimurium*
M3	40 ± 0.0	57.5 ± 3.5	30 ± 0.0	40 ± 0.0
M4	54 ± 0.0	30 ± 14.1	19.5 ± 0.7	58 ± 2.8
M5	40 ± 0.0	39.5 ± 2.1	16 ± 0.0	57.5 ± 3.5
M6	40 ± 0.0	38 ± 0.0	63 ± 4.2	50 ± 0.0
M7	44 ± 0.0	32 ± 1.4	20 ± 0.0	40 ± 0.0
M8	40 ± 0.0	45 ± 7.1	60 ± 0.0	45 ± 1.4
M9	44 ± 0.0	36 ± 4.2	40 ± 0.0	50 ± 0.0
M10	22 ± 2.8	41 ± 1.4	22 ± 2.8	52 ± 2.8
M11	49 ± 1.4	55 ± 7.1	34 ± 0.0	55.5 ± 0.7
M12	46 ± 0.0	38 ± 0.0	34 ± 0.0	50 ± 0.0
B13	45 ± 0.0	36.5 ± 0.7	26 ± 8.5	50 ± 0.0
B14	44 ± 0.0	54 ± 0.0	34 ± 0.0	22 ± 0.0
B16	55 ± 7.1	42 ± 0.0	38 ± 2.8	60 ± 0.0
B17	62 ± 2.8	38.5 ± 0.7	53 ± 1.4	50 ± 0.0
B18	50 ± 0.0	38 ± 0.0	49 ± 1.4	60 ± 0.0
B19	46 ± 0.0	39 ± 1.4	36 ± 0.0	52 ± 2.8
B20	40 ± 0.0	26 ± 4.2	24 ± 0.0	50 ± 0.0

3.4. Resistance to Bile Salts

Most isolates were highly resistant to bile salts at the range of 0.3-2% after 24 h of exposure with little viable count reduction to the level of less than 1 log cycle (Table 5). However, *L. pentosus* M9, *L. pentosus* B20 and M12 were drastically affected after exposure to 1% and 2% for 24 h where both M9 and B20 lost viability totally after 24 h at 2% bile concentration. Since bile plays a role in the defenses of the gut, hence, bile tolerance is a paramount marker in choosing probiotic bacterial strains (Charteris *et al.*, 2000). Sanders *et al*. (1996) demonstrated the ability of lactobacilli to grow and metabolize at normal physiological bile concentrations of the gastrointestinal environment. Ganzel *et al.* (1999) reported the effect of food nature and components in the intestine in enhancing probiotics resistance to bile salts.

3.5. Antibiotic Susceptibility

Table 6 presents the Minimum Inhibitory Concentration (MIC) breakpoints for the isolates. Strains were considered resistant if they had higher breakpoints compared with that of the European Food Safety Authority (EFSA, 2008). Most isolates showed resistance to kanamycin, ampicillin, erythromycin and tetracycline. However, resistance varied within the strains of the same species especially isolates of *L. plantarum* 1. The most sensitive isolate *L. plantarum* 1 M8 was resistant only to ampicillin, erythromycin and kamanycin and sensitive towards the 5 other antibiotics used, while B19 was also sensitive to 4 antibiotics. *L. pentosus* M5 and B20 strains were the most resistant isolates exhibiting resistance to 6 antibiotics of the 8 tested. The observed resistance for kanamycin in this study was in agreement with previous reports about *Lactobacillus* in general (Temmerman *et al.*, 2003). Tetracycline resistance among isolates was higher than that reported by others (Choi *et al.*, 2003). Ammor *et al*. (2008) reported high resistance among *Lactobacillus* isolates towards aminoglycosides (gentamicin and kanamycin) which was observed in this study. Resistance to such antibiotics is considered natural and intrinsic in lactobacilli due to it being chromosomally encoded (Charteris *et al.*, 2001; Morrow *et al.*, 2012).

Table 5. Tolerance of *Lactobacillus* isolates to varying concentrations of bile salts after 24 h of anaerobic incubation (results are presented as mean ± S.D. of viable counts at zero and 24 h exposure to bile).

	Bile concentration							
Isolate	0.3		0.5		1.0		2.0	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
M3	6.73 ± 0.05	8.90 ± 0.18	6.52 ± 0.22	8.36 ± 0.10	6.33 ± 0.16	8.03 ± 0.14	5.26 ± 0.24	5.02 ± 0.55
M4	6.37 ± 0.06	6.83 ± 0.16	6.23 ± 0.10	8.70 ± 0.03	6.42 ± 0.07	6.70 ± 0.17	6.10 ± 0.09	7.16 ± 0.02
M5	6.30 ± 0.07	8.97 ± 0.49	6.40 ± 0.16	8.22 ± 0.32	6.27 ± 0.10	9.62 ± 0.20	5.94 ± 0.12	7.61 ± 0.09
M6	6.37 ± 0.15	8.74 ± 0.23	6.14 ± 0.38	9.07 ± 0.20	6.30 ± 0.06	8.95 ± 0.0	6.32 ± 0.09	7.81 ± 0.20
M7	6.55 ± 0.09	8.40 ± 0.17	6.66 ± 0.10	7.97 ± 0.03	6.53 ± 0.08	7.97± 0.03	6.35 ± 0.03	7.66 ± 0.05
M8	6.43 ± 0.06	7.03 ± 0.05	6.56 ± 0.03	5.60 ± 0.23	6.53 ± 0.02	3.49 ± 0.10	6.34 ± 0.09	3.44 ± 0.03
M9	6.05 ± 0.19	8.35 ± 0.69	6.35 ± 0.11	8.25 ± 0.51	4.98 ± 0.03	5.69 ± 0.36	5.00 ± 0.0	0.00 ± 0.0
M10	6.35 ± 0.03	8.58 ± 0.27	6.34 ± 0.05	8.63 ± 0.06	6.39 ± 0.10	8.10 ± 0.17	6.35 ± 0.06	7.74 ± 0.13
M11	6.51 ± 0.08	8.37 ± 0.08	6.59 ± 0.07	8.37 ± 0.09	6.44 ± 0.16	8.42 ± 0.15	6.44 ± 0.02	8.41 ± 0.03
M12	5.49 ± 0.48	0.00 ± 0.0	5.32 ± 0.26	0.00 ± 0.0	5.10 ± 0.17	0.00 ± 0.0	5.07 ± 0.20	0.00 ± 0.0
B13	6.54 ± 0.06	8.57 ± 0.05	6.81 ± 0.14	8.45 ± 0.05	6.73 ± 0.05	9.17 ± 0.08	6.68 ± 0.04	8.40 ± 0.02
B14	6.18 ± 0.07	8.10 ± 0.35	6.06 ± 0.11	7.83 ± 0.30	6.02 ± 0.20	7.97 ± 0.03	6.30 ± 0.25	8.13 ± 0.24
B16	6.62 ± 0.06	8.43 ± 0.12	6.69 ± 0.08	8.35 ± 0.04	6.53 ± 0.04	8.41 ± 0.03	6.62 ± 0.08	8.08 ± 0.0
B17	6.83 ± 0.06	8.64 ± 0.14	6.72 ± 0.11	8.30 ± 0.03	6.69 ± 0.02	8.45 ± 0.09	6.60 ± 0.08	8.08 ± 0.0
B18	6.62 ± 0.07	8.37 ± 0.19	6.73 ± 0.04	8.62 ± 0.13	6.78 ± 0.01	8.54 ± 0.06	6.66 ± 0.01	8.40 ± 0.05
B19	6.51 ± 0.06	9.17 ± 0.10	6.55 ± 0.07	8.92 ± 0.06	6.42 ± 0.09	8.55 ± 0.04	6.55 ± 0.08	8.38 ± 0.05
B20	6.20 ± 0.08	7.08 ± 0.0	5.97 ± 0.18	4.66 ± 0.02	5.74 ± 0.23	3.46 ± 0.19	5.07 ± 0.20	2.27 ± 0.07

Table 6. Antibiotic susceptibility profiles of *Lactobacillus* isolates of probiotic potential.

	Antibiotic breakpoint[a] (µg/ml)							
Isolate	A (2)	C (4)	E (4)	G (1)	K (32)	S (16)	Te (16)	Tr (16)
M3	R	S	R	R	R	R	R	R
M4	R	R	R	S	R	S	R	S
M5	S	R	R	R	R	R	R	S
M6	S	R	R	S	R	R	R	R
M7	R	S	R	R	R	S	R	S
M8	R	S	R	S	R	S	S	S
M9	R	S	R	S	R	R	R	S
M10	R	S	R	R	R	S	R	S
M11	R	R	S	R	R	S	R	R
M12	R	R	R	R	R	S	R	S
B13	R	R	S	S	R	S	R	R
B14	R	R	R	R	R	R	R	R
B16	R	R	S	S	R	R	R	R
B17	R	R	R	S	S	S	R	R
B18	R	R	R	S	S	R	R	R
B19	R	R	S	S	S	S	R	R
B20	R	S	S	R	R	R	R	R
L. reuteri DSMZ 20056	S	R	S	S	S	S	R	S

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

In conclusion, it is becoming increasingly obvious that the traditional fermented foods offer unlimited reservoir of probiotics. However, *in vitro* results should be substantiated by *in vivo* studies.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research of The University of Jordan, Amman, for their financial support. Thanks are also due to Ms. Sara Hamdan for her efforts as a research assistant and for typing the manuscript.

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