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Probiotic Characteristics of Lactobacilli Isolated from Various Native Yoghurts Made by Local and Traditional Dairy Producers of Isfahan, Iran

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Yoghurt is an important source of Lactobacilli and other lactic acid bacteria. In Iran, native yoghurts naturally contain some valuable probiotic lactobacilli. The aim of this study was to isolate and identify *Lactobacilli* strains with high quality probiotic potentials from all kinds of yoghurts made by traditional dairy producers of Isfahan, Iran. *Lactobacillus* strains were isolated from various traditional yoghurts. Then probiotic properties of the selected lactobacilli were determined. Strong acid and bile salt tolerant strains were considered as high quality probiotics, and identified at the species level by biochemical tests and were further identified according to16s rRNA specific sequences. A total of 82 *Lactobacillus* strains were isolated. Fourteen strains were graded as high quality probiotic lactobacilli (strong acid and bile tolerants). The majority of high quality probitics were sensitive to the most commonly used antibiotics. The phenotypic characterization of high quality probiotics resulted in identification of different lactobacillus species including 3 *Lactobacillus casei,* 8 *Lactobacillus plantarum, and* 3 *Lactobacillus pentosus.* The results of 16s rRNA gene sequencing assay confirmed the biochemical tests. The results indicated that, the Lactobacilli isolated from native yoghurts of Isfahan have great probiotic potentials, and could be used for production of different probiotic products.

Key words: Probiotics, Lactobacillus, Native and traditional yoghurts.

Probiotics are live, non-pathogenic, safe microorganisms that when administrated in adequate amount could exert positive health effects to the host[1-3]. Probiotics are chiefly consisted of lactic acid bacteria (LAB), such as *Lactobacilli* spp., *Bifidobacterium* spp., some *Streptococci* spp., a few non-lactic acid bacteria, and some yeast[2-4]. The essential in-vitro criteria for any microorganism to be considered probiotic are: resistance to acid and bile condition similar to gastrointestinal tract, production of antimicrobial substances and organic acids such as lactic acid, susceptibility to important antibiotics such as tetracycline, amoxicillin, tetracycline, cephalexin, vancomycin and chloramphenicol[3-6]. In-vivo essential criteria include: ability to adhere the epithelial tissue, persistence within gastrointestinal tract (GIT), no side effects in host and some other capabilities[3-5]. It has been suggested that probiotics are capable to exert beneficial health effects such as resistance to infections, improvement of immune responses, prevention of diarrhea due to antibiotic therapy, reduction of allergy, reduction of serum cholesterol and decreasing colon cancers[4,5]. Traditionally probiotic bacteria such as *lactobacilli* species including *L. acidophilus, Lactobacillus casei, Lactobacillus*

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rhamnousus, Lactobacillus brevis, Lactobacillus plantarum, and some *Bifidobacteria* have been utilized in dairy products such as milk and yoghurt in combination with some yeasts, but the most important part of almost all probiotic products are *Lactobacilli* strains[3-4, 6-8].

Lactobacilli are rod-shaped, gram positive, non-spore forming, non-pigmented bacteria with negative catalase and oxidase reaction. They are all anaerobic to micro-aerophilic, and are able to grow at low pH of acidic media. Most species are homofermonter, some are facultative heterofermenters, and some others are obligate heterofermenters[4, 6]. They are all member of LAB, and are considered as generally recognized as safe (GRAS) bacteria. *Lactobacilli* are naturally found in many dairy products, such as yoghurt, cheese and other dairies as starter culture or non-starters[4, 6, 9]. In Isfahan like other cities of Iran, various kinds of local and native yoghurts are made by different kinds of cattle milks such as cow, goat, and ewe's milk as plain yoghurts[4, 6, 7, 9]. Moreover some traditional yoghurts including mixed yoghurt, yoghurt and celery, yoghurt and scallion(shallot), and drained yoghurts are produced by many local dairy producers that are supposed to contain a wide range of non-starter lactobacilli with probiotic potentials[10, 11, 12]. Scallion yoghurt and celery yoghurt are made using native varieties of scallion and celery with scientific name of *Kelussia odoratissima and Allium hirtifolium* respectively.

The aims of this study were to isolate high quality probiotic lactobacilli present in traditional yoghurts such as scallion, celery, drained, mixed and plain yoghurts made by local dairy producers of Isfahan-Iran and then to identify them at species and strain level.

MATERIAL AND METHODS

Sample collection

Thirty two various native yoghurt samples including ten plain cow yoghurt (PY), seven plain cow-ewe mixed yoghurt (MY), ten vegetable yoghurts including five celery yoghurts (CY), five scallion yoghurts (SY), and also five drained yoghurts (DY), were purchased from local and traditional dairy producers and dairy stores of Isfahan. Samples were transported to laboratory in cold ice box and kept in the refrigerator (2°C) for a few hours before cultivation procedure.

Media

In order to maximize the chances of lactobacilli species to be isolated from different yoghurt samples, enrichment, modified, selective and differential media such as MRS (deMan Rogosa Sharpe) agar and MRS broth (Merck, Germany), acidified MRS agar (AMRS), *Lactobacillus* selection agar (LBS) , Lee's agar (Himedia, India), were respectively employed throughout the isolation steps[8,10,11]. In identification procedure, glucose and meat extract free MRS broth containing 1-2% specific carbohydrate and 0.04% bromocresol purple were used for fermentation assay. In addition nitrate broth, arginine and aesculin broth were made using MRS broth base (Merck, Germany) for other identification tests[12, 13, 14].

Isolation procedure

Initially all sample were homogenized by vigorous agitation and mixing by spoon, in order to give a soft viscous texture. Then the homogenized samples were diluted up to 10000 fold. In order to get single colony and avoid massive growth, 1ml of each diluted sample was inoculated by streak plate method to MRS, AMRS, LBS, and Lee's agar. Plates were then incubated in specific conditions according to the following procedures. For isolation of mesophilic *Lactobacilli* such as *L. casei, L. plantarum* and *L. brevis* plates were incubated anaerobically at 27-30°C for 3-5 days. For isolation of thermophilic *Lactobacilli* strains such as *L. acidophillus, L. bulgaricus* and *L. lactis* plates were incubated anaerobically at 43-45°C for 24-72 hours. After incubation period all well grown single colonies were visually investigated, and small to medium, non pigmented colonies on MRS agar, AMRS, LBS, and pigmented colonies on Lee's agar were selected as *Lactobacilli* strains for further steps[9-12, 14-16].

Primary phenotypic characterization

More than 133 purely isolated colonies were tested phenotypically by gram staining followed by catalase and oxidase tests which led to identification of 82 lactobacilli strains at genus level. Pure culture of rod shaped, catalase and oxidase negative bacteria were prepared on MRS agar. At the same time nitrate reduction, motility, anaerobic growth, and growth at pH 4 were carried

out [6, 9, 11, 12].

Probiotic chracterization

Purely cultured *Lactobacilli* (82 strains) were investigated for various probiotic properties including acid and bile tolerance, antimicrobial activity, antibiotic sensitivity, gelatinase and haemolysine activity. Strains demonstrated strong acid and bile tolerance potential were considerd high quality probiotics and selected for further probiotic properties assessments including, antimicrobial activity, sensitivity to antibiotics, gelatinase and haemolysine activity[6, 7, 8, 11].

Acid tolerance assay

Initially all isolates were screened for their acid tolerance potentials according to modified method described by Wang, *et al*.[8]. To evaluate the survival of all isolated *Lactobacilli* in acidic condition, one ml of fresh MRS broth culture of each isolate was added to 9 ml sterile MRS broth with adjusted pH of 3, and repeated for pH 2. Tubes were incubated in 37°C for 2, 3 and 4 hours. Viable numbers of survived bacteria were measured at each time interval and for each pH by using pour plate method and then colony counting carried out. Any isolate which showed viable number equal or greater than 50% of 0 hour test culture (control) was considered as tolerant strain. Tolerant strains were variably classified to weak, moderate and strong at both pH conditions according to their survival time[7, 8, 11, 17].

Bile salt tolerance assay

Screening for bile salt tolerant strains were carried out according to modified method of Yateem, *et al*.[6]. To evaluate the survival of strong acid tolerant strains selected in previous screening assay in the presence of bile salt, one ml of the fresh MRS broth culture of the selected strain was inoculated to 9 ml MRS broth containing bile salt at different concentrations of 0.2% and 0.3% (V/V). The cultures were incubated at 37°C for 6, 8 and 12 hours. Viable counts of each strain were determined at each time interval and for different bile salt concentrations by using pour plate method and then colony counting. Any strain that demonstrated viable number equal or greater than 50% of 0 hour culture or 0% of bile salt (controls) was considered as bile salt tolerant strain. Tolerant strains were classified to three groups, weak, moderate and strong according to their survival times[5, 7, 8, 11, 18]. Finally, strong acid and bile salt tolerant strains were considered as high quality probiotics and selected for further trails.

Antimicrobial activity

Antimicrobial activity of strong acid and bile tolerant strains (high quality probiotics) were determined against indicator bacteria including *S. aureus* PTCC 1431, *Pseudomonas aeruginosa* PTCC 1707, *Salmonella typhi* PTCC 1609 *and E. coil* PTCC 1338, using agar well diffusion method according to modified method of Sielaidie, *et al*.[17]. Initially the test bacteria were cultured in nutrient broth and incubated at 37°C for 24 hours. The turbidity of cultures was adjusted to McFarland No. 2 before antagonistic assay. At the same time the *Lactobacillus* strains were cultured in MRS broth and incubated anaerobically at 37°C for 24 h. Then 0.1 ml of each test bacteria was inoculated into 25 ml, melted 45°C Muller Hinton Agar and poured into sterile plates. Once inoculated MHA solidified, five wells with 5mm diameter were made and filled with 100µl centrifuged cell free supernatant of each *Lactobacilli* culture. Plates were kept in 2°C for 4 hours and then were incubated at 37°C for 24 hours. The diameter of inhibition zones were measured after incubation period. Strains demonstrated inhibition zone diameter greater than 9 mm (IZD>9mm) were considered as strong inhibitors[6, 7, 8, 11, 17, 18].

Antibiotic susceptibility assay

Antibiotic sensitivity of high quality probiotic strains that previously selected was also tested by disk diffusion method according to modified method of Palop and Narbad[5]. In this procedure 100 ul fresh 24h MRS broth culture of selected isolate with turbidity equal to 0.5 scale of McFarland was inoculated to soft MRS agar containing 0.7% agar. The antibiotic discs including penicillin G, amoxicillin, tetracycline, erythromycin, gentamycin, kanamycin, cephalexin, clindamycin, streptomycin and chloramphenicol (Oxoide, England) were applied. Plates were incubated at 37°C for 24 hours. Then the inhibition zone diameters (IZD) were measured and the results were defined as resistance (R), sensitive (S), and intermediate (I) [5, 7, 8, 11, 18].

Gelatinase activity

High quality probiotic strains that previously selected as strong acid and bile tolerants and showed good antimicrobial activity were also tested for their gelatinase activity

according to the modified method deseribed by Sieladie, *et al.*,[7,17] using nutrient gelatin agar containing: pepton 5%, yeast extract 3gr, gelatin 30 gr and agar 15 gr. To evaluate the gelatinase activity, 5ul of fresh MRS broth culture of each strain was spotted on gelatin agar, and incubated anaerobically at 37°C for 24-48 h. Surroundings of well grown colonies were observed for probable clear zones. For better observation, saturated ammonium sulfate solution was poured on colonies. *Bacillus cereus* PTCC 1665 was used as positive control.

Haemolysin activity

Haemolysine activity of selected strains was tested according to modified method described by Sieledie, *et al.*[17]. 5 µl of fresh MRS broth culture of each strain was spotted on 7% sheep blood agar and incubated anaerobically at 37°C for 24–48 h. Then surroundings of well grown colonies were investigated visually for clear zones.

Phenotypic characterization

All strains that previously identified at genus level and demonstrated high quality probiotic potentials were tested for their biochemical and physiological characteristics including growth at different temperatures (15-45°C), arginine and esculin hydrolysis, gas production from glucose fermentation. Carbohydrate fermentation profile of all selected strains was also tested for L-arabinose, lactose, sucrose, D-glucose, sorbitol, melibiose, D-xylose, D-raffinose, melezitose, D-ribose, mannitol, D-rhamnose, Ramnose and trehalose[9, 10, 12].

Genetic identification test

Isolates that already were identified at species level by biochemical and carbohydrate fermentation tests and also demonstrated high quality probiotic properties were differentiated to strain level by 16S rRNA gene sequencing using PCR method. According to modified method of Wang *et al.*[8] the nucleic acids of each isolate were extracted using a DNA purification kit (QI Aamp DNA mini Kit made by KIA gene Co. USA), by following the manufacturer's instructions. Two universal primers, including forward primer (3-AGGAGGTGATCCAACCGC-5) and 3-AACTGGAGGAAGGTGGGG-5 as reverse primer were used for amplification of 370 bp of 16s rRNA gene[13]. PCR products were then separated by electrophoresis at 50 V on a 2% (v/v) agarose and then DNA was observed. Sequencing of amplicons was carried out by Bioneer co. of Korea. The BLAST was performed on the obtained sequences using the NCBI database.

RESULTS

Isolation and primary identification

A total of 82 strains were selected due to their excellent growth in different selective, differential and also semi- selective media used for *Lactobacilli* isolation. 22 strains were isolated from plain yoghurts (PY), 28 strains were isolated from mixed yoghurts (MY), 13 strains isolated from drained yoghurts (DY), 9 strains isolated from celery yoghurts (CY), and 10 strains isolated from scallion yoghurts (SY) (Table 1). Colonies of isolates on MRS agar were non-pigmented, small to medium, circular with entire or undefined edges. All isolates were gram positive, spore less, long or short rods with single, pair or filamentous arrangement .They all were catalase, oxidase and nitrate negative.

Acid tolerance assay

Screening for selection of acid tolerant strains at pH3 and pH2 indicated that 37 isolates were non-tolerant, while 45 strains demonstrated a range of tolerance from weak to strong at both pH conditions. All 45 strains could survive at pH3 for 2h and evaluated as tolerant strains. Twenty one strains did not tolerate pH 3 after 2 hours and evaluated as weak acid tolerant while 10 strains

Table 1. The number and origin of isolated lactobacilli

Origin	Frequency	Designations	Mesophillic	Thermophyllic
Plain yoghurts (PY)	22	PY1-PY22	14 isolate	8 isolate
Mixed yoghurt (MY)	28	MY1-MY28	19 isolate	9 isolate
Drained yoghurts (DY)	13	DY1-DY13	8 isolate	3 isolate
Celery yoghurts (CY)	9	CY1-CY9	5 isolate	4 isolate
Scallion yoghurts (SY)	10	SY1-SY10	6 isolate	3 isolate

could survive 4 h at pH 3 and 2 h at pH 2 and evaluated as moderates. Further data demonstrated that 14 strains were able to survive 4 h at pH 3 and 3-4 h at pH 2. The latter strains were qualified as strong tolerant and selected for bile salts assay (Table 2).

Table 2. Acid and bile tolerance of high quality probiotic strains

Strains	Survival time(hour)				Tolerance
	pH3	pH2	0.2%bile	0.3%bile	quality
Py8	4h	4h	12h	12h	Excellent
Py13	4h	3h	12h	8h	Good
My4	4h	3h	8h	8h	Good
My16	4h	3h	12h	12h	Very good
My24	4h	4h	8h	8h	Good
My27	4h	3h	12h	12h	Very good
Dy7	4h	4h	12h	12h	Excellent
Dy10	4h	4h	12h	12h	Excellent
Dy11	4h	3h	8h	8h	Good
Cy2	4h	3h	12h	8h	Good
Cy3	4h	4h	8h	8h	Good
Sy4	4h	3h	12h	8h	Very Good
Sy5	4h	3h	12h	8h	Good
Sy9	4h	4h	8h	8h	good

Plain yoghurts (PY), Mixed yoghurt (MY), Drained yoghurts (DY), Celery yoghurts (CY), Scallion yoghurts (SY)

Bile salt tolerance assay

Assessment of strong acido-tolerants for their bile salts tolerance at 0.2% and 0.3% concentrations demonstrated that all 14 strains were able to survive 6-12 h variably at both concentrations and evaluated as strong bile tolerant. Finally, a total of 14 strong tolerant strains were qualified as high quality probiotic strains (Table 2).

Table 3. Antimicrobial activity of high quality probiotic strains

Strains	Inhibition zone diameter (mm)				Qualification
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	
Py8	9	8	9	8	good
Py13	9	9	8	4	Good
My14	7	9	8	5	Good
My16	9	9	7	4	Good
My24	11	10	11	10	Excellent
My27	10	9	9	5	very good
Dy7	11	8	10	4	Good
Dy10	10	9	8	10	very good
Dy11	7	10	9	4	Good
Cy2	10	9	10	9	Excellent
Cy3	8	6	9	5	Good
Sy4	11	10	11	5	very good
Sy5	8	11	10	5	Good
Sy9	9	9	11	4	very good

Plain yoghurts (PY), Mixed yoghurt (MY), Drained yoghurts (DY), Celery yoghurts (CY), Scallion yoghurts (SY)

Antimicrobial activity

The results of antimicrobial potential of strong acid and bile tolerant strains (high quality probiotics) against indicator bacteria demonstrated variable inhibitory activities. The majority of strains including PY13, MY16, MY27, DY7, CY2, SY4, SY5 and SY9 showed strong inhibitory potential against *E. coli*, *S. aureus* and *S. typhi*, but only PY8 and DY10 could inhibit *P. aeruginosa* growth. Some strains including MY4, DY7, DY11 and CY3, demonstrated moderate antimicrobial activity (Table 3).

Antibiotic susceptibility assay

Antibiotic susceptibility of high quality probiotic strains demonstrated that the majority of the strains were sensitive or intermediate to the most commonly used antibiotics for medical purposes such as penicillin, amoxicillin, tetracycline, erythromycin, chloramphenicol, cephalexin, gentamicin, kanamycin, streptomycin, clindamycin. But five strains including MY27, MY4, DY11, SY5 and CY3, were resistant to four of the most commonly used antibiotics (Table 4).

Gelatinase and haemolysin activity

None of the high quality probiotic strains demonstrated gelatinase and haemolysin activity. As the result of probitic characterization only four strains including PY8, MY24, DY10, and CY2 showed all essential probiotic properties and selected as the most valuable probiotic lactobacilli.

Phenotypic identification

Physiological and biochemical assesment of high quality probiotic strains revealed that most of them were *L. plantarum* species (8 strains), three strains were *L. pentosus* and three strains were *L. casei* (Table 5).

Genotypic identification

In PCR, 370 bp bands were observed. Different sequences obtained showed high homology with 3 different species of the lactobacilli. So, the Py8 was identified as *L. casei*, My24 was identified as *L. plantarum*, Dy10 identified as *L. plantarum*, and CY2 was identified as *L. pentosus*.

DISCUSSION

In Iran a wide range of native and traditional yoghurts are made by many local and small producers with variable taste and various textures with undefined, native yoghurt culture under non-standard conditions. Traditional yoghurts contain not only starter culture bacteria, but also they may harbor non-starter lactobacilli

Table 4. Antibiotic susceptibility of high quality probiotic strains

Strains	Antibiotics									
	P	Am	T	E	C	CL	GM	S	K	CD
Py8	S	S	S	S	S	I	S	S	S	I
Py13	I	S	S	I	S	S	S	S	S	S
My14	S	S	S	R	R	S	R	S	R	S
My16	I	S	S	S	S	I	I	S	S	S
My24	S	S	S	I	I	S	S	I	S	I
My27	S	S	S	R	R	S	R	S	R	S
Dy7	S	S	I	S	S	I	S	S	S	S
Dy10	I	I	S	S	I	S	I	S	S	I
Dy11	S	S	S	R	R	S	R	S	R	S
Cy2	S	S	S	S	S	S	S	S	S	S
Cy3	I	I	S	R	R	S	R	S	R	I
Sy4	S	S	S	S	S	I	I	S	S	I
Sy5	S	S	S	R	S	S	S	S	S	S
Sy9	S	I	S	S	S	S	S	S	S	S

Plain yoghurts (PY), Mixed yoghurt (MY), Drained yoghurts (DY), Celery yoghurts (CY), Scallion yoghurts (SY)
P: penicillin, Am: amoxicillin, C: chloramphenicol, CD: clindamycin, Cl: cephalexin, E: erythromycin, GM: gentamicin, K: kanamycin, S: streptomycin, T: tetracycline

Table 5. Phenotypic characteristics of the high quality probiotic strains

Characteristics	Strains													
	PY8	PY13	MY4	MY16	MY24	MY27	DY7	DY10	DY11	CY2	CY3	SY4	SY5	SY9
Gr. at: 10°C	+	+	+	-	+	+	+	+	+	+	+	-	-	+
Gr. at: 25°C	+	+	+	-	+	+	+	+	+	+	+	-	-	+
Gr. at: 35°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gr. at: 45°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arg.Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Gas from G:	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	-	±	+	-	+	+	-	-	+	-	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Manitole	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Melibiose	-	+	+	±	+	+	-	-	+	-	-	+	+	+
Raffinose	-	+	+	±	+	-	+	+	+	-	-	+	+	+
Rhamnose	+	-	-	+	-	+	-	+	-	-	-	+	-	-
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	±	+	+	±	+	+	+	+	+	+	+
Xylose	-	±	-	-	+	-	-	-	±	-	-	+	+	-
Presumptive Species	*L. casei*	*L.plant-rarmm*	*L. pent-osus*	*L. plant-rum*	*L. plan-trum*	*L. plan-trum*	*L. plan-trum*	*L. plan-trum*	*L. plan-trum*	*L. casei*	*L. casei*	*L.pen-tosus*	*L.pen-tosus*	*L. plant-ourum*

Plain yoghurts (PY), Mixed yoghurt (MY), Drained yoghurts (DY), Celery yoghurts (CY), Scallion yoghurts (SY), Gr.(Growth), Arg. (Arginine), G (Glucose)

and other LAB strains with probiotic potentials[10, 12, 20-23]. Recently many efforts have been made to identify high quality probiotic lactobacilli such as *L. acidophillus*, *L. plantarum*, *L. casei* and *L. brevis* from these traditional yoghurts by many Iranian researchers[9, 11].

In this study 35 native and traditional yoghurt samples were tested, from which 82 lactobacilli strains were identified primarily at genus level and then their probiotic properties were determined through screening method. Probiotic bacteria should be able to encounter and tolerate the acidity of stomach and bile salts of small intestine as first two antimicrobial barriers for at least 2-3 hours and 6-12 hours respectively[3-5, 7, 8]. Therefore in this study all isolated lactobacilli were initially tested for their acid tolerance at pH 3 and pH 2, and then for bile salt concentration of 0.2% and 0.3% for different time intervals. In pH trails, 45 strains demonstrated a range of tolerance from weak to strong with variable survival time from 2-4 h, but only 14 strains could strongly tolerate both pH with survival time of 4 h at pH 3 and 3-4 h at pH 2. These results were in agreement with the results obtained in the study of Wang *et al*.[8] and Yateem *et al*.[6] in which some Lactobacilli strains were resistant to pH 2 for 3-4 hours while some other strains were non tolerant. Since the pH of human gastric juice ranges from 1-4 before and after meal[3, 4, 7, 18], therefore it seems that, some strains such as PY8, MY24, DY7, DY10, CY3, and SY9, in our study are able to survive in stomach for at least 3-4 h, and this is enough for successful passage through stomach.

The next barrier for probiotic bacteria is bile salt concentration of small intestine that ranges from 0.2% to 0.3% [5, 7, 8, 18]. The results obtained from the bile salt assay of acidotolerant strains in our study were very promising, because some of the stains such as PY8, MY16, MY27, DY7 and DY10, could resist 12h in 0.3%. The result obtained here were in agreement with the findings of Wang *et al*.[8], and Chowdhury *et al*.[23], in which Lactobacilli strains could survive in 0.3% bile salts for 6 to 12 hours. Therefore it seems some strains in our study such as PY8, MY16, MY27, DY7, and DY10 are able to survive in small intestine for 12 hours which indicates that these strains could be good candidates as high quality probiotics.

Another important feature of probiotic bacteria is their antibacterial potentials against many pathogenic bacteria that chiefly exerted by lactic acid and some other substance such as H_2O_2, acetic acid, bacteriocins, and antimicrobial peptides[3-5, 7, 8]. In present experiment all 14 strong acid and bile tolerant (high quality probiotics) demonstrated variable antimicrobial activity against four food born pathogenic bacteria as indicators. *E. coli*, *S. typhi*, and *S. aureus* were inhibited variably by all 14 strains, while MY24 and CY2 could strongly inhibit not only the mentioned indicators, but also inhibited *P. aeruginosa* as well as other indicators. These data indicate that antimicrobial activity is strain dependent and also demonstrated that many lactobacilli strains present in traditional yoghurts are able to kill a wide range of intestinal pathogens[7, 18]. These results were also in agreement with experiments done by Wang *et al*.[8] and Yateem *et al*.[6] in which Lactobacilli strains demonstrated strong inhibitory activities against some indicator bacteria such as *E. coli*, *S. typhi*, and *S. aureus*, while *B. cereus* and *Vibrio parahaemolyticus* were variably resistant. In our experiment *P. aeruginosa* which is one the most well known resistant bacteria was inhibited by strains MY24 and CY2, so it seems that these two strains can be considered as good alternative for treatment of superficial *P. aeruginosa* infections. Another essential aspect of probiotics is their safety for human consumption[8, 18, 19]. Therefore several guidelines have been suggusted by FAO and WHO for selection of safe microorganisms[19]. These guidelines emphasize on the use of various in vitro tests to evaluate the safety of probiotics, including antibiotic susceptibility tests, and also gelatin hydrolysis and heamolysis tests[18, 19].

The results of antibiotic susceptibility assay in our study revealed that the majority of selected strains were sensitive to antibiotics, but some of the strains including MY27 and DY11 were resistant to kanamycin, erythromycin, chloramphenicol, and gentamicin, while MY4 and SY5 were resistant to kanamycin and gentamicin and CY3 was resistant to clindamycin which indicates the presence of resistance genes among some of the selected strains. These results were in agreement with some similar studies, including the experiments performed by Sieladie *et al*.[18] and Hoque *et al*.[10], in which some Lactobacilli strains such as *L. plantarum* and *L. casei* were resistant

to some important antibiotics. Since some of the resistant genes are able to be transferred to pathogenic bacteria via plasmids in conjugation process, so it can be a critical threat to health and very important issue in medical community[8, 19]. Therefore the mentioned strains could not be considered as probiotics and should not include for use as probiotic or starter culture and also the starters used routinely should be tested for the presence of these bacteria.

In conclusion, this study revealed that the native and traditional yoghurts of Isfahan like many other traditional yoghurts are important source of high quality probiotic lactobacilli such as *L. casei*, *L. plantarum*, and *L. pentosus*. Moreover, this research showed some of these strains are highly resistant to acid and bile, with potent antimicrobial activity, and acceptable safety potentials, so they can be used as excellent probiotics in probiotic products such as various kinds of probiotic yoghurts. This study also demonstrated that some of the traditional yoghurts contain lactobacilli with resistant genes to some of the most important antibiotics, so they could threaten the public health.

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