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Diversity and probiotic potentials of lactic acid bacteria isolated from gilaburu, a traditional Turkish fermented European cranberrybush (*Viburnum opulus* L.) fruit drink

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ABSTRACT

The aim of the present study was to characterize lactic acid bacteria (LAB) strains isolated from traditional fermented gilaburu fruit juice and their probiotic potential. The LAB counts of the fermented gilaburu fruit juice were in the range of 3.92–8.30 log cfu/g. Total of 332 isolates belonging to *Lactobacillus* and *Leuconostoc* species were characterized from traditional fermented gilaburu juice by genotypic methods. It was also determined that the major LAB strains belong to *Lactobacillus plantarum* (173 isolates), *Lactobacillus casei* (52 isolates) and *Lactobacillus brevis* (24 isolates), while *Lactobacillus buchneri, Lactobacillus parabuchneri, Lactobacillus pantheris, Leuconostoc pseudomesenteroides* and *Lactobacillus harbinensis* were the least in isolated LAB strains. In terms of the probiotic potentials, *Lb. plantarum* strains were able to grow at pH 2.5, but 3 of *Lb. casei* strains, one of each *Lb. brevis* and *Lb. buchneri* strains could not grow at the same pH. All selected LAB stains were resistant to bile salt at ≤0.3% concentration. While all the LAB species grew at 15 °C, two *Lactobacillus hordei* strains could also grow at 45 °C. The highest cell hydrophobicity degrees were for *Lb. casei* (G20a) and *Lb. plantarum* (G19e) as 87.5 and 86.0%, respectively. *Listeria monocytogenes* and *Bacillus cereus* were the most sensitive bacteria against the selected LAB strains, while *Escherichia coli* and *Staphylococcus aureus* were the most resistant. Again all the isolated LAB species were resistant to three antibiotics; kanamycin, streptomycin and vancomycin. Characterization and probiotic potentials of the LAB isolated from fermented gilaburu (*Viburnum opulus)* juice were studied first time, and further research needs to be done on their behaviors in similar food formulations as a probiotic.

1. Introduction

European cranberrybush (*Viburnum opulus* L.) is the fruit of a deciduous shrub, which belongs to Caprifoliaceae family that originated in Europe, North Africa and North Asia. However, it is also frequently found in the central zone of western Russia (Sedat Velioglu, Ekici, & Poyrazoglu, 2006). In the world, it is commonly used for ornamental purposes and known with some other names such as European cranberrybush, American cranberrybush, cranberry tree, guelder rose, wild guelder rose, gueldres-rose, cherry-wood, rose elder, snowball bush, crampbark tree and whitten tree. It produces pendulous clusters of bright red berries that contain one seed in late autumn (Aksoy, Guvensan, Akcicek, & Ozturk, 2004; Anonymous, 2003, 2008). Barks and fruits of European cranberrybush tree are widely used in pharmacology. European, Native American and Asian people, discovered its antispasmodic properties independently. Also, it has been used for relief of asthma, cold, fever, nervousness, water retention problems, cough, cramps, stomachache, menstrual cramps, uterine infections, blood pressure and infertility (Anonymous, 2003; Nellessen, 2006). Antimicrobial properties of European cranberrybush fruit's extracts and seed oils previously were determined in some research (Sagdic, Aksoy, & Ozkan, 2006; Yilmaz, Yayli, Misir, Çoskunçelebi, & Karaoglu, 2008). It is indigenously grown in the Central Anatolia Region of Turkey and in some regions, its fruits are traditionally collected at the end of the autumn, washed with water, put in plastic jars, filled with water and allowed to spontaneously ferment at room temperature for three–five months. After the fermentation period, fruit juice is obtained by squashing of the fruits and consumed by adding some water and/or sugar if desired. This juice is not very acceptable due to its astringent taste that is a tactile sensation (Sedat Velioglu et al., 2006). It is believed that this taste can be reduced with a long fermentation by the local people who consume it, and it is fondly consumed compared to fresh counterparts.

Fermented vegetables and fruits are one of the most popular food consumed throughout the world. Some microorganisms such as lactic acid bacteria, acetic acid bacteria and yeast are involved in the fruit and vegetable fermentation (FAO., 1998). For centuries, LAB have been used to produce fermented food products and LAB in fermented foods have a long history of application in the industry for their beneficial

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influence on nutritional, organoleptic and shelf-life of food and feedstuffs (Kalantzopoulos, 1997; Stiles & Holzapfel, 1997). They rapidly acidify the raw material through the production of organic acids, mainly lactic acid. Furthermore, they produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes (Leroy & De Vuyst, 2004). In recent years, LAB are the focus of interest for both food industry and international researches because of their functional properties such as production of various antimicrobial compounds, reduction of serum cholesterol, alleviation of lactose intolerance, stimulation of the immune system, stabilization of gut microflora and antitumoral activities (Khedid, Faid, Mokhtari, Soulaymani, & Zinedine, 2009).

No study has been found on the characterization of LAB in the fermented gilaburu juice in the literature. Thus, the aims of the current study were (i) to isolate and identify LAB by genotypic methods from the traditional Turkish fermented European cranberrybush (*Viburnum opulus* L. Turkish name is gilaburu) fruit juice, and (ii) to determine their functional properties and probiotic potentials of the isolated species.

2. Materials and methods

2.1. Microbiological and pH analyses

In this research, twenty different fermented gilaburu (European cranberrybush) fruit samples that are traditionally fermented by local people and/or producer for 3 or 5 months were obtained in original packages (5 kg) from different region of Kayseri, Turkey. Firstly, the fermented gilaburu fruits were removed from the original packages and homogenized under sterile conditions. Then, the gilaburu samples were used for pH and microbiological analyses.

The pH values were measured electrometrically with a pH meter (InoLab 720, WTW GmbH, Weilheim, Germany), according to the standard procedures.

For microbiological analyses, 25 g of gilaburu juice sample was homogenized with 225 mL Maximum Recovery Diluent (Merck, GmbH, Darmstadt, Germany) and other serial dilutions were prepared. Total aerobic plate counts (TMAB), coliform bacteria, *Escherichia coli*, and *Staphylococcus aureus* counts were determined according to standard operating procedures described by the US Food and Drug Administration manual (Anonymous, 2011). The samples were examined for total aerobic plate counts using Plate Count Agar at 35 °C for 48 h. Coliform bacteria and *E. coli* were determined on Violet Red Bile Agar and Eosin Metilen Blue Agar for 24 h at 37 °C, and *S. aureus* on Baird Parker Agar were determined after incubation at 35 °C for 48 h. Yeasts and molds were enumerated on Dichloran Rose Bengal Chloramphenicol Agar after 3–5 days of incubation at 25 °C. For the detection of thermo-acidophilic spore forming bacteria, *Alicyclobacillus* sp., the plates of BAT Agar adjusted to 3.9 pH were incubated at 45 °C for 3–5 days (Murray, Gurtler, Ryu, Harrison, & Beuchat, 2007). Lactic acid bacteria (LAB) isolation was performed from serial dilutions of the samples by plating on MRS agar added with 10 ppm of cycloheximide to prevent growing of yeasts and molds. Then plates were incubated under anaerobic conditions at 30 °C for 48 h. After incubation period and counting the colonies, representative colonies were selected and purified by replanting on MRS broth and then on agar medium. Colonies were reselected and initially Gram-stained and tested for production of catalase. Only Gram-positive and catalase-negative strains were selected for LAB identification (De Man, Rogosa, & Sharpe, 1960; Sagdic, Arici, & Simsek, 2002).

2.2. Genotypic characterization by rep-PCR and Box-PCR analysis

In order to determine total genomic DNA from each LAB isolate, a commercial DNA extraction kit (Invitrogen, Carlsbad, CA, USA) was used. LAB isolates were grown overnight on MRS agar and the DNA of pure LAB was extracted according to the manufacturer's protocol. Concentrations and purity of obtained LAB genomic DNA were determined using a NanoDrop (ACT Gene UV-99, USA). The rep-PCR analysis with primer $(GTG)_5$ was used to discriminate LAB at the level of strain. Amplification reactions were performed in a final volume of 50 μL containing 24 μL commercial PCR master mix (Qiagen GmbH, Germany), 20 μL nuclease-free water, 4 μL (50 pmol) of $(GTG)_5$ primer and 2 μL (about 100 ng) DNA. DNA samples were amplified in the thermal cycler (Applied Biosystems, Veriti, Foster City, California, USA) which was programmed as follows: Initial denaturation of DNA for 10 min at 95 °C, 35 cycles at 94 °C for 60 s, 40 °C for 60 s, and 65 °C for 8 min; and followed by a final elongation step of 65 °C for 16 min. The rep-PCR products were separated using horizontal gel electrophoresis system (Thermo Scientific) on a 1.5% (wt/vol) agarose gel containing 0.5 μg/mL ethidium bromide in 1 × TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8 2) at a constant voltage of 50 V at a constant temperature (4 °C) for 20 h. The rep-PCR fingerprinting obtained from the LAB isolates was visualized and compared by using the pattern analysis software package Gel Compare II, version 6.1 (Applied Maths, Kortrijk, Belgium).

The Box-PCR analysis with primer BOX A1R (CTACGGCAAGGCGACG CTGACG) was used to discriminate the LAB at the level of strain as a second molecular identification method. For this purpose, the program was used as follows: Initial denaturation of DNA for 7 min at 95 °C, 35 cycles at 94 °C for 60 s, 53 °C for 60 s, and 65 °C for 8 min; and followed by a final elongation step of 65 °C for 16 min. Then, electrophoresis conditions were practiced at a constant voltage of 40 V at 4 °C for 200 min (Adiguzel & Atasever, 2009).

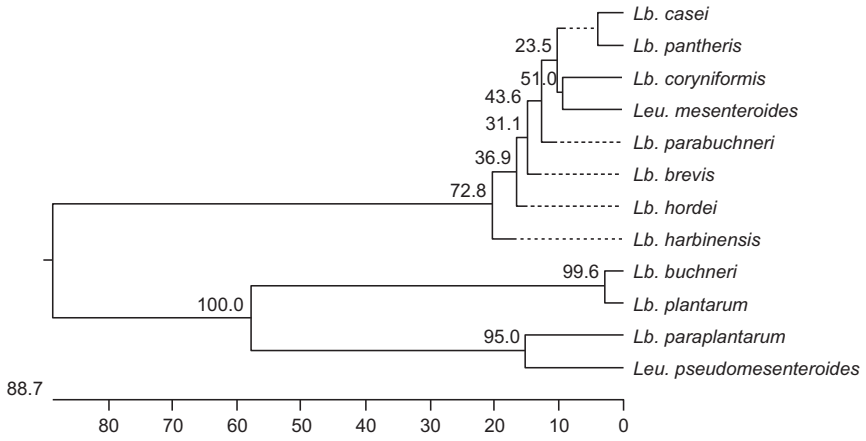

Fig. 1. The 16S rRNA gene based phylogenetic tree for LAB isolates. The tree was constructed using the Neighbor-Joining method. A total of 2000 bootstrap replications were performed; only bootstrap values higher than 85 are shown. The bar indicates the length representing 0.1 nucleotide substitution per site.

Table 1
Fermented gilaburu juice microbiota (log cfu/g) and pH values.

Samples	pH	TMAB	Yeast-Mold	LAB	*S. aureus*	Coliform	*E. coli*	*Alicyclobacillus* sp.
FG1	4.00 ± 0.00	7.30 ± 0.07	5.00 ± 0.02	6.71 ± 0.08	<1	<1	<1	<1
FG2	3.66 ± 0.00	6.32 ± 0.16	5.03 ± 0.04	6.57 ± 0.19	<1	<1	<1	<1
FG3	3.61 ± 0.00	5.73 ± 0.05	5.11 ± 0.01	3.92 ± 0.11	<1	<1	<1	<1
FG4	3.64 ± 0.01	6.94 ± 0.02	5.64 ± 0.23	6.07 ± 0.11	<1	<1	<1	<1
FG5	3.44 ± 0.01	7.47 ± 0.15	5.99 ± 0.07	6.52 ± 0.12	<1	<1	<1	<1
FG6	3.57 ± 0.01	7.15 ± 0.01	5.89 ± 0.03	5.80 ± 0.01	<1	<1	<1	<1
FG7	3.56 ± 0.00	7.84 ± 0.04	5.59 ± 0.10	6.99 ± 0.02	<1	<1	<1	<1
FG8	4.25 ± 0.00	6.93 ± 0.08	6.55 ± 0.12	6.74 ± 0.06	<1	<1	<1	<1
FG9	4.29 ± 0.00	6.34 ± 0.08	6.48 ± 0.01	4.98 ± 0.03	<1	<1	<1	<1
FG10	4.44 ± 0.01	6.43 ± 0.12	7.02 ± 0.02	8.03 ± 0.04	<1	<1	<1	<1
FG11	3.49 ± 0.00	7.45 ± 0.35	7.24 ± 0.02	6.39 ± 0.12	<1	<1	<1	<1
FG12	4.05 ± 0.00	8.97 ± 0.10	6.91 ± 0.18	8.30 ± 0.25	<1	<1	<1	<1
FG13	4.43 ± 0.00	8.24 ± 0.09	7.58 ± 0.18	7.83 ± 0.10	<1	<1	<1	<1
FG14	3.85 ± 0.01	7.27 ± 0.02	6.31 ± 0.17	8.10 ± 0.15	<1	<1	<1	<1
FG15	3.51 ± 0.00	8.27 ± 0.10	7.17 ± 0.05	6.65 ± 0.07	<1	<1	<1	<1
FG16	4.21 ± 0.00	7.10 ± 0.02	6.32 ± 0.03	6.36 ± 0.08	<1	<1	<1	<1
FG17	3.69 ± 0.00	5.85 ± 0.21	7.01 ± 0.01	5.31 ± 0.43	<1	<1	<1	<1
FG18	3.43 ± 0.00	6.25 ± 0.07	7.22 ± 0.11	6.53 ± 0.35	<1	<1	<1	<1
FG19	3.50 ± 0.01	6.68 ± 0.03	6.31 ± 0.09	4.45 ± 0.64	<1	<1	<1	<1
FG20	3.36 ± 0.00	8.53 ± 0.15	6.75 ± 0.04	8.44 ± 0.13	<1	<1	<1	<1

FG1-20: Fermented gilaburu juice samples, TMAB: Total mesophilic aerob bacteria. LAB: Lactic acid bacteria.

2.3. 16S rRNA gene sequencing

A fragment of 16S rRNA gene for LAB was amplified by PCR using the primers: LPW57 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW205 (5′-CTTGTTACGACTTCACCC-3′). PCR amplification reactions were carried out in a final volume of 50 μL, containing 24 μL commercial PCR master mix (Qiagen GmbH, Germany) and 18 μL nuclease-free water 0.5 μM (3 μL) for each primer and 2 μL (about 100 ng) total template DNA, and under the following conditions: the initial denaturation of DNA for 10 min at 95 °C was followed by 35 cycles of denaturation at 95 °C for 60 s, annealing at 58 °C for 60 s, and extension at 72 °C for 2 min, and a final extension of incomplete products at 72 °C for 10 min. The presence of specific PCR products was checked by agarose 1.5% (w/v) gel electrophoresis (1× TAE, 70 V, 1 h). The PCR products were purified using a PureLink PCR Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the supplier's instructions. The PCR products purified were transferred to the laboratory (Iontek, Istanbul, Turkey) for sequencing. The sequence results obtained were aligned with the NCBI database using the BLAST algorithm. The LAB isolates were identified according to similarity criterion of 97–100%. The 16s rRNA gene sequences for all of the LAB species were arranged in MegAlign® (DNAstar®, Lasergene). Phylogenetic trees were constructed using the neighbor-joining (NJ) method with 2000 bootstrap replicates. All phylogenetic analyses were performed using PAUP version 4.0 beta 10 (Fig. 1).

2.4. Functional properties and probiotic potentials of selected LAB isolates

Some LAB strains were selected to determine their functional and probiotic potentials, after the identification of LAB isolates. For this purpose, a total of 40 strains, which two same or different LAB strains from each gilaburu sample were selected. Selected LAB cultures were activated two times in MRS broth (MRS, Merck, GmbH, Darmstadt, Germany) and inoculated (1% v:v) in two series of MRS tubes, which were subsequently incubated at 15 and 45 °C for 24 h. Growth of LAB isolates was determined by visual observation. In order to estimate growth capabilities of selected LAB isolates at different pH, activated cultures (1% v:v) were inoculated into MRS broth tubes with pH adjusted at 2.5 and 3.5 (with 3 N HCl). Then the tubes were incubated at 30 °C for 24 h, and the results were determined visually (Psomas, Andrighetto, Litopoulou-Tzanetaki, Lombardi, & Tzanetakis, 2001). To determine NaCl tolerance of selected LAB isolates, activated cultures were inoculated in MRS broth tubes adjusted at concentrations of 2 and 4% with NaCl. The tubes were incubated at 30 °C for 24 h and the growth was determined visually. The experiments were duplicated on two separate occasions. Bile salt tolerance of selected LAB was determined using bile salt (Sigma-Aldrich, Germany). Activated cultures were inoculated in MRS broth tubes adjusted at concentrations of 0.15 and 0.3% with bile salt. The tubes were incubated at 30 °C for 24 h and the growth was determined visually.

Production of gas from D-glucose was determined in MRS broth inserted with inverted Durham tubes (Sagdic et al., 2002).

Hydrolysis of arginine test was carried out in MRS broth containing no glucose and meat extract. In addition to that, arginine (0.3% w/v) and sodium citrate (0.2% w/v) were added to the medium replacing ammonium citrate. Ammonia production was detected using Nessler's reagent (Arici, Bilgin, Sagdic, & Ozdemir, 2004).

Acid productions of the selected LAB strains were determined in MRS broth. Activated culture (1% v/v) was added to MRS broth. Then the tubes were incubated for 24 h at 30 °C and pH of mediums was measured with a pH meter at 0th, 6th, 12th and 24 th h (Arici et al., 2004).

Lactate isomers including D (−), L (+) and DL lactic acid producing ability of selected LAB isolates were also determined by using enzyme test kit according to the manufacturer's protocol (Roche Diagnostic, Germany) (Boehringer-Mannheim, 1989).

The ability of the selected LAB strains hydrophobicity was performed according to Vinderola and Reinheimer (2003). The LAB cultures were harvested in the stationary phase by centrifugation at 12,000 *g* at 4 °C for 5 min, washed carefully twice using 50 mM K_2HPO_4 (pH 6.5) buffer

Table 2
Diversity and prevalence of LAB isolated from fermented gilaburu juice.

LAB strains	The number of isolates	Frequency (%)	The number of samples
Lb. plantarum	173	52.1	15
Lb. casei	52	15.7	5
Lb. brevis	24	7.2	3
Leu. mesenteroides	20	6.0	1
Lb. hordei	19	5.7	3
Lb. paraplantarum	16	4.8	1
Lb. coryniformis	13	3.9	1
Lb. buchneri	5	1.5	1
Lb. parabuchneri	4	1.2	1
Lb. pantheris	3	0.9	2
Leu. pseudomesenteroides	2	0.6	1
Lb. harbinensis	1	0.3	1

Lb: *Lactobacillus*, *Leu*: *Leuconostoc*.

and finally resuspended by the same buffer. The cell suspension prepared was adjusted to an A560 nm value of approximately 1.0 with the 50 mM K_2HPO_4 buffer and 3 mL of the LAB suspensions were incorporated with 0.6 mL of n-hexadecane. Then, these suspensions were thoroughly mixed by vortex for 2 min. The mixes were incubated to separate into two phases for 20 min at 37 °C. The aqueous phase obtained was carefully removed and its absorbance was measured with a spectrophotometer at the A560 nm. Hydrophobicity of (%) selected LAB strains was calculated with the formula:

$$H\% = [(A1-A2)/A1]100 \quad (1)$$

where H is hydrophobicity %, and A1 and A2 are the absorbance before and after the treatment with n-hexadecane, respectively.

Resistance of selected LAB isolates against 8 antibiotic substances including ampicillin (Amp, 10 μg), chloramphenicol (C, 30 μg), erythromycin(E, 15 μg), kanamycin (K, 30 μg), penicillin (P, 10 μg), streptomycin (S, 10 μg), tetracycline hydrochloride (TE, 30 μg), and vancomycin (VA, 30 μg) (Oxoid, UK) was determined in the present study. LAB isolates were activated in MRS and added to MRS 1% at 45–50 °C and poured 20 mL of agar into plate dishes. Then, disks were placed at the center of the medium and plates were incubated at 30 °C for 24–48 h. As a result formed inhibition zones around the holes were measured and expressed as millimeter (mm).

Antimicrobial activities of selected LAB strains were performed using the agar well diffusion method. Seven microorganisms namely *Bacillus cereus* ATCC 33019, *E. coli* ATCC 25922, *E. coli* O157:H7 ATCC 33150, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028, *S. aureus* ATCC 25923 and *Yersinia enterocolitica* ATCC 27729 strains were used as test organisms. Bacteria strains were inoculated in nutrient broth and incubated at 35 °C for 24 h. Then, 1% the bacteria cultures were added to nutrient agar at 45–50 °C and poured 25 mL of agar into petri dishes. Meanwhile, selected LAB strains were activated in MRS broth at 30 °C for 24 h. LAB strains were centrifuged to obtain supernatant at 9000 ×*g* for 10 min. Then the supernatant was filtered using sterile filter (0.22 μm, Millipore, MA, USA). Holes were bored into solidified medium with help of sterile cork borers (Ø = 6 mm). Each sterile LAB supernatant (50 μL) was added to the holes using a micropipette. The plates prepared were incubated at 35 °C for 24 h. At the end of the incubation, inhibition zones were measured, and the results were expressed as mm.

3. Results and discussion

3.1. Microbiological and pH results

The results of pH values, LAB, yeast–mold, TMAB, *Alicyclobacillus* sp. *S. aureus,* total coliform and *E. coli* counts conducted in fermented gilaburu (European cranberrybush) were presented in Table 1. The pH values of the samples were in the range of 3.36 and 4.44 log cfu/g. LAB counts of the samples ranged from 3.92 to 8.44 log cfu/g in the fermented gilaburu juice samples, and it was lower than 6.00 (log cfu/g) in only five samples. Again, TMAB and mold–yeast counts of the samples were ranged from 5.73 to 8.97 and from 5.00 to 7.58 log cfu/g, respectively, and *Alicyclobacillus* sp., *S. aureus,* total coliform and *E. coli* could not be detected in any samples.

It has been well known that LAB is the largest group of bacteria associated with fermented dairy, meat and vegetables products, and it has a great importance in the formation of aroma and flavor in these foods (Carr, Chill, & Maida, 2002). Our microbiological results indicated that the numbers of LAB are different and quite high in the gilaburu juice samples (Table 1). Therefore, it might be presumed that fermented gilaburu (European cranberrybush) fruit juices may safely be consumed due to their microbiological characteristics and the absence of pathogen bacteria.

Table 3
Distribution of LAB strains in the gilaburu juice samples.

Samples	Total Isolates	*Lb. plantarum*	*Lb. casei*	*Lb. brevis*	*Leu. mesenteroides*	*Lb. hordei*	*Lb. paraplantarum*	*Lb. coryniformis*	*Lb. buchneri*	*Lb. parabuchneri*	*Lb. pantheris*	*Leu. pseudomesenteroides*	*Lb. harbinensis*
FG1	16	–	16	–	–	–	–	–	–	–	–	–	–
FG2	16	–	–	–	–	–	16	–	–	–	–	–	–
FG3	20	–	–	–	20	–	–	–	–	–	–	–	–
FG4	13	–	–	–	–	–	–	13	–	–	–	–	–
FG5	16	16	–	–	–	–	–	–	–	–	–	–	–
FG6	14	11	–	3	–	–	–	–	–	–	–	–	–
FG7	16	14	–	–	–	–	–	–	–	–	–	2	–
FG8	15	15	–	–	–	–	–	–	–	–	–	–	–
FG9	16	15	1	–	–	–	–	–	–	–	–	–	–
FG10	16	16	–	–	–	–	–	–	–	–	–	–	–
FG11	16	16	–	–	–	–	–	–	–	–	–	–	–
FG12	14	7	–	7	–	–	–	–	–	–	–	–	–
FG13	16	–	–	–	–	16	–	–	–	–	–	–	–
FG14	18	18	–	–	–	–	–	–	–	–	–	–	–
FG15	18	4	–	14	–	–	–	–	–	–	–	–	–
FG16	19	10	1	–	–	2	–	–	–	4	2	–	–
FG17	18	2	9	–	–	–	–	–	5	–	1	–	1
FG18	16	7	9	–	–	–	–	–	–	–	–	–	–
FG19	19	18	–	–	–	1	–	–	–	–	–	–	–
FG20	20	4	16	–	–	–	–	–	–	–	–	–	–
Total	332	173	52	24	20	19	16	13	5	4	3	2	1

FG1–20: Fermented gilaburu samples, *Lb: Lactobacillus*, *Leu: Leuconostoc.*

3.2. Characterization of LAB in the gilaburu juice

First of all, the LAB isolates were identified for some properties such as Gram reaction, catalase activity and growth ability at anaerobic conditions. A totally of 332 isolates from 12 different LAB species were identified with genotypic methods from traditional fermented gilaburu juice collected in the current work. Typing tests showed that these isolates were *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus brevis, Leuconostoc mesenteroides, Lactobacillus hordei, Lactobacillus paraplantarum, Lactobacillus coryniformis, Lactobacillus buchneri, Lactobacillus parabuchneri, Lactobacillus pantheris, Leuconostoc pseudomesenteroides* and *Lactobacillus harbinensis* (Table 2), and most of them belonged to lactobacilli strains (95.4%). It was also found that the major LAB were composed by *Lb. plantarum* (173 isolates), *Lb. casei* (52 isolates) and *Lb. brevis* (24 isolates), while, *Lb. buchneri, Lb. parabuchneri, Lb. pantheris, Leu. pseudomesenteroides* and *Lb. harbinensis* were the least. While *Lb. plantarum* was isolated from 15 gilaburu samples, *Lb. casei, Lb. brevis and L. hordei* strains were only isolated from 6, 3 and 3 gilaburu samples, respectively (Table 3).

Any research related to characterization of LAB microbiota of fermented gilaburu juice could not be found, again there was not much research with regard to fermented or non-fermented fruit juice in the literature. Yien Ong, Siang Tan, Rosfarizan, Chan, and Ti Tey (2012) determined only *Enterococcus* sp. in all LAB species from fermented red dragon fruit (*Hylocereus polyrhizus*) juices that is a local originating from Mexico, South America and some Southeast Asian countries. In a similar study, tempoyak (fermented durian fruit (*Durio zibethinus*)), is widely consumed product in Malaysia and Indonesia was studied. Major LAB species of tempoyak was found to be *Enterococcus* sp. followed by *Lactobacillus* sp. (Pato & Surono, 2013). However, Yuliana and Dizon (2011) analyzed the same type of product made in the Philippines and reported *Lactobacillus*, *Weissella*, *Pediococcus* sp. as main species. These findings indicate that even though the same type of fermented products is used for the studies, the microbiota can vary based on several factors including the origin of the fruits and production methods. Saguir, Campos, Maturano, and Manca de Nadra (2009) also reported that *Oenococcus oenii* was the predominant LAB strains in grape juice that is not fermented. In the same study, other LAB species were reported as *Lactobacillus*, *Leuconostoc* and *Pediococcus* sp. Hardaliye is a Turkish beverage which is produced from red grape or grape juice with the addition of crushed mustard seeds, and the characterization of LAB of this product was performed by Arici and Coskun (2001). In the results, *L. paracasei* subsp. *paracasei* and *Lb. casei* subsp. *pseudoplantarum* strains were predominantly found in this product. In another work, characterization of LAB was conducted in different fruits like apple, orange, grape strawberry, peach, papaya and melon (Naeem, Ilyas, Haider, Baig, & Saleem, 2012). As a result, *Lb. plantarum* was the major LAB species, and *Leu. mesenteroides* showed a sporadic presence (Naeem et al., 2012). In a similar study, *Weissella cibaria* and *Lb. plantarum* were the predominant LAB species in ripe mulberries (Chen, Wu, & Yanagida, 2010). Duangjitcharoen, Kantachote, Ongsakul, Poosaran, and Chaiyasut (2008) also reported that a strain of *Lb. plantarum* with a probiotic potential in the fermented star fruit beverage. Moreover, *Lb. plantarum* can be found during the fermentation of some vegetable products including kimchi, sauerkraut pickles produced from different materials and as well as in fermented dairy, meat and baked food products (Rodríguez et al., 2009).

Lb. plantarum is a resilient and multifaceted LAB species that is fallen with in different environmental conditions like fermented dairy, meat, and vegetable products (Kleerebezem et al., 2003). Furthermore, it is commonly encountered in the human gastrointestinal tract which is a complex metabolic ecosystem, and some strains are known and marketed as probiotic lactobacilli due to their beneficial effects on human health (Kleerebezem et al., 2003). *Leu. mesenteroides, Lb. casei* and *Lb. brevis* species except of *Lb. plantarum,* are frequently encouraged in the fermented foods. *Lb. pantheris* isolated from fermented beet (Nguyen et al., 2013), tea leaves (Tanasupawat, Pakdeeto, Thawai, Yukphan, & Okada, 2007) and cereal product (Oguntoyinbo, Tourlomousis, Gasson, & Narbad, 2011) is one of new lactobacilli species. Again, it was reported that reuterin can be produced by *Lb. coryniformis* which is one of the less studied Lactobacilli in the literature, and it is frequently encountered in the fermented vegetable products (Martín et al., 2005). In this research, we also identified these recently reputed lactobacilli in gilaburu juices (Table 2 and 3).

3.3. Functional properties and probiotic potentials

A total of 40 strains from 9 LAB species were selected to determine functional and probiotic potentials in the present study. Selected LAB strains are given in Table 4. Half of selected strains were *Lb. plantarum.* One strain was selected from each of *Lb. buchneri* and *Leu. pseudomesenteroides*, while two strains of *Lb. hordei*, *Lb. coryniformis*, *Lb. paraplantarum* and *Leu. mesenteroides* were selected to determine probiotic potentials. In the results, *Lb brevis*, *Lb. buchneri, Leu. mesenteroides* and *Leu. pseudomesenteroides* produced gas from glucose, and *Lb. brevis* and *Lb. buchneri* produced ammonia as well. Except these species the selected LAB strains did not have neither gas nor ammonia production (Table 5). These strains are known to be heterofermentative LAB and therefore, they can produce products such as CO_2, acetic acid, ethanol, mannitol from hexose sugars as well as lactic acid(McDonald, Mcfeeters, Daeschel, & Fleming, 1987).

Table 4
Acid production properties of selected LAB strains.

Strains	Isolate no	Acid production (pH-time (h))			
		0th	6th	12th	24th
Lb. casei	G1a	5.64	5.59	5.47	4.08
Lb. casei	G1b	5.64	5.56	5.39	4.04
Lb. paraplantarum	G2a	5.64	5.46	4.95	3.86
Lb. paraplantarum	G2b	5.64	5.54	5.31	3.96
Leu. mesenteroides	G3a	5.64	5.52	5.17	4.40
Leu. mesenteroides	G3d	5.64	5.52	5.20	4.41
Lb. coryniformis	G4a	5.64	5.52	5.38	4.14
Lb. coryniformis	G4d	5.64	5.56	5.38	4.21
Lb. plantarum	G5a	5.64	5.56	5.32	3.86
Lb. plantarum	G5d	5.64	5.56	5.18	3.83
Lb. plantarum	G6a	5.64	5.55	5.23	3.91
Lb. brevis	G6d	5.64	5.57	5.47	4.14
Lb. plantarum	G7a	5.64	5.58	5.25	3.86
Leu. pseudomesenteroides	G7f	5.64	5.57	5.29	3.84
Lb. plantarum	G8a	5.64	5.56	5.26	3.84
Lb. plantarum	G8c	5.64	5.52	5.15	3.84
Lb. plantarum	G9a	5.64	5.50	5.15	3.85
Lb. casei	G9f	5.64	5.52	5.15	3.82
Lb. plantarum	G10a	5.64	5.44	4.89	3.79
Lb. plantarum	G10j	5.64	5.52	5.30	4.21
Lb. plantarum	G11a	5.64	5.50	5.07	3.82
Lb. plantarum	G11d	5.64	5.50	5.08	3.81
Lb. plantarum	G12a	5.64	5.55	4.99	3.84
Lb. brevis	G12f	5.64	5.54	5.38	4.93
Lb. hordei	G13a	5.64	5.53	5.26	4.11
Lb. hordei	G13f	5.64	5.56	5.29	4.13
Lb. plantarum	G14a	5.64	5.51	5.01	3.84
Lb. plantarum	G14f	5.64	5.47	4.97	3.82
Lb. brevis	G15a	5.64	5.48	5.37	4.90
Lb. plantarum	G15b	5.64	5.48	5.05	3.80
Lb. plantarum	G16a	5.64	5.47	5.15	4.08
Lb. casei	G16r	5.64	5.52	5.23	4.11
Lb. casei	G17a	5.64	5.53	5.29	4.04
Lb. buchneri	G17b	5.64	5.59	5.50	5.24
Lb. casei	G18b	5.64	5.51	5.24	3.92
Lb. plantarum	G18f	5.64	5.55	5.33	3.83
Lb. plantarum	G19a	5.64	5.44	4.94	3.91
Lb. plantarum	G19e	5.64	5.51	5.15	3.81
Lb. casei	G20a	5.64	5.53	5.32	3.95
Lb. plantarum	G20c	5.64	5.54	5.16	3.80

Lb: *Lactobacillus*, *Leu*: *Leuconostoc*, G1a....G20c: The isolates no of LAB strains isolated from fermented gilaburu.

Table 5
Some functional and probiotic characteristics of selected LAB strains.

Strains	GP	AP	Lactate isomers	Bile salt (%)		pH		Temperature (°C)		NaCl (%)	
				0.15	0.3	2.5	3.5	15	45	2	4
Lb. plantarum	0/20	0/20	DL	20/20	20/20	20/20	20/20	20/20	0/20	20/20	20/20
Lb. casei	0/7	0/7	L (+)	7/7	7/7	4/7	6/7	7/7	0/7	7/7	7/7
Lb. brevis	3/3	3/3	DL	3/3	3/3	2/3	3/3	3/3	0/3	3/3	3/3
Leu. mesenteroides	2/2	0/2	D (−)	2/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
Lb. coryniformis	0/2	0/2	DL	2/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
Lb. paraplantarum	0/2	0/2	DL	2/2	2/2	2/2	2/2	2/2	0/2	2/2	2/2
Lb. hordei	0/2	0/2	L (+)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Leu. pseudomesenteroides	1/1	0/1	D (−)	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
Lb. buchneri	1/1	1/1	DL	1/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1

Lb: *Lactobaillus*, *Leu*: *Leuconostoc*, GP: Gas production, AP: Ammonia production.

Lactate isomers from the selected LAB were determined as L(+), DL and D(−) (Table 5). Most of them were DL isomers, while *Lb. casei, Lb. hordei, Leu. mesenteroides* and *Leu pseudomesenteroides* were L(+), L(+), D(−) and D(−), respectively. Acid production ability of selected LAB strains was determined by measuring the pH values in 24 h (Table 4). The pH values of the strains were about pH 4 at the end of 24 h. As can be seen in Table 5, acid production ability of two *Lb. brevis* (G12f and G15a) and *Lb buchneri* strains were not good enough and the pH values for most of the strains were lower than pH 4.

Some functional and probiotic characteristics of the LAB strains were presented in Table 5. For this purpose, bile resistance, pH tolerance, and growth ability at different temperatures and NaCl concentrates of selected LAB strains were tested. To test the viability of the LAB strains at low pH, (pH 2.5 and 3.5) were also studied. All *L plantarum* strains tested grew at pH 2.5 and 3.5. However, three of *Lb. casei* (G1a, G1b, G16r) strains, each one of *Lb. brevis* G15a and *Lb. buchneri* G17b strain could not grow at pH 2.5, while one *Lb. casei* G16r and *Lb. buchneri* G17b strain could not grow at pH 3.5. Cebeci and Gürakan (2003) reported that some strains of *Lb. plantarum* tested can grow in acidified MRS agar (pH 3.5) after 96 h. Tolerance to low pH is one of the most important criteria to accept the microorganisms it as a probiotic property. Since the probiotic microorganisms should be able to survive at the pH values of the human gastrointestinal tract(Salminen et al., 1998). Fruits are important nutrition sources for LAB due to their rich nutrition components such as carbohydrate, mineral and nitrogen compounds. Additionally, the natural low pH environment of the fruit juices promotes LAB to grow (Naeem et al., 2012). Also, LAB can survive passing through the digestive tract since it is resistant and can grow at low pH values.

While all the selected LAB grew at 15 °C, only two *Lb. hordei* strains could be able to grow at 45 °C. This result shows the thermophilic properties of *Lb. hordei* strains. All the selected LAB species did not have any problem to grow at both 2 and 4% NaCl concentrations.

Bile resistance of microorganisms is also one of the most important criteria to determine their probiotic potential(Ibrahim & Bezkorovainy, 1993). The microorganisms that are resistant to bile salt can survive and grow in the natural bile content of the animal and human gastrointestinal tract (Psomas et al., 2001). All the selected LAB strains showed resistance against bile salt and grew at 0.15 and 0.3% bile salt concentrations. This means that all the selected LAB species could be potential probiotic bacteria. Similar to our study, some *Lb. plantarum* strains were determined to be resistance against high bile salt in some studies (Adamberg et al., 2014; García-Ruiz et al., 2014; Peres et al., 2014).

Again, hydrophobicity is one of the important parameter to determine probiotic potential of bacteria. The adhesion of microorganisms to hydrocarbons such as chloroform, ethyl acetate, xylene, toluene and hexadecane has been widely used to measure the cell surface hydrophobicity of LAB's (Divya, Varsha, & Nampoothiri, 2012; Vinderola & Reinheimer, 2003). The cell hydrophobicity properties of selected LAB strains are shown in Fig. 2. The cell hydrophobicity degree of the strains ranged from 0.5 to 87.5%. Nine of the LAB isolates including 5 *Lb. plantarum* (G5a, G5b, G15b, G19a and G19e), 3 of *Lb. brevis* (G6d, G12f and D15a) and 1 of *Lb. casei* (G20a) showed the higher hydrophobicity properties. Moreover, the highest cell hydrophobicity values were found with *Lb. casei* (G20a) and *Lb. plantarum* (G19e) as 87.5 and 86.0%, respectively. However, the hydrophobicity values were quite low (<10%) for 5 *Lb. plantarum* (G8a, G8c, G14f, G16a and G20c) strains, 1 *Lb. paraplantarum* (G2b), 1 *Lb. coryniformis* (G4d), 1 *Lb. casei* (G9f) and 1 *Lb. hordei* (G13a) strain. Meira, Helfer, Velho, Lopes, and Brandelli (2012) reported that the highest hydrophobicity was in *Lb. brevis* strain (88%) which isolated from Brazilian ovine cheese. However, Tamang, Tamang, Schillinger, Guigas, and Holzapfel (2009) reported that the hydrophobicity degrees of *Lb. brevis* and *Lb. plantarum* were 94 and 94.5%, respectively. In another study, the highest hydrophobicity was determined for *Lb. acidophilus* and *Bifidobacterium bifidium* as 67 and 64%, respectively (Vinderola & Reinheimer, 2003). It is concluded that the cell hydrophobicity degrees of LAB changes according to the LAB strains.

Antimicrobial activity test results of selected LAB strains are given in Table 6. While *Lb. brevis* did not show any antimicrobial activity against the any bacteria tested, *Leu. mesenteroides* (G3a and G3b), *Leu. pseudomesenteroides* (G7f), *Lb. casei* (G17a) and *Lb. buchneri* (G17b) strains showed antimicrobial activity against only *L. monocytogenes*. However, antimicrobial activity of some *Lb. plantarum* (G5a, G5b, G11a, G11d, G12 etc.) strains was quite high against indicator bacteria tested (Table 6). While *L. monocytogenes* and *B. cereus* were the most

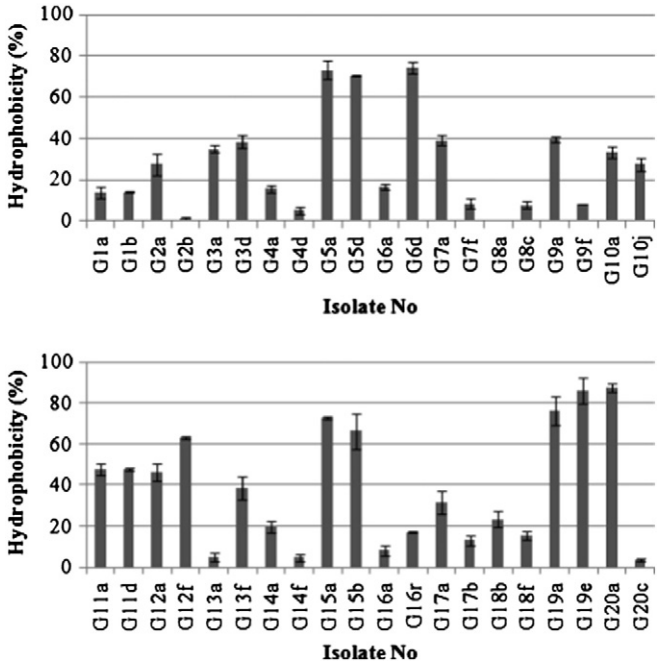

Fig. 2. Hydrophobicity (%) degrees of selected LAB strains. The isolate no of LAB strains stated in the Table 4.

Table 6
Antimicrobial activity of selected LAB strains against some pathogenic bacteria (inhibition zone, mm).

Isolate No	BC	EC	EC7	LM	ST	SA	YE
G1a	10.68 ± 0.54	–	8.97 ± 0.68	9.42 ± 2.07	8.32 ± 0.18	–	7.36 ± 0.55
G1b	11.67 ± 1.05	–	9.33 ± 2.11	10.53 ± 0.17	8.55 ± 1.12	–	6.98 ± 0.49
G2a	8.81 ± 0.95	8.45 ± 0.66	9.19 ± 0.34	18.24 ± 3.19	9.18 ± 1.06	–	–
G2b	8.57 ± 1.29	8.12 ± 0.37	8.98 ± 0.59	16.87 ± 1.94	10.25 ± 0.82	–	–
G3a	–	–	–	18.47 ± 0.82	–	–	–
G3d	–	–	–	17.30 ± 0.61	–	–	–
G4a	6.73 ± 0.27	–	–	14.11 ± 2.21	8.23 ± 0.47	–	–
G4d	9.46 ± 1.86	–	–	12.30 ± 0.76	9.38 ± 0.41	–	–
G5a	9.57 ± 0.76	8.99 ± 0.51	–	18.50 ± 2.14	10.70 ± 1.51	–	8.33 ± 0.77
G5d	10.45 ± 0.22	–	–	15.42 ± 2.74	7.31 ± 0.65	–	7.90 ± 1.14
G6a	7.52 ± 0.80	–	8.71 ± 0.62	15.87 ± 0.53	–	–	9.52 ± 0.35
G6d	–	–	–	–	–	–	–
G7a	10.96 ± 1.20	–	8.43 ± 0.64	11.08 ± 1.18	8.23 ± 0.66	12.26 ± 1.16	–
G7f	–	–	–	11.98 ± 2.18	–		–
G8a	10.14 ± 1.07	–	–	13.30 ± 0.86	–	9.52 ± 1.29	8.10 ± 0.91
G8c	11.99 ± 1.39	–	–	13.63 ± 2.93	–	–	7.93 ± 1.58
G9a	9.05 ± 1.39	–	–	8.31 ± 1.03	–	–	–
G9f	9.17 ± 0.39	–	–	11.47 ± 1.75	–	–	–
G10a	9.42 ± 0.71	–	8.50 ± 0.62	14.95 ± 2.66	–	–	–
G10j	9.32 ± 0.98	–	7.41 ± 0.66	9.84 ± 1.50	–	–	–
G11a	10.26 ± 1.72	–	–	15.32 ± 2.13	–	11.03 ± 1.49	10.62 ± 0.64
G11d	10.85 ± 1.60	–	–	18.42 ± 3.58	–	9.63 ± 1.10	9.62 ± 0.25
G12a	8.33 ± 0.76	9.00 ± 0.59	–	18.03 ± 1.91	–	9.65 ± 1.24	9.27 ± 1.45
G12f	–	–	–	–	–	–	–
G13a	8.18 ± 0.85	–	–	12.77 ± 1.46	–	–	–
G13f	8.38 ± 0.56	–	–	10.78 ± 0.69	–	–	–
G14a	9.07 ± 0.62	–	–	15.80 ± 1.48	–	9.23 ± 1.02	8.45 ± 1.83
G14f	9.03 ± 1.68	–	–	13.90 ± 2.35	–	8.67 ± 0.69	7.57 ± 0.69
G15a	–	–	–	–	–	–	–
G15b	9.36 ± 1.81	–	9.35 ± 2.02	13.72 ± 3.83	7.80 ± 0.49	–	8.42 ± 0.23
G16a	9.86 ± 1.78	–	–	16.17 ± 2.77	–	–	8.18 ± 1.05
G16r	7.95 ± 0.90	–	–	14.47 ± 0.88	–	–	8.13 ± 0.20
G17a	–	–	–	13.17 ± 2.04	–	–	–
G17b	–	–	–	9.01 ± 1.17	–	–	–
G18b	9.29 ± 0.50	–	–	13.15 ± 2.28	–	–	10.33 ± 0.72
G18f	10.20 ± 1.35	–	7.76 ± 0.45	15.34 ± 1.44	8.55 ± 0.04	–	10.32 ± 1.77
G19a	11.11 ± 1.44	–	7.79 ± 0.33	11.33 ± 0.90	–	–	8.26 ± 0.87
G19e	12.85 ± 0.74	–	6.95 ± 0.52	15.78 ± 1.36	–	–	8.80 ± 1.39
G20a	9.74 ± 0.40	–	7.53 ± 0.83	12.80 ± 2.98	–	–	7.26 ± 0.78
G20c	12.16 ± 0.44	–	8.34 ± 0.71	13.99 ± 0.45	–	–	10.38 ± 0.87

BC: *B. cereus*, EC: *E.coli*, EC7: *E.coli* O157:H7, LM: *L. monocytogenes*, ST: *S. typhimurium*, SA: *S. aureus*, YE: *Y. enterocolitica*,–: No inhibition zone, G1a…G20c: The isolates no of LAB strains stated in the Table 4.

sensitive bacteria against the selected LAB species, *E. coli* and *S. aureus* showed the most resistant. Four and 7 of LAB showed inhibitor effect against *E. coli* and *S. aureus*, respectively. Antimicrobial activity of LAB can show variations based on the strains. Some LAB may produce antimicrobial products including bacteriocin (nisin), hydrogen peroxide, diacetyl, ethanol and organic acids such as lactic, acetic, formic, benzoic, phenyllactic acid and caproic acid(Leroy & De Vuyst, 2004). Antimicrobial activity of LAB may differ depending on the source isolated and indicator microorganisms used. Çakır (2010) reported that six *Lb. plantarum* strains isolated from naturally fermented herbs showed different antimicrobial activities against some pathogen bacteria. In the same work, *Lb. brevis* showed strong activity against *L. monocytogenes*. However, *Lb. brevis* has shown no antimicrobial activity against *L. monocytogenes* in our work.

Resistance of selected LAB isolates against some antibiotics is shown in Table 7. All the LAB strains tested had resistance against 3 of the antibiotics kanamycin, streptomycin and vancomycin (Table 7). These antibiotics could not show any inhibition zones on any of the LAB strains tested. *Lb. hordei* was the most sensitivity LAB strain against ampicillin, chloramphenicol, penicillin, erythromycin and tetracycline hydrochloride. However, some *Lb. plantarum* (especially G7a and G10j) strains were quite resistant to the antibiotics (Table 7). Our results were similar to the results of previous studies, where *Lactobacillus* sp. and *Leuconostoc* sp. were sensitive against ampicillin and penicillin, and resistant to kanamycin and vancomycin (Argyri et al., 2013; Solieri, Bianchi, Mottolese, Lemmetti, & Giudici, 2014). Nguyen, Kang, and Lee (2007) reported *Lb. plantarum* PH04 that is a LAB isolated from infant feces was resistant to chloramphenicol, penicillin, kanamycin, tetracyline, while it was sensitive to erythromycine and ampicillin. These results are different from our results. However, antibiotic resistance or sensitivity of *L. plantarum* tested in our study was similar to those of Zhou, Pillidge, Gopal, and Gill (2005). They determined that probiotic *Lb. plantarum* was resistant against streptomycin, kanamycin and vancomycin. In another study, resistance and sensitivity of *Lb. plantarum* strains were reported as different against penicillin, vancomycin, ampicillin and tetracycline (Cebeci & Gürakan, 2003). Overall, these results show that resistance or sensitivity of LAB may vary based on the strains.

4. Conclusion

As a conclusion, LAB microbiota of traditional fermented gilaburu (European cranberrybush) juice was first time identified and characterized in this study. *Lb. plantarum* was the predominant LAB species. Other than this strain, 11 different LAB strains were determined in the fermented gilaburu juice. The results of current study showed also that some LAB strains might be used as probiotic bacteria. Some of *Lb. plantarum* strains showed quite high hydrophobicity, antimicrobial activity and bile salt resistance. Fermented gilaburu juice may also be considered as a functional food. Since, it contains some antioxidant compounds at high levels like phenolic acids, flavonoids, ascorbic acid and anthocyanins. Moreover, due to the high numbers of LAB in the fermented gilaburu juice, it may be considered as a possible probiotic

Table 7
Resistance of selected LAB strains against antibiotics (inhibition zone, mm).

Isolate No	AMP	C	E	K	P	S	TE	VA
G1a	14.84 ± 0.66	18.25 ± 1.36	20.63 ± 0.71	–	20.94 ± 0.94	–	27.15 ± 0.83	–
G1b	16.37 ± 0.86	21.30 ± 0.44	18.43 ± 0.11	–	20.49 ± 0.18	–	27.23 ± 1.68	–
G2a	19.30 ± 1.45	21.06 ± 0.27	17.50 ± 0.06	–	21.88 ± 0.23	–	15.77 ± 0.75	–
G2b	20.43 ± 1.00	23.67 ± 1.94	19.57 ± 0.23	–	18.28 ± 1.64	–	18.25 ± 1.08	–
G3a	17.62 ± 0.07	15.89 ± 1.05	17.41 ± 0.48	–	17.02 ± 0.04	–	18.73 ± 0.64	–
G3d	16.61 ± 0.46	18.56 ± 0.49	16.82 ± 0.84	–	17.61 ± 0.69	–	19.37 ± 0.58	–
G4a	17.03 ± 0.52	24.53 ± 2.52	17.91 ± 0.69	–	21.56 ± 1.90	–	24.55 ± 0.42	–
G4d	23.92 ± 0.88	31.08 ± 0.81	20.80 ± 0.43	–	26.87 ± 0.25	–	25.57 ± 0.85	–
G5a	19.78 ± 0.71	17.65 ± 1.01	16.08 ± 0.40	–	19.44 ± 0.98	–	16.30 ± 0.22	–
G5d	18.74 ± 0.41	18.19 ± 0.26	17.69 ± 0.72	–	17.56 ± 1.77	–	15.88 ± 0.43	–
G6a	19.87 ± 0.67	20.63 ± 0.03	17.44 ± 1.07	–	17.88 ± 1.66	–	18.21 ± 1.78	–
G6d	17.26 ± 0.33	22.07 ± 1.15	17.11 ± 0.36	–	17.89 ± 0.95	–	15.95 ± 1.08	–
G7a	19.91 ± 1.00	18.48 ± 0.62	15.66 ± 0.16	–	18.75 ± 0.86	–	13.48 ± 0.45	–
G7f	18.55 ± 0.52	19.14 ± 0.12	16.40 ± 0.23	–	17.92 ± 1.00	–	14.62 ± 0.60	–
G8a	17.80 ± 1.37	17.88 ± 1.05	16.64 ± 0.43	–	15.95 ± 0.39	–	15.34 ± 0.80	–
G8c	21.02 ± 0.21	18.72 ± 0.66	16.87 ± 0.33	–	18.72 ± 1.18	–	16.07 ± 1.03	–
G9a	22.00 ± 1.94	21.24 ± 0.71	16.80 ± 0.42	–	16.49 ± 0.69	–	14.58 ± 0.57	–
G9f	19.60 ± 0.38	19.85 ± 0.59	15.56 ± 0.43	–	18.02 ± 0.47	–	16.38 ± 0.43	–
G10a	21.04 ± 1.57	19.78 ± 0.34	15.72 ± 0.42	–	19.09 ± 0.99	–	15.04 ± 0.12	–
G10j	18.20 ± 0.49	16.50 ± 0.66	14.68 ± 0.66	–	11.22 ± 0.57	–	13.85 ± 0.53	–
G11a	21.28 ± 1.32	18.86 ± 0.27	15.71 ± 0.60	–	21.74 ± 0.78	–	15.90 ± 0.72	–
G11d	21.22 ± 0.62	19.39 ± 0.94	16.14 ± 0.25	–	20.13 ± 1.18	–	13.74 ± 1.01	–
G12a	22.07 ± 0.87	19.17 ± 0.69	16.70 ± 0.43	–	19.11 ± 1.24	–	15.55 ± 0.78	–
G12f	17.88 ± 1.03	23.01 ± 0.64	18.68 ± 0.42	–	15.77 ± 0.28	–	17.16 ± 0.49	–
G13a	24.60 ± 1.45	38.98 ± 0.62	26.44 ± 1.26	–	35.92 ± 0.57	–	35.06 ± 0.92	–
G13f	25.54 ± 1.48	32.14 ± 0.52	24.94 ± 0.22	–	21.50 ± 0.60	–	32.11 ± 1.56	–
G14a	21.92 ± 0.54	19.58 ± 0.76	17.38 ± 0.86	–	19.59 ± 1.97	–	16.27 ± 0.30	–
G14f	23.53 ± 0.07	22.37 ± 0.24	17.36 ± 1.59	–	14.85 ± 0.60	–	19.48 ± 0.84	–
G15a	20.49 ± 1.10	25.48 ± 0.13	20.40 ± 1.42	–	13.04 ± 0.95	–	16.62 ± 0.30	–
G15b	21.97 ± 1.27	19.53 ± 0.37	16.89 ± 0.55	–	18.13 ± 0.67	–	16.01 ± 0.17	–
G16a	21.25 ± 0.44	21.34 ± 0.64	16.81 ± 0.66	–	13.35 ± 0.31	–	14.04 ± 0.13	–
G16r	23.98 ± 1.75	22.56 ± 0.39	16.64 ± 1.27	–	14.09 ± 0.59	–	13.73 ± 1.80	–
G17a	20.88 ± 0.95	26.13 ± 1.60	22.67 ± 0.31	–	19.69 ± 0.05	–	25.55 ± 1.27	–
G17b	25.22 ± 0.11	28.96 ± 0.16	28.34 ± 1.19	–	29.21 ± 1.47	–	20.68 ± 1.51	–
G18b	20.18 ± 0.42	24.20 ± 0.49	21.38 ± 0.03	–	23.29 ± 1.12	–	27.50 ± 1.29	–
G18f	19.61 ± 1.51	22.86 ± 0.38	18.42 ± 2.23	–	19.02 ± 0.13	–	16.26 ± 0.37	–
G19a	21.17 ± 0.98	19.62 ± 0.06	16.58 ± 0.81	–	19.43 ± 0.70	–	15.67 ± 1.03	–
G19e	21.74 ± 0.74	20.67 ± 0.69	18.02 ± 0.54	–	19.25 ± 1.45	–	15.42 ± 0.73	–
G20a	19.55 ± 0.64	21.35 ± 0.44	21.36 ± 0.94	–	20.98 ± 0.22	–	26.97 ± 2.45	–
G20c	19.12 ± 0.87	20.08 ± 1.01	17.93 ± 0.18	–	18.04 ± 1.19	–	17.21 ± 1.21	–

Amp: Ampicillin, C: Chloramphenicol, E: Erythromycin, K: Kanamycin, P: Penicillin, S: Streptomycin, TE: Tetracycline hydrochloride, VA: Vancomycin, —: No inhibition zone, G1a…G20c: The isolates no of LAB strains stated in the Table 4.

fruit juice. Further different tests measuring the ability of probiotic potential should be performed on these strains isolated from the gilaburu juices, and their health effects should also be studied. The isolated LAB strains, having high probiotic potential can be used to produce the probiotic gilaburu juice or some other fruit juices as well.

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References

Adamberg, S., Sumeri, I., Uusna, R., Ambalam, P., Kondepudi, K. K., Adamberg, K., et al. (2014). Survival and synergistic growth of mixed cultures of *Bifidobacteria* and lactobacilli combined with prebiotic oligosaccharides in a gastrointestinal tract simulator. *Microbial Ecology in Health and Disease*, *25* (23062 (1–9)).

Adiguzel, G. C., & Atasever, M. (2009). Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermented sausage. *Romanian Biotechnological Letters*, *14*(1), 4130–4138.

Aksoy, A., Guvensan, A., Akcicek, E., & Ozturk, M. (2004, September 7–9). Etnoecology of *Viburnum opulus* L. *Paper presented at the International Symposium on Medicinal Plants: Linkages Beyond National Boundaries, Islamabad-Pakistan*.

Anonymous (2003). *Highbush-cranberry, Viburnum opulus L. var. Americanum Ait.* USDA, NRCS, National Plant Data Center (http://Plant-Materials nrcs.usda.gov.).

Anonymous (2008). *Viburnum opulus*—European cranberrybush, University of Arkansas horticulture 3103 summary sheet. http://www.uark.edu/campus-resources/cotinus/plants3_html/vibuopulhtml

Anonymous (2011). U.S. Food and Drug Administration bacteriological analytical manual online. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071363.htm

Argyri, A. A., Zoumpopoulou, G., Karatzas, K. -A. G., Tsakalidou, E., Nychas, G. -J. E., Panagou, E. Z., et al. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiology*, *33*(2), 282–291.

Arici, M., Bilgin, B., Sagdic, O., & Ozdemir, C. (2004). Some characteristics of *Lactobacillus* isolates from infant faeces. *Food Microbiology*, *21*(1), 19–24.

Arici, M., & Coskun, F. (2001). Hardaliye: Fermented grape juice as a traditional Turkish beverage. *Food Microbiology*, *18*(4), 417–421.

Boehringer-Mannheim (1989). UV-method for the determination of L-lactic acid and D-lactic acid in foodstuffs and other materials. *Methods of biochemical analysis using single reagents, Boehringer-Mannheim GmbH* (pp. 78–81). Mannheim: Biochemica.

Çakır, İ. (2010). Antibacterial and antifungal activities of some lactic acid bacteria isolated from naturally fermented herbs. *Journal of Food, Agriculture & Environment*, *8*, 223–226.

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: A literature survey. *Critical Reviews in Microbiology*, *28*(4), 281–370.

Cebeci, A., & Gürakan, C. (2003). Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiology*, *20*(5), 511–518.

Chen, Y. -S., Wu, H. -C., & Yanagida, F. (2010). Isolation and characteristics of lactic acid bacteria isolated from ripe mulberries in Taiwan. *Brazilian Journal of Microbiology*, *41*, 916–921.

De Man, J. C., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*, *23*, 130–135.

Divya, J., Varsha, K., & Nampoothiri, K. (2012). Newly isolated lactic acid bacteria with probiotic features for potential application in food industry. *Applied Biochemistry and Biotechnology*, *167*(5), 1314–1324. http://dx.doi.org/10.1007/s12010-012-9561-7.

Duangjitcharoen, Y., Kantachote, D., Ongsakul, M., Poosaran, N., & Chaiyasut, C. (2008). Selection of probiotic lactic acid bacteria isolated from fermented plant beverages. *Pakistan Journal of Biological Sciences*, *11*, 652–655.

FAO. (1998). *Basic principles of fermentation fermented fruits and vegetables: A global perspective.* FAO agricultural services bulletin n. 134 (Roma, Italy.).

García-Ruiz, A., González de Llano, D., Esteban-Fernández, A., Requena, T., Bartolomé, B., & Moreno-Arribas, M. V. (2014). Assessment of probiotic properties in lactic acid bacteria isolated from wine. *Food Microbiology*, *44*, 220–225.

Ibrahim, S. A., & Bezkorovainy, A. (1993). Survival of bifidobacteria in the presence of bile salt. *Journal of the Science of Food and Agriculture*, *62*(4), 351–354. http://dx.doi.org/10.1002/jsfa.2740620407.

Kalantzopoulos, G. (1997). Fermented products with probiotic qualities. *Anaerobe*, *3*(2–3), 185–190.

Khedid, K., Faid, M., Mokhtari, A., Soulaymani, A., & Zinedine, A. (2009). Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. *Microbiological Research*, *164*(1), 81–91.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O. P., Leer, R., et al. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Sciences*, *100*(4), 1990–1995.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, *15*(2), 67–78.

Martín, R., Olivares, M., Marín, M. L., Xaus, J., Fernández, L., & Rodríguez, J. M. (2005). Characterization of a reuterin-producing *Lactobacillus coryniformis* strain isolated from a goat's milk cheese. *International Journal of Food Microbiology*, *104*(3), 267–277.

McDonald, L. C., Mcfeeters, R. F., Daeschel, M.A., & Fleming, H. P. (1987). A differential medium for the enumeration of homofermentative and heterofermentative lactic acid bacteria. *Applied and Environmental Microbiology*, *53*, 1382–1384.

Meira, S. M. M., Helfer, V. E., Velho, R. V., Lopes, F. C., & Brandelli, A. (2012). Probiotic potential of *Lactobacillus* spp. isolated from Brazilian regional ovine cheese. *Journal of Dairy Research*, *79*(01), 119–127.

Murray, M. B., Gurtler, J. B., Ryu, J. -H., Harrison, M.A., & Beuchat, L. R. (2007). Evaluation of direct plating methods to enumerate *Alicyclobacillus* in beverages. *International Journal of Food Microbiology*, *115*(1), 59–69.

Naeem, M., Ilyas, M., Haider, S., Baig, S., & Saleem, M. (2012). Isolation characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pakistan Journal of Botany*, *44*, 323–328.

Nellessen, J. E. (2006). *Viburnum opulus* L. var. americanum (Mill.) Ait. (American cranberrybush): *A technical conservation assessment. Prepared for the USDA Forest Service, Rocky Mountain Region, species conservation project, May 8, 2006.*

Nguyen, T. D. T., Kang, J. H., & Lee, M. S. (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. *International Journal of Food Microbiology*, *113*(3), 358–361.

Nguyen, D. T. L., Van Hoorde, K., Cnockaert, M., De Brandt, E., Aerts, M., Binh Thanh, L., et al. (2013). A description of the lactic acid bacteria microbiota associated with the production of traditional fermented vegetables in Vietnam. *International Journal of Food Microbiology*, *163*(1), 19–27.

Oguntoyinbo, F. A., Tourlomousis, P., Gasson, M. J., & Narbad, A. (2011). Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. *International Journal of Food Microbiology*, *145*(1), 205–210.

Pato, U., & Surono, I. S. (2013). Bile and acid tolerance of lactic acid bacteria isolated from tempoyak and their probiotic potential. *International Journal of Agricultural Technology*, *9*, 1849–1862.

Peres, C. M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M. R., et al. (2014). Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. *LWT - Food Science and Technology*, *59*(1), 234–246.

Psomas, E., Andrighetto, C., Litopoulou-Tzanetaki, E., Lombardi, A., & Tzanetakis, N. (2001). Some probiotic properties of yeast isolates from infant faeces and Feta cheese. *International Journal of Food Microbiology*, *69*(1–2), 125–133.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., et al. (2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology*, *132*(2–3), 79–90.

Sagdic, O., Aksoy, A., & Ozkan, G. (2006). Evaluation of the antibacterial and antioxidant potentials of cranberry (gilaburu, *Viburnum opulus* L.) fruit extract. *Acta Alimentaria*, *35*(4), 487–492.

Sagdic, O., Arici, M., & Simsek, O. (2002). Selection of starters for a traditional Turkish yayik butter made from yoghurt. *Food Microbiology*, *19*(4), 303–312.

Saguir, F. M., Campos, I. E. L., Maturano, C., & Manca de Nadra, M. C. (2009). Identification of dominant lactic acid bacteria isolated from grape juices. Assessment of its biochemical activities relevant to flavor development in wine. *International Journal of Wine Research*, *1*, 175–185.

Salminen, S., von Wright, A., Morelli, L., Marteau, P., Brassart, D., de Vos, W. M., et al. (1998). Demonstration of safety of probiotics — A review. *International Journal of Food Microbiology*, *44*(1–2), 93–106.

Sedat Velioglu, Y., Ekici, L., & Poyrazoglu, E. S. (2006). Phenolic composition of European cranberrybush (*Viburnum opulus* L.) berries and astringency removal of its commercial juice. *International Journal of Food Science & Technology*, *41*(9), 1011–1015.

Solieri, L., Bianchi, A., Mottolese, G., Lemmetti, F., & Giudici, P. (2014). Tailoring the probiotic potential of non-starter Lactobacillus strains from ripened Parmigiano Reggiano cheese by in vitro screening and principal component analysis. *Food Microbiology*, *38*, 240–249.

Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, *36*(1), 1–29.

Tamang, J. P., Tamang, B., Schillinger, U., Guigas, C., & Holzapfel, W. H. (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *International Journal of Food Microbiology*, *135*(1), 28–33.

Tanasupawat, S., Pakdeeto, A., Thawai, C., Yukphan, P., & Okada, S. (2007). Identification of lactic acid bacteria from fermented tea leaves (miang) in Thailand and proposals of *Lactobacillus thailandensis* sp. nov., *Lactobacillus camelliae* sp. nov., and *Pediococcus siamensis* sp. nov. *The Journal of General and Applied Microbiology*, *53*(1), 7–15.

Vinderola, C. G., & Reinheimer, J. A. (2003). Lactic acid starter and probiotic bacteria: A comparative "in vitro" study of probiotic characteristics and biological barrier resistance. *Food Research International*, *36*(9–10), 895–904.

Yien Ong, Y., Siang Tan, W., Rosfarizan, M., Chan, E. S., & Ti Tey, B. (2012). Isolation and identification of lactic acid bacteria from fermented red dragon fruit juices. *Journal of Food Science*, *77*(10), M560–M564.

Yilmaz, N., Yayli, N., Misir, G., Çoskunçelebi, K., & Karaoglu, S. (2008). Chemical composition and antimicrobial activities of the essential oils of *Viburnum opulus*, *Viburnum lantana* and *Viburnum orientala*. *Asian Journal of Chemistry*, *20*(5), 3324–3330.

Yuliana, N., & Dizon, E. I. (2011). Phenotypic identification of lactic acid bacteria isolated from tempoyak (fermented durian) made in the Philippines. *International Journal of Biology*, *3*, 145–152.

Zhou, J. S., Pillidge, C. J., Gopal, P. K., & Gill, H. S. (2005). Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *International Journal of Food Microbiology*, *98*(2), 211–217.