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Some Technological and Functional Properties of Lactic Acid Bacteria Isolated from Hardaliye

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ABSTRACT

Hardaliye is a lactic acid fermented beverage produced from red grape or grape juice with addition of crushed mustard seeds and benzoic acid and it is widely produced and consumed in the Thrace region of Turkey. The aim of this study was to determine the dominant lactic acid bacteria (LAB) species found in hardaliye and to investigate their technological properties related to probiotic action and potential use as a starter culture for production of hardaliye. For this aim; LAB were isolated from 28 hardaliye samples (23 hardaliye samples that were obtained from different regions of Kirklareli, Turkey and 5 hardaliye samples were produced by using traditional methods in laboratory conditions). After carrying out conventional and molecular biological methods, it was found that all LAB species isolated belonged to genus *Lactobacillus*. The dominant species in the microbiota was found to be *Lactobacillus plantarum* while around 98% of the isolates were similar to each other. Therefore, it was well understood that a small diversity of LAB strains played role during the fermentation process. The results of this study revealed that the isolates had the potential to be used as starter cultures in hardaliye production due to their antimicrobial effects and acid production capabilities.

Keywords: Hardaliye; *Lactobacillus*; Probiotic properties; Technological properties

Hardaliyeden İzole Edilen Laktik Asit Bakterilerinin Bazı Teknolojik ve Fonksiyonel Özellikleri

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ÖZET

Hardaliye; benzoik asit ve ezilmiş hardal tohumu ilavesiyle kırmızı üzüm ya da üzüm suyundan üretilen laktik asit fermentasyonuna uğramış bir içecektir ve yaygın olarak Türkiye'nin Trakya Bölgesi'nde üretilmekte ve tüketilmektedir. Bu çalışmanın amacı hardaliyedeki baskın laktik asit bakterisi türlerini belirlemek ve onların teknolojik ve probiyotik özellikleri ile hardaliye üretiminde starter kültür olarak potansiyel kullanımını araştırmaktır. Bu amaçla toplam 28 hardaliye örneğinden (Türkiye'de Kırklareli'nin farklı bölgelerinden toplanan 23 hardaliye örneği ve laboratuvar şartlarında geleneksel yöntemler kullanılarak üretilen 5 hardaliye örneği) laktik asit bakterileri (LAB) izole edilmiş ve tanımlanmıştır. Geleneksel ve moleküler biyolojik yöntemler uygulandıktan sonra, tüm LAB türlerinin *Lactobacillus* cinsine ait olduğu bulunmuştur. İzolatların yaklaşık % 98'i birbirine benzerken mikrobiyotadaki baskın tür *Lactobacillus plantarum* olarak bulunmuştur. Bundan dolayı, fermentasyon sırasında etkili LAB çeşitliliğinin düşük olduğu anlaşılmıştır. LAB'nin antimikrobiyal etkileri ve asit üretim yetenekleri göz önünde bulundurulduğunda, bu çalışmanın sonuçları izolatların hardaliye üretiminde kullanılmak için starter kültür olarak potansiyele sahip olduklarını göstermiştir.

Anahtar Kelimeler: Hardaliye; *Lactobacillus*; Probiyotik özellikler; Teknolojik özellikler

1. Introduction

Lactic acid bacteria (LAB) are the main technological group of microorganisms that are responsible for the production of fermented products with a great economic importance.

Fermentation is one of the oldest methods for increasing the shelf life of food products (Battock & Azam-Ali 1998). Today, LAB have attracted attention of researches due to their essential role in most fermented foods and their abilities to produce various antimicrobial compounds as well as their probiotic properties (Temmerman et al 2003) including antitumoral activity (De Vuyst & Degeest 1999; Ostile et al 2003), reduction of serum cholesterol (Desmazeaud 1996; Jackson et al 2002), alleviation of lactose intolerance (De Vrese et al 2001), stimulation of the immune system (Isolauri et al 2001) and stabilization of gut microbiota. The LAB are food grade microorganisms and are in generally recognized as safe (GRAS) status that increases their technological potentials in food industry (Trias et al 2008). LAB are widely used in the production of dairy products such as yohurts and fermented milks because of their health benefits. Recently, researches have increased for the isolation of LAB from non-dairy products.

Hardaliye is a lactic acid fermented beverage produced from red grape or grape juice with the addition of crushed mustard seeds, benzoic acid. It is widely produced in the Thrace region of Turkey. Grape microbiota plays the major role in the quality of hardaliye. Hardaliye is a valuable, beneficial traditional beverage that is consumed with pleasure and has been produced for centuries in this region. Hardaliye offers a very nice opportunity for the consumption of grape in cold seasons.

Since consumers demand for more varieties of food products in the marketplace, many indigenous and traditional foods have been industrially produced. Hardaliye is one of the traditional products that have potential to become widespread in the market (Coşkun 2005). Therefore, we aimed to determine technological and functional properties of LAB isolated from hardaliye, as a potential functional product in this study.

2. Material and Methods

2.1. Materials

Twenty-three hardaliye samples were obtained from different households in Kirklareli province of Turkey and used for the isolation of LAB In this study. LAB strains were also isolated from five hardaliye samples that are produced under laboratory conditions using different grape varieties obtained from Tekirdağ Viticulture Research

Institute, namely Dingil Kara, Kara Üzüm, Cabernet Sauvignon, Izabella and Siyah Üzüm.

2.2. Methods

2.2.1. Production of hardaliye samples

Grapes were washed and pressed in oak barrels having a tap at the position 10 cm above the bottom of the barrel. Then 0.2% of the crushed raw mustard seed and 0.1% of benzoic acid were mixed with the grape and the mixture was fermented at room temperature (~22 ºC) for 7 days.

2.2.2. Isolation and identification of LAB

Serial dilutions of the fermented mixtures were prepared using Ringer solution and spreaded onto MRS plates. Then, the plates were incubated in anaerobic conditions at 30±1 ºC for 72 h (De Man et al 1960). Isolates were tested in terms of colony morphology, cell morphology, Gram staining, catalase reaction, gas formation from glucose, NH_4 production from arginine, growth in different salt concentrations (2% or 4%) and growth at different temperatures (15 °C or 45 °C) (Schillinger & Lucke 1989). Total 50 isolates were selected and tested in API 50 CHL kits (Bio Merieux, France) to determine the ability to use carbohydrates.

2.2.3. Genotypic identification of LAB

2.2.3.1. Isolation of genomic DNA

For DNA isolation, bacterial strains were grown overnight in MRS broth at 30 °C. An aliquot of 1 mL bacterial suspension was centrifuged at 14,000 *g* for 20 min and from each bacterial culture supernatant weight by weighing about 20 mg was obtained from biomass (Pitcher et al 1989). DNA isolation was performed using commercial isolation kit (Quiagen, Taiwan).

2.2.3.2. The determination of DNA concentration and preparation of working solution

The amount of DNA was determined using spectrophotometer (Hitachi U-5100 UV/VIS, Japan) at 260 nm wavelength. The resulting amount of the genetic material, protein was determined by measurement carried out at 280 nm (Marmur 1961).

2.2.3.3. Genotypic identification of isolates

16s rRNA gene of the isolates were amplified by following primers: forward primer, 5'-CCGTCAATTCCTTTGAGTTT- 3' and reverse primer, 5'-CCGTCAATTCCTTTGAGTTT- 3' (Beasley & Saris 2004).

Amplification was achieved by programmable thermal controller, after a pre denaturation step at 94 ºC for 5 min, by the 30 incubation cycles of denaturation at 94 ºC for 45 s, primer annealing at 53 ºC for 1 min, and polymerization at 72 ºC for 1 min. Final DNA extension was performed at 72 ºC for 2 min. Sterile water was used as negative control.

Aliquots of PCR products (10 µL) were analyzed by electrophoresis (30 min at 100 V) through 1% (w v^{-1}) agarose gel in tris-acetate buffer (Meyers et al 1976). A 5 µL of a 1 kb DNA ladder marker was used as a size marker. Then amplification products were stained with ethidium bromide and analyzed by gel documentation system.

2.2.3.4. Genetic relationship by BOX-PCR

A PCR-reaction mixture (30 µL) containing 5 µL 5x Gitschier buffer (for 200 mL; 16.6 mL 1 M $(NH_4)_2SO_4$, 67 mL 1 M Tris-HCl (pH 8.8), 6.7 mL 1 M $MgCl_2$, 1.3 mL 0.5 M EDTA (pH 8.8) and 2.08 mL 14.4 M-mercapto-ethanol and was incorporated with 200 mL of distillated water), 2.5 µL DMSO, 1.25 µL BSA, 1.25 µL dNTP mix, 4 µL primer BOX AIR (5' CTA CGG CAA GGC GAC GCT GAC G-3'), 0.3 µL taq DNA polimerase, 11.7 µL sterile distilled water was added to 4 µL g-DNA (75 ng μL^{-1}) of each sample. PCR cycling conditions were 7 min at 95 ºC, 30 cycles of 1 min at 95 ºC, 1 min at 53 ºC and 8 min at 65 ºC and final step of 15 min at 65 ºC (Louws et al 1994; Norman et al 2003).

Aliquots of BOX-PCR products (10 µL) were analyzed by electrophoresis and visualized as described above.

2.2.3.5. DNA sequence analysis

LAB isolates isolated from hardaliye were analyzed to determine the structure of DNA. DNA purification of the samples and DNA sequence analysis were made by the company of IONTEK (Istanbul). Formed by the band patterns of each isolate was noted and analyzed using SPSS (Statistical Package for Social Sciences 2009, 18:0 Edition for Windows) and their genetic relationship rate was determined with dendogram. BLAST analysis was conducted according to Altschul et al (1997).

2.2.4. Determination of technological and functional properties of LAB isolates

Isolates were inoculated in MRS broth and incubated at 30 ^{o}C to test the acid production capabilities. pH measurement of cultures were made at the end of 3rd, 6th, 18th and 24th h periods during the incubation (Sağdıç et al 2002).

LAB strains were tested for their ability to produce hydrogen sulphide from Triple Sugar Iron agar (TSI, Oxoid). Sterilized TSI agar plate was inoculated by streaking the slant and incubated at 30 °C for 2 weeks. The reactions were examined daily for the formation of the blackness in the media (Lee & Simard 1984). For determination of hydrogen peroxide production capabilities, measurements were conducted at a wavelegth of 360 nm with a spectrophotometer (Toksoy 1996). The configuration of the lactic acid enantiomers was determined enzymatically using d-lactate and l-lactate dehydrogenase (Biopharm 2014).

Antibacterial activities were tested against *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922 and *E. coli* O157:H7 NCTC 12900 using agar diffusion method as described for LAB strains using 10^{7} cfu mL^{-1} concentrations of the tested strains (Toksoy 1996). In order to test bacteriocin and bacteriocin-like substance production capability of the isolates, *S. aureus* ATCC 25923, *L. monocytogenes* ATCC 7644, *S. enteritidis* ATCC 13076, *E. coli* ATCC 25922, *E. coli* O157:H7 NCTC 12900 were used (Toksoy 1996).

LAB isolates were subcultured in 10 mL of fresh MRS broth adjusted to different pH values (1 and 3) with HCl (3M) for the determination of acid tolerance after grown in MRS broth at 37 °C overnight (Hyronimus et al 2000)

Five antibiotic discs were used to determine the antibiotic resistance of LAB strains. These antibiotic discs were chloramphenicol (CHL), kanamycin (KAN), penicillin G (PEN), streptomycin (STR) and tetracyclin hydrochloride (TET) (Kheadr et al 2007). Results were evaluated according to the criteria of Clinical and Laboratory Standards Institute (CLSI 2012). The sensitivity of the strains to bile salts was determined according to the method described by Vinderola & Reinheimer (2003). Cell surface hydrophobicity was determined by the method of Perez et al (1998).

3. Results and Discussion

3.1. Phenotypic properties of LAB isolates

It was detected that as 28 isolates grown at 45 ^{o}C produced gas from glucose whereas no gas production was detected for 73 isolates grown at 15 ^{o}C. All isolates showed growth in 2% NaCl, except sample one. However, no growth was observed in 4% NaCl. Thirty isolates were able to produce NH_4 from arginine.

3.2. Carbohydrate metabolism (API 50 CHL), identification of LAB isolates

Phenotypic properties of *Lactobacillus* isolates, which were identified, were selected and API 50 CHL test was performed. According to API 50 CHL test, it was identified that 23 isolates (46%) were *Lactobacillus plantarum,* 20 isolates (40%) were *Lactobacillus pentosus,* 4 isolates (8%) were *Lactobacillus brevis* and the remaining 3 isolates (6%) were *Lactobacillus collinoides*.

As a result of API 50, 23 isolates (HR2, HR6, HR9, HR13, HR21, HR27, HR29, HR30, HR34, HR35, HR42, HR43, HR48, HR49, HR51, HR54,

HR60, HR64, HR77, HR88, HR91, HR93, HR96) were identified as *Lactobacillus plantarum*, 22 of the isolates (HR10, HR14, HR31, HR33, HR36, HR37, HR38, HR39, HR50, HR56, HR57, HR59, HR62, HR75, HR84, HR86, HR89, HR92, HR94, HR97) were identified as *Lactobacillus pentosus,* 4 of the isolates (HR1, HR22, HR26, HR40) were identified as *Lactobacillus brevis* and 3 of the isolates (HR88, HR90, HR95) identified as *Lactobacillus collinoides.*

3.3. Genotypic identification of isolates

Species of all the strains were identified based on the DNA sequence analysis results. In the results, 26 isolates (HR1, HR2, HR6, HR9, HR13, HR22, HR27, HR29, HR34, HR35, HR37, HR40, HR42, HR43, HR49, HR51, HR54, HR57, HR59, HR60, HR64, HR85, HR86, HR89, HR93, HR97) were identified as *Lactobacillus plantarum* with similarity indices from 92 to 99%, 17 of the isolates (HR10, HR26, HR31, HR33, HR36, HR38, HR39, HR62, HR75, HR84, HR88, HR90, HR91, HR92, HR94, HR95, HR96) were identified as *Lactobacillus brevis* with similarity indices from 90 to 99%, one isolate (HR50) were not identified. Identification results according to API50 and 16S riposomal RNA gene sequencing using NCBI BLASTn (Basic Local Alignment. Search Tool nucleotide).

16S riposomal RNA gene sequencing and BLAST search showed that twenty four isolates were identified as *Lactobacillus plantarum* NC_004567.2 (HR1, HR2, HR6, HR9, HR13, HR22, HR27, HR29, HR35, HR37, HR40, HR42, HR43, HR49, HR51, HR54, HR57, HR59, HR60, HR64, HR85, HR86, HR93, HR97), one isolate identified as *L. plantarum* AY_590777 (HR34), one isolated identified as *L. plantarum* CP012122.1 (HR89), fifteen isolates identified as *L. brevis* NC_008497.1 (HR10, HR26, HR31, HR36, HR38, HR39, HR62, HR84, HR88, HR90, HR91, HR92, HR94, HR95, HR96) and two *L. brevis* LC 062897.1 (HR33, HR75).

These findings supported the aim, which was the determination of the main LAB strains playing roles in hardaliye fermentation in which low numbers of hardaliye investigated in our study can also be explained with this aim.

3.3.1. Genetic relationship among LAB strains

BOX-PCR genotypic characterization of the isolates from occurring polymorphisms has been utilized. As shown in Figure 1, all strains generated bands of 2500 with BOX-PCR. Some of them, however, exhibited additional bands with sizes of 1000 bp (14 strains), 1200 bp (8 strains), 1700 bp (5 strains), 500 bp (2 strains) or 2000 bp (only one strain). BOX-PCR profiles of the strains generated two main clusters. The similarity level among strains determined with BOX-PCR was found as 98% (Figure 2).

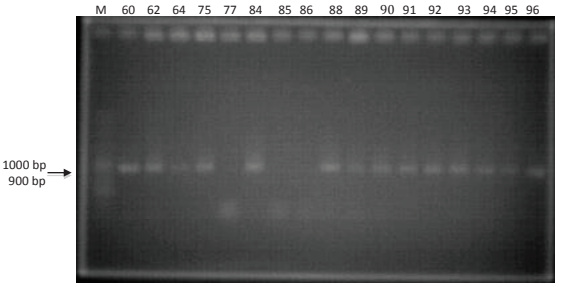

Figure 1- PCR amplification with 16S rRNA genes

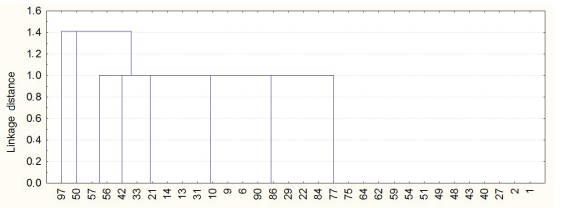

Figure 2- Cluster analysis of BOX-PCR

3.4. Technological and probiotic properties of LAB isolates

To test acid production ability, the isolates were inoculated one by one to tubes containing MRS broth and incubated at 30±1 °C. pH was measured at the end of 3rd, 6th, 18th and 24th hours of incubation period. pH of medium changed between 3.91 and 4.56 at the end of the incubation (24 h). Lactic acid production level of the isolates was found to be between 0.949 and 3.901 g L^{-1} at the end of the incubation period. None of the isolates tested produced hydrogen sulfide. Hydrogen peroxide

(H_2O_2) produced by isolates changed between 1.57-3.75 µg mL^{-1}. Only one strain produced L-lactate while the other isolates produced DL-lactate or D-lactate. Total lactic acid production of LAB isolates after 24 h was determined between 0.949 and 3.901 g L^{-1}.

Effects of LAB isolates on different pathogens are shown in Table 1. In general, the isolates showed the highest antibacterial effect on *S. aureus* while effect on *L. monocytogenes* and *E. coli* O157:H7 was generally lower than those of others. All isolates except HR27 showed inhibitory effects on the tested bacteria (Table 1).

None of the isolates produced a visible inhibition zone. Therefore, it was found that LAB isolates did not produce bacteriocin or bacteriocin-like substances.

Table 1- Antibacterial activity of LAB isolates (Zone diameter; mm)

Isolate numbers	*S. aureus*	*L. monocytogenes*	*S. enteritidis*	*E. coli*	*E. coli* O157:H7	*Isolate numbers*	*S. aureus*	*L. monocytogenes*	*S. enteritidis*	*E. coli*	*E. coli* O157:H7
HR1	28	9	13	15	6	HR49	24	8	9	9	7
HR2	25	11	13	13	7	HR50	26	8	7	8	8
HR6	23	10	13	12	6	HR51	27	7	4	9	8
HR9	24	9	12	14	8	HR54	26	8	5	10	8
HR10	23	8	11	12	7	HR56	34	9	18	15	14
HR13	24	8	12	12	8	HR57	29	5	11	13	11
HR14	27	9	12	11	9	HR59	28	7	13	13	10
HR21	25	9	11	10	10	HR60	26	8	12	12	12
HR22	30	7	9	10	11	HR62	27	6	12	11	7
HR26	28	8	9	11	8	HR64	29	8	13	13	12
HR27	26	8	10	11	-	HR75	29	8	13	12	11
HR29	26	9	11	11	10	HR77	28	6	15	12	11
HR30	24	9	10	10	9	HR84	29	7	13	13	11
HR31	25	8	8	10	9	HR85	29	8	11	13	13
HR33	32	9	10	9	9	HR86	26	8	10	11	9
HR34	27	8	11	9	8	HR88	25	6	9	12	9
HR35	29	8	10	10	9	HR89	28	-	11	14	7
HR36	29	6	11	10	8	HR90	30	9	13	12	8
HR37	31	8	9	9	7	HR91	26	8	11	9	4
HR38	26	9	6	9	6	HR92	27	8	5	8	7
HR39	28	11	7	11	8	HR93	26	6	3	59	7
HR40	28	9	7	12	9	HR94	25	7	5	11	7
HR42	26	5	8	12	8	HR95	25	6	5	9	5
HR43	26	8	7	7	7	HR96	24	7	5	6	4
HR48	26	8	7	10	8	HR97	25	9	12	12	8

S. aureus, ATCC 25923; *L. monocytogenes*, ATCC 7644; *S. enteritidis*, ATCC 13076; *E. Coli*, ATCC 25922; *E. Coli* O157:H7, NCTC 12900

Tolerances of the isolates against acidity were investigated in two different pH values (pH 1 and 3). None of the isolates could maintain their viability at pH 1 at the end of 180 min incubation while viability rate of the isolates changed between 37.39 and 90% at pH 3.

The viability of LAB strains isolated from different hardaliye samples was substantially influenced from tetracyclin hydrocloride, chloramphenicol and penicillin. Antibiotic resistance of the isolates is presented in the Table 2. As can be seen, 18% of the isolates showed resistance against tetracyclin hydrocloride. 78% of the isolates intermediate susceptible, 6% of the isolates susceptible against tetracyclin hydrocloride. None of the isolates showed resistance against chloramphenicol. 10% of the isolates intermediate susceptible, 90% of the isolates susceptible against chloramphenicol. 38% of the isolates showed resistance against penicillin. 38% of the isolates intermediate susceptible, 24% of the isolates susceptible against penicillin. All isolates resistance against kanamycin and streptomycin.

Isolates showed moderate tolerance to bile salts at 0.3% concentration. The isolates grew slowly in medium containing 0.5% and 1% bile salts while six isolates were not be able to grow in medium with 1% bile salts.

It is clear from the results that in spite of low levels, all isolates except for HR 6 had hydrophobicity

Table 2- Antibiotic resistances of LAB isolates

Isolate numbers	*TET*	*CHL*	*PEN*	*KAN*	*STR*	*Isolate numbers*	*TET*	*CHL*	*PEN*	*KAN*	*STR*
HR1	I	S	S	R	R	HR49	I	S	I	R	R
HR2	I	S	S	R	R	HR50	I	S	S	R	R
HR6	I	S	I	R	R	HR51	I	S	R	R	R
HR9	I	I	I	R	R	HR54	I	S	S	R	R
HR10	I	I	I	R	R	HR56	I	S	R	R	R
HR13	I	S	I	R	R	HR57	I	S	R	R	R
HR14	I	S	S	R	R	HR59	I	S	I	R	R
HR21	R	S	I	R	R	HR60	I	S	I	R	R
HR22	I	S	S	R	R	HR62	I	S	R	R	R
HR26	I	S	I	R	R	HR64	I	S	I	R	R
HR27	R	S	R	R	R	HR75	S	S	R	R	R
HR29	R	S	R	R	R	HR77	I	S	S	R	R
HR30	I	S	R	R	R	HR84	I	S	S	R	R
HR31	I	S	R	R	R	HR85	I	S	I	R	R
HR33	R	I	R	R	R	HR86	I	S	S	R	R
HR34	R	S	I	R	R	HR88	I	S	R	R	R
HR35	I	I	I	R	R	HR89	I	S	R	R	R
HR36	I	S	I	R	R	HR90	I	S	S	R	R
HR37	I	S	S	R	R	HR91	I	S	R	R	R
HR38	R	S	I	R	R	HR92	I	S	R	R	R
HR39	S	S	S	R	R	HR93	I	S	R	R	R
HR40	I	S	I	R	R	HR94	S	S	R	R	R
HR42	I	S	R	R	R	HR95	R	S	I	R	R
HR43	R	I	I	R	R	HR96	I	S	R	R	R
HR48	R	S	I	R	R	HR97	I	S	R	R	R

TET, tetracyclin hydrocloride (30 µg); CHL, chloramphenicol (30 µg); PEN, penicillin (10 U); KAN, kanamycin (30 µg); STR, streptomycin (10 µg) (Bioanalyse)

against xylene. Hydrophobicity value of the isolates changed between 1.01 and 15.82% suggesting some strains might adhere better than the others to the gastrointestinal tract, which is a crucial functional characteristic in probiotic action.

Lactic acid-producing ability of the isolates was partly similar to those reported by Arici et al (2004).

Hydrogen sulfide production capability of LAB isolates was not in accordance with those reported by Lee & Simard (1984) and Arici et al (2004). These studies showed that some LAB did not generate hydrogen sulfide and hydrogen sulfide production depends on the sulfite reductase and cysteine desulfohydrase enzyme activity of LAB strains (Fernandes et al 1987).

Toksoy et al (1999) found the H_2O_2 production ranged between 1.80-3.45 µg mL^{-1} while Raccach & Baker (1978) found as 0.85 µg mL^{-1}. In another study, it was concluded that *L. plantarum* isolates produced 0.59-0.65 µg mL^{-1} hydrogen peroxide (Beyatlı & Akbari 1996). In this research, hydrogen peroxide production of the isolates was similar to findings of Toksoy et al (1999) and higher than those of Raccach & Baker (1978) and Beyatlı & Akbari (1996), respectively. LAB produced different amounts of hydrogen peroxide, which can be related with their different oxygen oxidoreductase activities (Reinheimer et al 1990).

D- and L-lactate production capabilities of LAB isolates in our study were in accordance with those of Arici et al (2004).

Arici et al (2004) determined that LAB (*L. rhamnosus, L. paracasei* subsp. *paracasei, L. fermentum, L. buchneri, L. brevis, L. curvatus* and *Lactobacillus* sp.) isolated from the faeces of baby formed inhibition zone against most pathogenic bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 2392, *S. aureus* ATCC 28213). Aslım & Beyatlı (2000) investigated inhibition effect of *S. thermophilus* and *L. bulgaricus* by 5 units from yohurt starter cultures on two different *E. coli* (I and II) and *S. aureus* (I and II). Generally all the starter strains showed the highest effect on *S. aureus* II. Toksoy et al (1999) investigated the inhibition effect of 39 different *L. plantarum* isolates isolated from sucuks and sausages on *E. coli* K12, coagulase positive *S. aureus* 4-43 and mutant coagulase negative *S. aureus*. Two strains did not show inhibitory effect on *E. coli*, while others resulted in different inhibitory effect level. Four strains did not show inhibitory effect on *S. aureus* positive. Tambekar & Bhudata (2010) investigated antimicrobial effect of *Lactobacillus* strains perceived as leading probiotics among 48 *Lactobacillus* strains isolated from goat milk on *E. coli* (MTCC443), *Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae* (MTCC 2653), *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 734) and *Shigella flexneri* (MTCC 1457). LAB showed different inhibitory effects on the tested bacteria.

Acid tolerances of the LAB isolates were similar to those determined by Iñiguez-Palomares et al (2007).

Antibiotic resistances of LAB isolates were consistence with results isolated Arici et al (2004) in which LAB strains isolated from the faeces of baby were found to be resistant against kanamycin and streptomycin, whereas they were sensitive against amoxycillin, chloramphenicol, erythromycin, penicillin and tetracyclin. Xanthopoulos et al (2000) isolated *Lactobacillus paracasei* subsp. *paracasei, Lb. rhamnosus, Lb. acidophilus, Lb. gasseri* and *Lb. reuteri* from the faeces of baby (new born) and they detected that these isolates were sensitive against chloramphenicol, erythromycin, penicillin G and tetracycline. Tambekar & Bhudata (2010) reported that probiotic strains of *L. plantarum* (G95a and G96a) and *L. rhamnosus* (G119b) isolated from goat milk were resistant to tetracycline while *L. rhamnosus* (G92 and G99c) was sensitive to tetracycline. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections although presence of the antibiotic resistance genes in these strains can also be problem in some aspects. Their resistance to antibiotics clarifies their potential in minimizing the negative effects of antibiotic therapy on the host bacterial ecosystem should be considered (El-Naggar 2004).

Raibaud (1992) reported that even low concentrations of bile salts (*in vitro*) were able to inhibit the microorganisms. Gilliland et al (1984)

claimed that bile salt at 0.3% concentration was decisive on the determination of resistance of microorganisms against these salts.

Hydrophobicity capability of the LAB isolates was in accordance with that of Mishra & Prasad (2005) who examined hydrophobicity ability of *Lactobacillus casei* strains and found that xylene hydrophobicity was between 5.81% and 42.52%.

4. Conclusions

All of the LAB strains isolated from hardaliye samples belonged to genus *Lactobacillus* and *Lactobacillus plantarum* was the dominant species in the microbiota. Around 98% of the isolates were similar to each other, indicating that, diversity of the starter LAB during the fermentation was very low. The isolates were found to have potentials to be used as starter cultures in the production of hardaliye due to their technological and functional properties. The antimicrobial effects and acid production capabilities of the isolates support this idea. The largest gain of our study was the determination of effective LAB during the fermentation of hardaliye and revealing their technological and functional properties.

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