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Research Article

Investigation of Probiotic Features of Bacteria Isolated from Some Food Products

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Abstract

Probiotics are used as natural supplements for good health and treatment of various diseases. Probiotics affect health in a positive way due to their activities in the gastrointestinal tract. There is a growing interest in using probiotic bacteria for their protective effects against diseases and an emerging trend towards consuming healthy foods. The aim of the present study was to reveal species with alternative probiotic properties from some food products, which are already known to have probiotic properties and whose natural properties are preserved. Probiotic characteristics of isolated bacteria strains from 130 food samples which include 10 boza, 40 cheese, 20 kefir and 60 raw milk samples were microbiologically analyzed in the present study.A total 144 strains including 127 *Enterococcus faecium,* 7 *Lactobacillus plantarum,* 5 *Lactobacillus para-plantarum* and 5 *Lactobacillus brevis* were typed with characterizing by mass spectroscopy (MALDI-TOF MS) to have probiotic effects.Then, all the tests required to comply with the probiotic properties of these bacteria were applied sequentially. Of the 144 bacterial strains identified, only 35 were resistant to gastric pH. In the next step, only 8 isolates from 35 isolates were able to survive under bile salt conditions. It has been determined that only 6 of bile salt-resistant isolates have the hydrophobicity ability. The remaining 6 isolates were examined for antimicrobial resistance and the presence of extended-spectrum beta-lactamases (ESBL) resistance and ESBL were not detected. At the end of analysis, only 6 (4.1%) bacteria of 144 isolates were found to have probiotic properties. Three of them were *Lactobacillus brevis* isolated from boza and 3 of them were *Lactobacillus plantarum* species isolated from kefir. However, no probiotics could be isolated from other food samples such as milk and cheese. Therefore, the present study demonstrated that probiotic bacteria could be produced as an alternative to industrial probiotics through non-transgenic microorganisms isolated from natural food products such as kefir and boza.

Keywords: *Probiotic Bacteria, Boza, Milk, Cheese, Kefir, Probiotic properties*

Bazı Gıda Ürünlerinden İzole Edilen Bakterilerin Probiyotik Özelliklerinin Araştırılması

Özet

Probiyotikler, çesitli hastalıkların tedavisi ve sağlık için doğal takviyeler olarak kullanılırlar. Probiyotikler gastrointestinal sistemde yaptıkları faaliyetler sonucunda sağlığı olumlu yönde etkilemektedirler. Günümüzde hastalıklara karşı koruyucu etkileri ve sağlıklı gıdaların tüketimine yönelik yoğun ilginin ortaya çıkışına bağlı olarak probiyotik bakterilere ilgi artmıştır. Bu araştırmada probiyotik özellikleri olduğu bilinen ve doğal özellikleri korunmuş bazı gıdalardan probiyotik özelliklere sahip türlerin ortaya çıkartılması amaçlanmıştır. Araştırmamızda 10 boza, 40 peynir, 20 kefir ve 60 çiğ süt olmak üzere toplam 130 gıda örneği mikrobiyolojik bakımdan incelenmiştir. Sonuçta; 127 *Enterococcus faecium,* 7 *Lactobacillus plantarum,* 5 *Lactobacillus para-plantarum* ve 5 *Lactobacillus brevis* olmak üzere toplam 144 probiyotik etkisi gösterebilecek bakteri kütle spektofotometre (MALDI-TOF MS) ile karakterize edilerek tiplendirilmiştir. Daha sonra bu bakterilerin probiyotik özelliklere uygunluğu konusunda gerekli olan tüm testler sırasıyla uygulanmıştır. Karakterize edilen 144 izolattan sadece 35 'inin mide pH'sına dayanıklı olduğu saptanmıştır. Bir sonraki basamakta ise yine 35 izolatdan sadece 8'i safra tuzu koşullarında canlılıklarını devam ettirebilmiştir. Safra tuzuna dayanıklı izolatlardan sadece 6'sının hidrofobisite yeteneğine sahip olduğu belirlenmiştir. Kalan 6 izolatın antimikrobiyel direnç durumları incelenmiş dirençliliğe ve ESBL varlığına rastlanmamıştır. İncelemeler sonunda 144 izolattan sadece 6 (4.1%) sının probiyotik özelliklere sahip olduğu görülmüştür. Bunlardan; 3'ü *Lactobacillus brevis* bozadan ve 3'ü *Lactobacillus plantarum* kefirden izole edilmişlerdir. Kefir ve bozadan probiyotik özellik gösteren bakteri izole edilirken diğer gıda örnekleri olan süt ve peynirden probiyotik özellik gösteren bakteri izole edilememiştir. Sonuç olarak, kefir ve boza gibi doğal gıda ürünlerinden izole edilen transgenetik olmayan mikroorganizmalar içinden probiyotik bakterilerin endüstriyel probiyotiklere alternatif olarak üretilebileceği bu araştırmada saptanmıştır.

Anahtar sözcükler: *Probiyotik bakteri, Boza, Süt, Peynir, Kefir, Probiyotik özellik*

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INTRODUCTION

Probiotics are microorganisms that improve the microbial balance of human and animal intestines and thus are beneficial for the digestive system. These may be used as a natural supplement for both the healthy development of the body and the treatment and prevention of diseases. The probiotic bacteria colonize on the surface of the intestine by competing against pathogen microorganisms for nutrients in the gastrointestinal system, thereby positively affect the health [1,2]. A study has reported that probiotics prevent *Escherichia coli* associated with diarrhea and death in newborns [3]. It has been reported that probiotics produce volatile fatty acids as a result of fermentation by digesting dietary fibers: oligosaccharide structures that remain undigested in the colon. In addition, the formation of butyric acid inhibits colon cancer [4]. Probiotics have been found to contribute to lactose digestion due to the production of lactase enzyme; stimulation of immune system with the enhancement of IgA production; and to reduce allergens by inhibiting the passage of antigen-presenting substances into the circulatory system. Similarly, several reports highlight the importance of preventive effects of probiotics on heart diseases, hypertension, and urogenital diseases due to anti-oxidant effects, the act of cell wall components like angiotensin 1 enzyme inhibitors, and colonization on urinary and vaginal surfaces [5]. Additionally, it has been reported that *Helicobacter pylori* exert gastritis and ulcer-inhibiting effects by the production of inhibitors and preventive effect on hepatic encephalopathy formation by inhibiting the urease-producing intestinal flora and reducing the serum ammonia levels [6,7]. Various studies report the use of *Lactobacillus rhamnosus,* a combination of *L. rhamnosus* and *Lactobacillus acidophilus* for the treatment of children with diarrhea, the prevention of intestinal diseases, colon cancer and for the treatment of heart diseases [8-10].

Therefore, it is essential to develop new probiotic strains with different effects against different diseases and to use them in preventive medicine.

There has been an increasing interest in the use of probiotics due to their protective effects against diseases. An expenditure of $28 million was reported on research related to probiotic market and consumption in USA in 2011 [11]. The increasing interest in probiotics has accelerated the studies on the development of new probiotic products.

The therapeutic results of probiotics have been found to treat diseases such as colon cancer, ulcers and gastritis, and allergies, diabetes. In one study, it was reported that a commercial culture mixture obtained from *Lactobacillus* species had the antiproliferative effect by inhibiting tumor cells causing colon cancer [12]. A study has reported the beneficial effects of *Lactobacillus* species, especially *L. rhamnosus* and *L. acidophilus* against the infections caused by *Helicobacter pylori* [13]. In an *in vivo* study with probiotic *L. brevis*, an anti-allergic effect on anaphylaxis reduction was observed [14]. Another study provided the evidence that hemoglobin A1C and fasting blood glucose decreased in diabetic patients after treatment with probiotic supplements [15].

Probiotics obtained from nutrients should be able to resist stomach acidity and bile salts and reach the intestinal system alive to exert their beneficial effects. Further, they should be able to colonize and survive on the epithelial cell surfaces of intestinal mucosa [16].

The current study aimed to discover the alternative species of bacteria with probiotic properties along with preserved natural characteristics. Bacteria isolated from boza, cheese, kefir, and raw milk samples were characterized by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (VITEK® MS) to examine the probiotic properties of the single species. The relationship between these parameters was established according to Pearson's nonparametric statistical correlation.

MATERIAL and METHODS

Materials

A total of 130 food samples consisting of 10 boza, 40 cheeses, 60 raw milk, and 20 kefir were obtained from Marmara, Central and Eastern Anatolia regions of Turkey between 2014 and 2016. The food samples were listed in *Table 1*.

Methods

Isolation of Bacteria

de Man, Rogosa and Sharpe (MRS) agar, MRS broth, M17 agar and M17 broth media were prepared and used to isolate and identify the pure cultures of probiotics [17].

Identification with MALDI-TOF MS

The microorganisms were identified by using a system formed by comparison with a reference spectrum obtained from colonies formed on M17 and MRS agar. Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (VITEK® MS) (bioMerieux, France) was utilized to identify the protein profiles of cell structures of the microorganisms [18].

Measurement of Acid Tolerance

In order to determine acid tolerance, the pH value of MRS and M17 broths was reduced to 2.5 by using hydrochloric acid (Sigma Aldrich, USA) for creating similar environment to stomach acidity conditions. The viability of cultures was then monitored at pH 2.5. Colony growth on solid media and broth turbidity were evaluated as presence of the development [19].

Determination of Bile Salt Tolerance of Isolates

For the bile salt tolerance test, 0.3% (w/v) Oxgall (Bile bovine, Sigma-Aldrich, USA) showing the antimicrobial effect and containing conjugated and deconjugated bile components was inoculated (1%) to 7 mL of MRS and M17 broths. The viability was analyzed by colony counting and broth turbidity after 48-72 h incubation at 37°C [20].

Determination of Hydrophobicity of Isolates

Active cultures in MRS and M17 broths were centrifuged for 15 min at 10.000 rpm. The resulting pellet was washed twice with phosphate buffer, dissolved in 0.1 M KNO_3 (pH 6.2) buffer, added to 96-well plates, and OD was set to 600 nm using a spectrophotometer (A_0). The cell suspension (1 mL) was mixed with 0.3 mL of xylene and incubated at room temperature for 4 h. Subsequently, the OD of the aqueous phase was measured again at 600 nm (A_1) and the microbial adhesion of isolates to hydrocarbons was determined using the formula $[(A_0-A_1)/A_0] \times 100$ [21].

Antibiotic Susceptibility Test

Disc diffusion method was utilized for antibiotic susceptibility analysis. Antibiogram verification and determination of MIC (Minimal Inhibitory Concentration) were performed by using Micronaut-S beta-lactamase VII Plate (Merlin Diagnostika, Germany) according to the phenotypic determination to identify the presence of ESBL with MIC parameters [22].

Statistical Analyses

Statistical analyses were performed by SPSS Inc. Software (22.0 Version, SPSS Inc., Chicago, IL). In the statistical analysis, Pearson's correlation was used to examine whether all the data correlated with each other.

RESULTS

Isolation of bacteria from a total of 130 food samples, including boza, cheese, kefir, and raw milk with MALDI-TOF MS resulted in as of *L. brevis, L. plantarum, L. para plantarum,* and *E. faecium* species. Among the 144 identified probiotic isolates (five *L. brevis*, seven *L. plantarum*, five *L. para plantarum,* and 127 *E. faecium*), 35 (five *L. brevis*, five *L. plantarum*, three *L. para plantarum*, and 22 *E. faecium*) passed the gastric pH resistance test. Out of the 35 isolates, eight isolates (four *L. brevis*, three *L. plantarum*, and one *E. faecium*) could resist stomach pH and maintain the viability in bile salt conditions in the gastrointestinal tract, whereas only six isolates (three *L. brevis* and three *L. plantarum*) displayed hydrophobicity. The remaining six isolates (three *L. brevis* and three *L. plantarum*) were analyzed for antimicrobial resistance according to the instructions of the Institute for Clinical and Laboratory Standards, and resistance or ESBLs were not detected. The study concluded that only six (4.1%) of a total of 144 probiotic bacteria exhibited probiotic properties. *L. brevis* and *L. plantarum*, the bacteria isolated from kefir and boza, were able to companced the criteria of probiotics [23,24].

The relationship between test parameters was determined according to Pearson's nonparametric statistical correlation, which revealed that there was a significant correlation between acid and bile salt tolerance of the isolates ($P<0.05$). Results are listed in *Table 2, Table 3, Table 4, Fig. 1, Fig. 2* and *Fig. 3*.

DISCUSSION

Only six (4.1%) isolates with probiotic properties were detected among 144 isolates obtained from food sources. Three of them were *L. brevis* strains isolated from boza, and the others were *L. plantarum* strains from kefir. In the previous studies, *Lactobacillus* spp. isolates with similar probiotic properties to our study were isolated from kefir and boza samples [23,24].

Similarly, Yadav (2016) isolated 54 strains belonging to *L. plantarum* which were obtained from a local fermented food from grain, stomach acidity and bile salts were checked. It was determined that all isolates showed poor resistance. Only 24 isolates (44%) were able to show good resistance. Six (11%) species that could remain viable were analyzed for probiotic properties, and *L. plantarum RYPR1* (1.9%) exhibited satisfying results [25].

In the present study, the bacteria obtained from raw

Table 1. *Distribution of food samples*

Region	Type of Food			
	Boza[a]	Cheese[b]	Raw Milk[c]	Kefir[d]
Marmara	10	10 *	10 *	5 *
Central Anatolia	-	5 * + 5 **	17 * + 13 **	10 *
Eastern Anatolia	-	9 * + 11 **	10 * + 10 **	5 *

[a,b,c,d] natural, non industrial type and non using starter culture
** Cow Milk, ** Goat Milk*

Table 2. *Distribution of isolates identified with MALDI-TOF MS (VITEK® MS)*

Isolate Name	Source			
	Boza	Cheese	Raw Milk	Kefir
Enterococcus faecium	-	43	83	1
Lactobacillus brevis	5	-	-	-
Lactobacillus plantarum	-	3	1	3
Lactobacillus para plantarum	-	3	-	2

Table 3. *Acid-tolerant isolates and bile salt tolerances of isolates, hydrophobicity results*

No	Product	Sample No	Isolate Code	Microorganism	Acid Tolerance	Bile Salt Tolerance	Hydrophobicity Ability
					Viability (+/-)	Viability (+/-)	Hydrophobicity (+/-)
1	Boza	10	5	*Lactobacillus brevis*	+	+	-
2	Boza	3	78	*Lactobacillus brevis*	+	-	-
3	Boza	1	79	*Lactobacillus brevis*	+	+	+
4	Boza	9	81	*Lactobacillus brevis*	+	+	+
5	Boza	8	84	*Lactobacillus brevis*	+	+	+
6	Cheese	32	60B	*Enterococcus faecium*	+	+	-
7	Cheese	25	81B	*Enterococcus faecium*	+	-	-
8	Cheese	35	69B	*Enterococcus faecium*	+	-	-
9	Cheese	23	24B	*Enterococcus faecium*	+	-	-
10	Cheese	30	65B	*Enterococcus faecium*	+	-	-
11	Cheese	29	80B	*Enterococcus faecium*	+	-	-
12	Cheese	38	43C	*Enterococcus faecium*	+	-	-
13	Cheese	27	77C	*Enterococcus faecium*	+	-	-
14	Cheese	21	54C	*Enterococcus faecium*	+	-	-
15	Cheese	26	G76	*Enterococcus faecium*	+	-	-
16	Cheese	31	G4	*Enterococcus faecium*	+	-	-
17	Cheese	33	G37	*Enterococcus faecium*	+	-	-
18	Cheese	46	E54	*Lactobacillus paraplantarum*	+	-	-
19	Cheese	22	13B	*Lactobacillus paraplantarum*	+	-	-
20	Cheese	48	8C	*Lactobacillus paraplantarum*	+	-	-
21	Cheese	33	A21	*Lactobacillus plantarum*	+	-	-
22	Raw milk	58	21B	*Enterococcus faecium*	+	-	-
23	Raw milk	98	70B	*Enterococcus faecium*	+	-	-
24	Raw milk	69	23B	*Enterococcus faecium*	+	-	-
25	Raw milk	72	8B	*Enterococcus faecium*	+	-	-
26	Raw milk	77	A19	*Enterococcus faecium*	+	-	-
27	Raw milk	64	G11	*Enterococcus faecium*	+	-	-
28	Raw milk	59	G37a	*Enterococcus faecium*	+	-	-
29	Raw milk	81	G1	*Enterococcus faecium*	+	-	-
30	Raw milk	98	E14	*Enterococcus faecium*	+	-	-
31	Raw milk	85	E75	*Enterococcus faecium*	+	-	-
32	Raw milk	96	4C	*Lactobacillus plantarum*	+	-	-
33	Kefir	111	44C	*Lactobacillus plantarum*	+	+	+
34	Kefir	112	74C	*Lactobacillus plantarum*	+	+	+
35	Kefir	128	12C	*Lactobacillus plantarum*	+	+	+

milk and cheese samples did not show any probiotic properties. It has been reported that *E. faecium* obtained from animal milk and cheese had good acidification and strong bile salt tolerance in the previous studies [26,27]. However, these studies may be considered as incomplete in terms of probiotic properties due to the lack of study about the ability of colony formation in the intestinal system.

In this study, the intestinal adhesion abilities, acid and bile tolerance of the isolates were examined to determine the probiotic properties of bacteria isolated from the food samples. A similar study was conducted by Sanni [28] for bacterial isolates from some regional food products derived from grain, in which *L. plantarum* showed a good and fast acid production capability. *L. plantarum* also showed similar results in our study *(Table 3)*.

A similar study was conducted by Banwo [29] for *E. faecium* isolated from raw milk, and the technological and

Table 4. *Antibiotic disc confirmation zones (mm) of the samples, antibiogram confirmation and MIC (µg/ml) results*

No	CAZ ZON	CAZ CV	CTX ZON	CTX CV	CPD ZON	CPD CV	CAZ	CAZ MIC	CAZ CV MIC	CTX	CTX MIC	CTX CV MIC	ESBL
3	16	18	24	25	23	23	R	32	>32/4	S	≤1	≤0.5/4	-
4	-	-	-	-	-	-	?	-	-	S	≤1	≤0.25/4	-
5	-	-	-	-	-	-	S	≤1	≤0.25/4	S	≤1	≤0.25/4	-
33	-	-	-	-	-	-	S	≤1	≤0.25/4	S	≤1	≤0.25/4	-
34	-	-	-	-	-	-	S	≤1	≤0.25/4	?	-	-	-
35	18	18	15	15	18	18	S	≤1	≤0.25/4	S	≤1	≤0.25/4	-

CAZ: Ceftazidime, CTX: Cefotaxime, CPD: Cefpodoxime, CV: Clavulanate
The isolates were identified as ESBL (-)

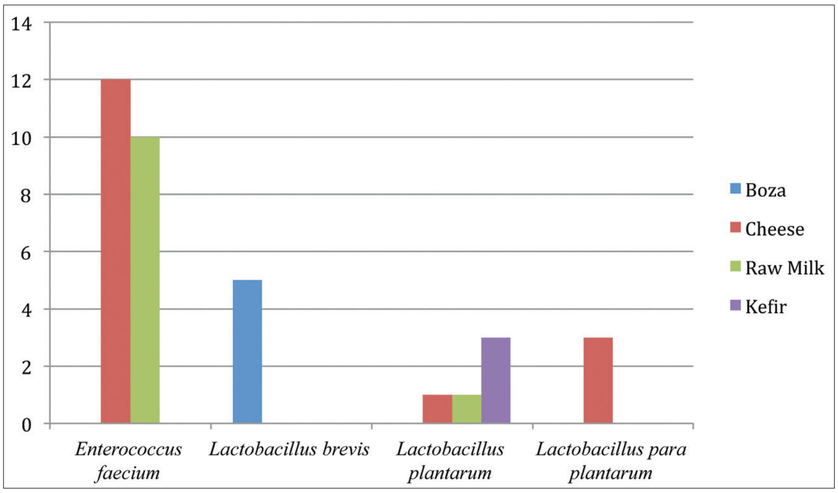

Fig 1. Distribution of isolates exceeding stomach resistance

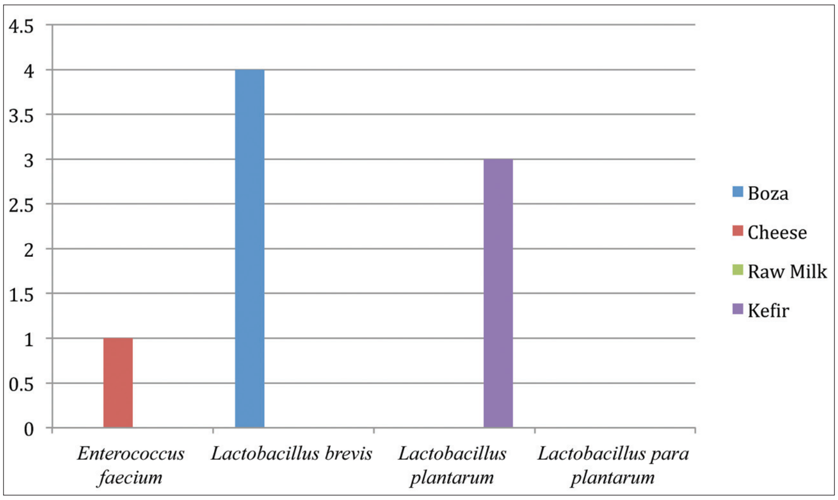

Fig 2. Distribution of bile-tolerant isolates

food safety characteristics of the species were investigated. *E. faecium* species have been detected in respect to resistance to bile salts and sensitive to antibiotics. In our study, *E. faecium* species isolated from raw milk did not show enough bile acid resistance.

In a study carried out by Gulel [30], lactobacilli strains isolated from kefir were able to resist both acid and bile salts, but their hydrophobicity remained low. In our study, the lactobacilli strains isolated especially from kefir and boza showed good hydrophobicity.

Probiotic bacteria must resist gastric acidity and bile salts and adhere to the epithelial surface of the intestinal mucosa. These properties are fundamental criteria for a bacterium to be a probiotic [31]. However, the relevant bacteria must be tested for antibiotic resistance and antibiotic resistance genes to ensure the safety for human consumption. In the studies carried out by Sanni and other researchers, the detection of antibiotic resistance status of the microorganisms seems to be missing [32-34]. It would be useful to consider these criteria, which should be examined in terms of food safety, among the probiotic properties. Thus, the probiotic character of examined the microorganisms should be the end result.

Acid tolerance is one of the most important criteria for probiotic bacteria as they are destroyed by the acidity of the stomach [35]. Probiotic bacteria are more resistant to stomach acidity than other microorganisms and are usually exposed to stomach acid with pH between 2.5 and 3.5 before arriving the colon. Acidic conditions are one of the important physiological challenges encountered by probiotic bacteria [20]. In our study, 13 strains of the 16 Lactobacilli showed resistance to pH 2.5. Besides, 22 (17%) of 126 *E. faecium* strains were able to show resistance to pH 2.5. However, there are technological methods recommended to analyze the probiotic bacteria for their

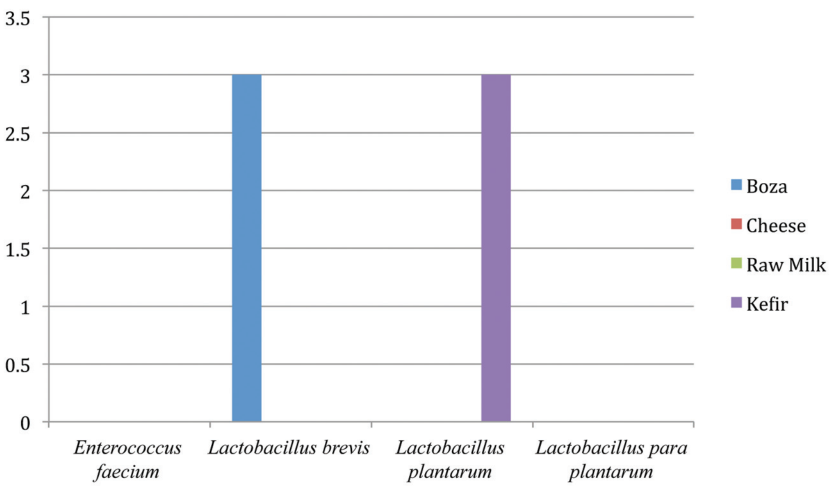

Fig 3. Distribution of isolates displaying high hydrophobicity

ability to pass through the stomach without being destroyed. The most commonly used method is micro-encapsulation. In principle, the powdered form of isolates is covered with a suitable material enabling bacteria to pass through the acidic environment of the stomach without getting killed [36]. In a study by Mishra and Prasad [37], three strains (43%) of seven lactobacilli were reported to be resistant to pH 2.0 or 3.0. It is indicated that the differences in pH resistance of different species and even of the same species are attributed to the differences in the multiplication stage of the bacteria [38].

The probiotic bacteria pass through the acidity of the stomach and then come into the contact with bile [39]. Bile salt tolerance is another important criterion used in the selection of probiotic bacteria [30]. Therefore, bacteria to be used as probiotics need to be resistant to bile to maintain their viability in the small intestine, a part of the gastrointestinal tract [40]. The present study found that *L. brevis* obtained from boza and *L. plantarum* obtained from kefir showed resistance to bile salts. *E. faecium* obtained from cheese and raw milk did not show enough resistance. In particular, the earlier studies on *L. brevis* and *L. plantarum* confirm the findings of our study. In the studies carried out by Ronka [41], Ramos [42], and Golowczyc [43], *L. brevis* and *L. plantarum* isolates exhibited good resistance to bile. However, in another study, 86 of the 122 *E. faecium* species isolated from traditional cheese samples (about 70%) were reported to be highly resistant to the medium containing 0.3% bile. In addition, *E. faecium* was reported to be more resistant to the harsh conditions of the gastrointestinal tract than other probiotic bacteria [44].

An important criterion for the selection of probiotic bacteria is their ability to colonize by attaching to the epithelial surfaces on the intestinal mucosa. A positive correlation has been observed between adhesion of bacterial cells and bacterial cell surface hydrophobicity [45,46]. In our study, *L. plantarum* obtained from kefir and *L. plantarum* obtained from boza showed high hydrophobicity. The probiotic properties of *L. plantarum* isolated from traditional Iranian dairy products and *L. brevis* obtained from Brazilian origin products were analyzed by Nejati [47] and Ramos [42] respectively, and the hydrophobicity abilities were found high. These studies conform to our findings on the high hydrophobicity of *L. brevis* and *L. plantarum* isolates obtained from different food samples [48].

According to the criteria established by Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) [31], bacteria with resistance to antibiotics and able to transfer the antibiotic resistance genes are considered unsafe for health and cannot be used as probiotics [49]. Therefore, transfer of antibiotic resistance genes by probiotics, especially to pathogenic bacteria is the most important risk factor and needs to be controlled [50]. Earlier studies have shown that transfer of antibiotic resistance genes to pathogenic bacteria from the *Lactobacillus* species found in the intestinal flora may be possible in limited numbers [51,52]. In our study, antibiotic resistance and especially the presence of ESBL were not observed in any bacterium. However, *Lactobacillus* strains carrying the genetic vancomycin resistance gene may be reliably used as probiotics, as no evidence has been shown for the transfer of this gene to other strains [53].

In a study by Gulel [30], although the *Lactobacillus* strains isolated from the kefir showed high resistance to nucleic acid synthesis inhibitors and cytoplasmic membrane inhibitors, a lower resistance to cell wall inhibitors and most of the protein synthesis inhibitors was observed. In our study, none of the isolated *Lactobacillus* strains displayed antibiotic resistance to nucleic acid synthesis, cytoplasmic membrane, and cell wall and protein synthesis inhibitors. In a study carried out by Zheng [54], *L. plantarum* isolated from kefir was susceptible to gentamicin, erythromycin, and chloramphenicol inhibitors, whereas it showed resistance to vancomycin.

In a study carried out by Forssten [55], the presence of ESBL was determined by administering a probiotic blend of *Lactobacillus* strains during antibiotic treatment, and ESBL negative results were obtained. *L. plantarum* and *L. brevis* strains isolated from kefir and boza yielded ESBL-negative results in a similar manner.

Kefir and boza have been produced by the fermentation of mixed cultures, including *Lactobacilli* species, could be regarded as beneficial microorganisms [23,24]. Especially, the *in vivo* studies on kefir have reported beneficial effects on health [56-59]. The result of our study show that the bacteria obtained from kefir and boza displayed probiotic properties. This explains the beneficial effects of probiotic-containing boza and kefir on health. However, the bacteria from cheese and milk samples did not show enough probiotic properties. These observations indicated that kefir and boza consist of more bacteria with probiotic bacteria as compared to cheese and milk.

The present study determined that *L. brevis* and *L. plantarum* isolated from kefir and boza were able to compansate the set probiotic criteria. The study indicates that the probiotic bacteria may be obtained as an alternative to industrial probiotics through non-GMO (non-genetically modified organism) isolated from natural fermented food products such as kefir and boza. Besides, probiotics of Turkish origin were identified from the bacteria isolated from kefir and boza samples.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Gülmez M, Güven A: Probiyotik, prebiyotik ve sinbiyotikler. *Kafkas Univ Vet Fak Derg,* 8 (1): 83-89, 2002.

2. Sarıpınar D, Sulu N: Ruminantlarda probiyotiklerin kullanımı ve rumene etkileri. *Kafkas Univ Vet Fak Derg,* 11 (1): 93-98, 2005.

3. Polewski MA, Krueger CG, Reed, JD, Leyer G: Ability of cranberry proanthocyanidins in combination with a probiotic formulation to inhibit *in vitro* invasion of gut epithelial cells by extra-intestinal pathogenic *E. coli. J Funct Foods,* 25, 123-134, 2016. DOI: 10.1016/j.jff.2016.05.015

4. Cui SW, Nie S, Roberts KT: Functional properties of dietary fibre. *Biotechnology,* 4, 517-525, 2011.

5. Butel MJ: Probiotics, gut microbiota and health. *Med Mal Infect,* 44, 1-8, 2014. DOI: 10.1016/j.medmal.2013.10.002

6. Nagpal R, Kumar A, Kumar M, Behare PV, Jain, S, Yadav H: Probiotics, their health benefits and applications for developing healthier foods: A review. *FEMS Microbiol Lett,* 334, 1-15, 2012. DOI: 10.1111/j.1574-6968.2012.02593.x

7. Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, Fakiri EM: Health benefits of pro-Ωbiotics: A review. *ISRN Nutr,* 2013. DOI: 10.5402/2013/481651

8. Goldenberg JZ, Ma SS, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, Johnston BC: Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst Rev,* 31, CD006095, 2013. DOI: 10.1002/14651858.CD006095.pub3

9. Gan XT, Ettinger G, Huang CX, Burton JP, Haist JV, Rajapurohitam V, Reid G: Probiotic administration attenuates myocardial hypertrophy and heart failure after myocardial infarction in the rat. *Circ Heart Fail,* 7, 491-499, 2014. DOI: 10.1161/CIRCHEARTFAILURE.113.000978

10. Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, Singh B: Probiotic metabolites as epigenetic targets in the prevention of colon cancer. *Nutr Rev,* 71, 23-34, 2013. DOI: 10.1111/j.1753-4887.2012.00542.x

11. Tall BD: We are what we eat: Should food microbiology take the lead on understanding how the homeostasis of the gut microbiome influences human health and disease? **In,** *IAFP 2016 Annual Meeting,* Jul 31-Aug 03, IAFP, 2016.

12. Georgiev K, Georgieva M, Iliev I, Peneva M, Alexandrov G: Antiproliferative effect of Bulgarian spring water probiotics (Laktera Nature Probiotics®), against human colon carcinoma cell line. *World J Pharm Pharm Sci,* 4 (6): 130-136, 2015.

13. Wang, ZH, Gao QY, Fang JY: Meta-analysis of the efficacy and safety of *Lactobacillus*-containing and *Bifidobacterium*-containing probiotic compound preparation in *Helicobacter pylori* eradication therapy. *J Clin Gastroenterol,* 47, 25-32, 2013. DOI: 10.1097/MCG.0b013e318266f6cf

14. Lee J, Bang J, Woo HJ: Effect of orally administered *Lactobacillus brevis* HY7401 in a food allergy mouse model. *J Microbiol Biotechnol,* 23, 1636-40, 2013. DOI: 10.4014/jmb.1306.06047

15. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi, M, Mofid V: Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition,* 28, 539-543, 2012. DOI: 10.1016/j.nut.2011.08.013

16. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E: Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int Dairy J,* 16, 189-199, 2006. DOI: 10.1016/j.idairyj.2005.02.009

17. ISO 11133:2014: Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media, Geneva, Switzerland, 2014.

18. Rifaat EA, Tekiner İH, Özpinar H: Halk sağlığı açısından içme ve kullanma sularında koliform ve fekal koliform bakterilerin varlıklarının klasik ve MASS spektrometresi yöntemleriyle incelenmesi. *Electronic J Food Technol,* 9 (2): 20-32, 2014.

19. Klingberg TD, Axelsson L, Naterstad K, Elsser D, Budde BB: Identification of potential probiotic cultures for Scandinavia-type fermented sausages. *Int J Food Microbiol,* 105, 419-431, 2005. DOI: 10.1016/j.ijfoodmicro.2005.03.020

20. Liong MT, Shah NP: Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *J Dairy Sci,* 88, 55-66, 2005. DOI: 10.3168/jds.S0022-0302(05)72662-X

21. Gusils C, Cuzzo S, Sesma F, Gonzalez S: Examination of adhesive determinants in three species of *Lactobacillus* isolated from chicken. *Can J Microbiol,* 48, 34-42, 2002. DOI: 10.1139/W01-122

22. Clinical and Laboratory Standards Institute (2015-2016 Catalog): http://clsi.org/wp-content/uploads/sites/14/2013/07/CLSI-2015-Catalog.pdf; *Accessed:* 29.07.2016

23. Todorov SD, Botes M, Guigas C, Schillinger U, Wiid I, Wachsman MB, Dicks LMT: Boza, a natural source of probiotic lactic acid bacteria. *J Appl Microbiol,* 104, 465-477, 2008. DOI: 10.1111/j.1365-2672.2007.03558.x

24. Leite AMO, Miguel MAL, Peixoto RS, Ruas-Madiedo P, Paschoalin VMF, Mayo, B, Delgado S: Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. *J Dairy Sci,* 98, 3622-3632, 2015. DOI: 10.3168/jds.2014-9265

25. Yadav R, Puniya AK, Shukla P: Probiotic properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage Raabadi. *Front Microbiol*, 7, 2016. DOI: 10.3389/fmicb.2016.01683

26. Kamruzzaman M, Jahan S, Fuadh-Al-Kabir M., Jahan MS, Rahman M, Khan MAK, Hossain MS: The investigation of probiotic potential of lactic acid bacteria isolated from cow milk. *Int J Biosci (IJB),* 3, 161-167, 2013. DOI: 10.12692/ijb/3.4.161-167

27. Ahmadova A, Todorov SD, Choiset Y, Rabesona H, Zadi TM, Kuliyev A, Haertlé T: Evaluation of antimicrobial activity, probiotic properties and safety of wild strain *Enterococcus faecium* AQ71 isolated from Azerbaijani motal cheese. *Food Control,* 30, 631-641, 2013. DOI: 10.1016/j.foodcont.2012.08.009

28. Sanni A, Franz C, Schillinger U, Huch M, Guigas C, Holzapfel W: Characterization and technological properties of lactic acid bacteria in the production of "Sorghurt," a cereal-based product. *Food Biotechnol,* 27, 178-198, 2013. DOI: 10.1080/08905436.2013.781949

29. Banwo K, Sanni A, Tan H: Technological properties and probiotic potential of *Enterococcus faecium* strains isolated from cow milk. *J Appl Microbiol,* 114, 229-241, 2013. DOI: 10.1111/jam.12031

30. Gülel Ş: Molecular identification and properties of 'Lactobacillus acidophilus group isolates from Turkish kefir. *PhD Thesis,* Middle East Technical Universit (METU), 2014.

31. FAO/WHO: Guidelines for the evaluation of probiotics in foods.

Report of a Joint FAO/ WHO Working Group. London Ontario, Canada, 2002.

32. Badis A, Guetarni D, Boudjema BM, Henni DE, Kihal M: Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiol,* 21, 579-588, 2004. DOI: 10.1016/j.fm.2003.11.006

33. Fernandez MF, Boris S, Barbes C: Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol*, 94, 449-455, 2003. DOI: 10.1046/j.1365-2672.2003.01850.x

34. García-Ruiz A, de Llano DG, Esteban-Fernández A, Requena T, Bartolomé B, Moreno-Arribas MV: Assessment of probiotic properties in lactic acid bacteria isolated from wine. *Food Microbiol,* 44, 220-225, 2014. DOI: 10.1016/j.fm.2014.06.015

35. Otles S, Cagindi O: Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan J Nutr,* 2 (2): 54-59, 2003.

36. Burgain J, Gaiani C, Linder M, Scher J: Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *J Food Eng,* 104, 467-483, 2011. DOI: 10.1016/j.jfoodeng.2010.12.031

37. Mishra V, Prasad DN: Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. *Int J Food Microbiol,* 103, 109-115, 2005. DOI: 10.1016/j.ijfoodmicro.2004.10.047

38. Yang X, Hang X, Zhang M, Liu X, Yang H: Relationship between acid tolerance and cell membrane in *Bifidobacterium*, revealed by comparative analysis of acid-resistant derivatives and their parental strains grown in medium with and without Tween 80. *Appl Microbiol Biotechnol,* 99, 5227-5236, 2015. DOI: 10.1007/s00253-015-6447-y

39. Pan D, Zeng X, Yan T: Characterization of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *J Sci Food Agric,* 91, 512-518, 2011. DOI: 10.1002/jsfa.4214

40. Maldonado NC, Nader-Macías MEF: Functional properties (acid and bile tolerance) and antibiotic susceptibility of lactic acid bacteria isolated from newborn calves for the design of a probiotic product. *Int J Vet Sci Res,* 1 (1): 11-22 , 2015.

41. Rönkä E, Malinen E, Saarela M, Rinta-Koski M., Aarnikunnas J, Palva A: Probiotic and milk technological properties of *Lactobacillus brevis*. *Int J Food Microbiol,* 83, 63-74, 2003. DOI: 10.1016/S0168-1605(02)00315-X

42. Ramos CL, Thorsen L, Schwan RF, Jespersen L: Strain-specific probiotics properties of *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. *Food Microbiol,* 36, 22-29, 2013. DOI: 10.1016/j.fm.2013.03.010

43. Golowczyc MA, Gugliada MJ, Hollmann A, Delfederico L, Garrote GL, Abraham A.G, De Antoni G: Characterization of homofermentative lactobacilli isolated from kefir grains: Potential use as probiotic. *J Dairy Res,* 75, 211-217, 2008. DOI: 10.1017/S0022029908003117

44. Bhardwaj A, Gupta, H, Kapila S, Kaur G, Vij S, Malik RK: Safety assessment and evaluation of probiotic potential of bacteriocinogenic *Enterococcus faecium* KH 24 strain under *in vitro* and *in vivo* conditions. *Int L Food Microbiol,* 141, 156-164, 2010. DOI: 10.1016/j.ijfoodmicro.2010.05.001

45. Collado MC, Meriluoto J, Salminen S, Development of new probiotics by strain combinations: Is it possible to improve the adhesion to intestinal mucus. *J Dairy Sci*, 90, 2710-2716, 2006. DOI: 10.3168/jds.2006-456

46. Collado MC, Meriluoto J, Salminen S: Adhesion and aggregation properties of probiotic and pathogen strains. *Eur Food Res Technol,* 226, 1065-1073, 2008. DOI: 10.1007/s00217-007-0632-x

47. Nejati F, Tobias O: *In vitro* characterization of *Lactococcus lactis* strains isolated from Iranian traditional dairy products as a potential probiotic. *Appl Food Biotechnol,* 3, 43-51, 2015.

48. Kaushik JK, Kumar A, Duary RK, Mohanty AK, Grover S, Batish VK: Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum. PloS one,* 4, e8099, 2009. DOI: 10.1371/journal.pone.0008099

49. Muñoz-Atienza E, Gómez-Sala B, Araújo, C, Campanero C, Del Campo R, Hernández PE, Cintas LM: Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiol,* 13, 15, 2013. DOI: 10.1186/1471-2180-13-15

50. Ouoba LII, Lei V, Jensen LB: Resistance of potential probiotic lactic acid bacteria and *Bifidobacteria* of African and European origin to nantimicrobials: Determination and transferability of the resistance genes to other bacteria. *Int J Food Microbiol,* 121; 217-224, 2008. DOI: 10.1016/j.ijfoodmicro.2007.11.018

51. Ammor MS, Flórez AB, Mayo B: Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiol,* 24, 559-570, 2007. DOI: 10.1016/j.fm.2006.11.001

52. Toğay SÖ, Mustafa AY, Altunatmaz SS: Antimicrobial activity potential of *Enterococcus* spp. isolated from some traditional Turkish cheeses. *Kafkas Univ Vet Fak Derg,* 22, 765-770, 2016. DOI: 10.9775/kvfd.2016.15369

53. Mathur S, Singh R: Antibiotic resistance in food lactic acid bacteria-A review. *Int J Food Microbiol*, 105, 281-295. 2005. DOI: 10.1016/j.ijfoodmicro.2005.03.008

54. Zheng Y, Lu Y, Wang J, Yang L, Pan C, Huang Y: Probiotic properties of *Lactobacillus* strains isolated from Tibetan kefir grains. *PloS one,* 8, e69868, 2013. DOI: 10.1371/journal.pone.0069868

55. Forssten S, Evans M, Wilson D, Ouwehand AC: Influence of a probiotic mixture on antibiotic induced microbiota disturbances. *World J Gastroenterol (WJG)*, 20, 11878, 2014. DOI: 10.3748/wjg.v20.i33.11878

56. Farnworth ER: Kefir- A complex probiotic. *Food Sci Technol Bull: Funct Food,* 2 (1): 1-17, 2006.

57. Özpınar H, Klasing KC, Tekiner İH: Interaction of mannan oligosaccharide with immune system "Transport of MOS in to the lamina propria". *Kafkas Univ Vet Fak Derg,* 18, 121-128, 2012. DOI: 10.9775/kvfd.2011.4539

58. Özpınar H, Tezmen G, Gökçe İ, Tekiner İH: Detection of animal species in some meat and meat products by comparatively using DNA microarray and real time PCR methods. *Kafkas Univ Vet Fak Derg,* 19, 245-252, 2013. DOI: 10.9775/kvfd.2012.7616

59. Tekiner İH, Özpınar H: Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. *Braz J Microbiol,* 47, 444-451, 2016. DOI: 10.1016/j.bjm.2015.11.034