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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of probiotic and some technological properties of lactic acid bacteria isolated from cheeses sold in the Kilis region, Turkey

Kilis bölgesinde satılan peynirlerden izole edilen laktik asit bakterilerinin probiyotik ve bazı teknolojik özelliklerinin belirlenmesi

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Ö Z E T / A B S T R A C T

Aims: The purpose of this study is to evaluate technological properties and probiotics potential of lactic acid bacteria (LAB) isolated from 15 raw milk Turkish Beyaz fresh cheese made in the Kilis region, Turkey.
Methods and Results: 287 colonies from 15 cheese samples were selected and 91 of them were analyzed as Gram-positive and catalase-negative. 19 strains of 91 colonies were accepted as potential probiotics since they stayed alive in pH 3.5 and 0.3% bile salt. They were identified as *Lactobacillus* sp. (11 strains), *Leuconostoc* sp. (3 strains), *Streptococcus* sp. (2 strains), *Lactococcus* sp. (2 strains), and *Enterococcus* sp. (1 strain) by biochemical tests and API test kit. It was determined that 19 strains with high acidification rate, 15 strains demonstrated weak proteolytic activity, 3 ones moderate, and 1 strong proteolytic activity. It was determined that 1 of the strains were resistant to chloramphenicol, 2 to tetracycline, and 6 to vancomycin. No strains resistant to penicillin and erythromycin could be detected. In the artificial gastric juice resistance test, although no viability was detected at pH 2.0. But it was determined that the viability values varied between 89.49-111.79% at pH 3.0. Also, these strains showed growth at bile salt. According to plasmid profiles of strains, 13 of 19 potential probiotic strains were determined to have plasmid DNA in the range of 1-5, while other strains were determined to not have plasmid DNA. The molecular sizes of the plasmid DNA of the isolated strains ranged from 2-16 kb.
Conclusions: In the research, it was determined that LABs isolated from Turkish white fresh cheese produced in Kilis have probiotic potential and can be used as starters in various fermented foods.
Significance and Impact of the Study: According to the results obtained, it was concluded that some strains can be used as probiotic starter culture in cheese production and others can be used for ripening of cheese.

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INTRODUCTION

Probiotics are defined as living microorganisms that provide beneficial effects on health as benefit the intestinal flora and activate the immune system when a sufficient amount is taken by humans and animals. A bacterium to be used as a probiotic; should maintain a high level of viability in the host's adverse conditions

(acid, bile salts), for example, in the gastrointestinal tract. In addition, the starter and probiotic bacteria cultures should be resistant to some antibiotics that are thought to adversely affect the growth of culture and are widely used in the clinic (Başyiğit, 2004).

For a microorganism to display its probiotic properties, it must be passed through the acidic medium (pH 1.5-3.0) of the stomach and reach the intestines. Therefore, it is most important in the selection of probiotic microorganisms resistant to acid (Dunne et al., 1999) and to bile salts (Başyiğit, 2004; Lee and Mahmoudi et al., 2021; Salminen, 1995; Pouwels and Leer, 1993).

Living microorganisms that are considered as probiotics are made of both bacteria and yeast. Common probiotic bacteria can include *Lactobacillus* spp. and *Bifidobacterium* spp. They are the natural flora of the gut microbiota and are the most studied and widely used bacteria in the probiotic field (Kaur and Das, 2011).

LAB are used in the production and maturation of many dairy products, especially cheese, as well as in fermented foods prepared from raw materials such as cereals, vegetables, fruits, meat, etc. Many factors are effective in determining the technological properties of LAB; growth temperature, development at low pH values, reproduction at high salt concentrations, and proteolytic activity. Strains, whose proteolytic activity is above 50 µl mL^{-1} tyrosine, cause high protein residues and amino acids, thus causing the development of bitter taste and thus not preferred in Turkish Beyaz fresh cheese production as a starter culture. In general, it is stated that the proteolytic activities of LAB are considered weak (Ertekin and Con, 2014; Mohammed and Con, 2021).

No studies on LAB isolation from food products produced in and around Kilis have been found. In Kilis, some cheeses are produced from raw milk and therefore have a rich LAB content.

The determination of probiotic and some technological properties of LABs are important in terms of knowing the characteristics of the cultures to be used as a starter culture. This way, it is possible to obtain cultures that were not previously isolated, as well as analyze their properties. At the same time, there is a possibility that the cheeses produced with these cultures will be suitable for Turkish taste.

Due to the increased interest in new products, it will be possible to produce new cheese varieties with different characteristics by using the cultures to be obtained as a starter culture. The aim of the study was to characterize potential probiotic cultures and to determine some technological properties of those isolates. So that isolates would be used as probiotic starter culture and for ripening of cheese.

MATERIALS and METHODS

Material

Turkish Beyaz fresh cheese samples obtained from Kilis province in southeast Turkey were used as the sources for lactic acid bacteria. Isolations were done from 15 Turkish Beyaz fresh cheese samples.

Method

Serial dilutions of samples were prepared with physiological salt water from cheese samples. M17 agar was used for isolation of cocci LAB, and Man, Rogosa and Sharpe (MRS) agar for *Lactobacilli* by spreading plate method and incubated at 30°C, 48 hours. Pure cultures obtained were stored at -20 °C in broth containing 20% glycerol. Gram staining and catalase tests of the isolates analyzed, and to determine potential probiotics, strains were evaluated for resistance to low pH (pH 3.5) and bile salts (Başyiğit, 2004; Kandler and Weiss, 1986; Öner et al., 2006). In order to identify the isolates; gas production from glucose, ammonia production from arginine, growing at different temperatures, growing at pH 9.6 and at 6.5% NaCl, 0.1% methylene blue reduction tests were performed. Analytical Profile Index (API) test kits were used to determine the carbohydrate fermentation properties of the strains. The method utilized by Citti et al. (1963) was used for the detection of proteolytic activities. The total acid production ability of cultures, pH value of isolates, and the ability to form % acidity (in lactic acid) were determined (Bradley et al., 1992; Ertürkmen and Öner, 2015). A method applied by many researchers was used to measure the resistance of isolates to artificial gastric juice at pH 3.0 and pH 2.0 (Gardiner et al., 1999; Sağlam, 2013; Vinderola and Reinheimer, 2003). Antibiotic resistance properties of isolates were determined by applying the disk diffusion method (Başyiğit, 2004; Sağlam, 2013) and the E-test method (Ammor et al., 2008; Danielsen and Wind, 2003; Franz et al., 2001). After obtaining the plasmid profile of the isolated cultures by QIAprep Spin Miniprep Kit, the plasmid DNA was loaded onto the 1% agarose gel and a gel image was taken after it was performed in electrophoresis (Egervarn et al., 2009; Sağlam, 2013; Smithies, 1955).

RESULTS and DISCUSSION

Isolation and Identification

As a result of isolation and purification, strains that Gram-positive, catalase-negative, non-spore, bacilli, or cocci were identified as Lactic Acid Bacteria (LAB). Various sizes of cocci-shaped, Gram-positive, and catalase-negative isolates are *Enterococci* and *Lactococcus*; rod-shaped, Gram-positive, and catalase-negative isolates are considered as lactobacilli (Ertürkmen and Öner, 2015; Schillinger and Lücke, 1987).

287 colonies from 15 cheese samples were selected and purified within the study. 91 strains showing Gram-positive and catalase-negative characteristics from the purified colonies were identified and were used to identify the probiotic properties. In the determination, resistance to low pH (3.5) and bile salt properties were investigated.

According to the results, 48 of 91 strains showed growth at pH 3.5 and 20 strain of them in bile salt, while 19 of these strains showed growth at both pH 3.5 and bile salt. Accordingly, 53% of isolated LAB were resistant to pH 3.5, 22% of them to bile salt, and 21% of them to both pH and bile salt. As a result, 19 strains are suitable to be potential probiotics.

According to the identification results, 19 strains of potential probiotics were identified as *Lactobacillus plantarum* (8 strains), *Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum* (3 strains), *Lactobacillus brevis* (3 strains), *Streptococcus* sp. (2 strains), *Lactococcus lactis* ssp *lactis* (1 strain), *Enterococcus* sp. (1 strain), and *Lactococcus* sp. (1 strain) (Table 1).

As shown in Table 1, it is seen that lactic acid bacteria isolated from cheese samples are of a five genus with *Lactobacillus* sp. 57.89%, *Leuconostoc* sp. 15.78%, *Streptococcus* sp. 10.52%, *Lactococcus* sp. 10.52%, and *Enterococcus* sp. 5.26%. In many studies, LABs isolated from fermented foods for starter culture use have been identified, LABs have been established to be at different genus and types (Arıcı et al., 2017; Azat et al., 2016; Ertürkmen and Öner, 2015; Gonzalez et al., 2000; Ouadghiri et al., 2005; Shangpliang et al., 2017). In one of these studies, 145 colonies from 7 cheese samples produced from raw milk isolated under laboratory conditions and determined that 77 of them were LAB and also 25, 22, and 30 of them were determined to be *Lactococcus* spp., *Enterococcus* spp., and *Lactobacillus* spp., respectively. In another study 164 LABs were isolated from soft white cheese. As a result of the identification of the isolates, 34% of all strains were *Lactobacillus*, 27% of all were *Lactococcus*, 27% were *Leuconostoc*, 10% of strains were *Enterococcus* and 1% belongs to the *Streptococcus* genus. Also, 2 strains with 1% could not be identified.

Table 1. Identification of strains

No	Strain	Identification
1	M170408	*Leuconostoc mesenteroides* ssp *mesenteroides/dextranicum*
2	MRS0501	*Lactobacillus plantarum*
3	M170702	*Leuconostoc mesenteroides* ssp *mesenteroides/dextranicum*
4	MRS0703	*Lactobacillus plantarum*
5	M170709	*Lactobacillus plantarum*
6	MRS0801	*Lactobacillus brevis*
7	M170803	*Lactobacillus plantarum*
8	M170806	*Lactobacillus plantarum*
9	MRS1001	*Streptococcus* sp.
10	MRS1003	*Lactococcus lactis* ssp *lactis*
11	M171103	*Streptococcus* sp.
12	MRS1103	*Lactobacillus brevis*
13	M171305	*Enterococcus* sp.
14	M171306	*Leuconostoc mesenteroides* ssp *mesenteroides/dextranicum*
15	M171307	*Lactobacillus plantarum*
16	M171310	*Lactococcus* sp.
17	M171313	*Lactobacillus brevis*
18	MRS1315b	*Lactobacillus plantarum*
19	MRS1317	*Lactobacillus plantarum*

According to the results of the 1st, 2nd, 3rd, and 7th days, pH values and % acidity ratios are given in Table 2. As shown in Table 2, the lowest pH value was determined in *L. plantarum* M171307 and the highest in *L. mesenteroides/dextranicum* M170408 on the 7th day. The average pH value obtained at the end of the 7th day was determined to be 4.41. The lowest total amount of acid production was determined at *L. mesenteroides/dextranicum* M170702 (0.24%) and the highest at *L. brevis* MRS0801 (0.40%) on the 7th day. The average acidity obtained at the end of the 7th day is 0.31%. LABs, which reduced the pH of the environment between 4.50-4.86 after 24 hours of incubation, were thought to have fast acidification properties of the environment and it was concluded that these cultures could be used as a starter culture.

In studies, LAB species and strains were found to produce different levels of acid (Bulut, 2003; Ertürkmen and Öner, 2015; Herreros et al., 2003; Turhan and Öner, 2014). The acidity values of cultures differed between 0.11-1.33% in the study in which 15% LAB 24-hour cultures isolated from Balıkesir region cheeses

determined by Dikbaş Yıldız (2013). Similar results were obtained as a result of 24-hour incubation in our study. The pH and % acidity values of LABs that Ertekin and Çon (2014) isolated from cheese, sausage, sourdough, and pickles were determined. It was confirmed that the pH values of the isolates on the 7th day were between 3.30-4.41 (average pH 3.85) and the acidity values between 1.07-2.47% (average 1.81%). In another study, it was determined that the pH production of the identified strains varied between 3.77-5.96 (Öner et al., 2006).

Proteolytic activity is involved in breaking down protein and peptides by microorganisms to obtain the necessary amino acids. It is one of the important factors in the selection of starter culture. In the production of fresh cheeses (Beyaz cheese etc.) proteolytic activity of cultures should not have high proteolytic activity. Cultures with high proteolytic activity are used for the ripening of hard cheeses (Turhan and Öner, 2014). Strains which have proteolytic activity value of 50 µg mL^{-1} tyrosine equivalent have a bitter taste due to the compounds formed as a result of breaking down the proteins and such strains are not preferred in cheese production (Atiles et al., 2000; Cogan et al., 1997). Proteolytic activity values (µg mL^{-1} tyrosine equivalent) (PAD) of strains isolated from white cheese studies are expressed as PAD<10 weak, 10<PAD<20 medium, and PAD> 20 strong proteolytic activity (Karakuş, 1994). While the types with high proteolytic activity are preferred in the production of ripened hard cheeses, types with low proteolytic activity are preferred in the production of Beyaz cheese that is consumed fresh without ripening. For example, strains with low proteolytic activity should be used in Beyaz cheese production. Proteolysis plays an important role in the formation of texture, taste, and aroma of cheese varieties. The proteolytic activity results of the isolates were calculated according to the standard curve. The tyrosine equivalents of the strains were given in Table 2.

Table 2. Total acid production ability and tyrosine equivalent (µg mL^{-1})

Culture	pH				titratable acidity %				Tyrosine equivalent
	1st day	2nd day	3rd day	7th day	1st day	2nd day	3rd day	7th day	
Leuconostoc mesenteroides ssp *mesenteroides/dextranicum* M170408	4.74	4.71	4.73	4.75	0.22	0.24	0.24	0.36	14.76
Lactobacillus plantarum MRS0501	4.74	4.55	4.47	4.41	0.22	0.25	0.28	0.32	<1
Leuconostoc mesenteroides ssp *mesenteroides/dextranicum* M170702	4.86	4.76	4.78	4.74	0.20	0.21	0.22	0.24	<1
Lactobacillus plantarum MRS0703	4.63	4.47	4.39	4.25	0.23	0.28	0.30	0.35	<1
Lactobacillus plantarum M170709	4.75	4.47	4.40	4.26	0.18	0.26	0.29	0.34	<1
Lactobacillus brevis MRS0801	4.80	4.57	4.61	4.55	0.24	0.26	0.25	0.40	<1
Lactobacillus plantarum M170803	4.79	4.62	4.53	4.34	0.18	0.24	0.25	0.30	<1
Lactobacillus plantarum M170806	4.76	4.63	4.54	4.33	0.20	0.23	0.25	0.30	<1
Streptococcus sp. MRS1001	4.55	4.45	4.56	4.40	0.24	0.27	0.26	0.30	<1
Lactococcus lactis ssp *lactis* MRS1003	4.79	4.55	4.48	4.39	0.22	0.24	0.27	0.31	<1
Streptococcus sp. M171103	4.73	4.59	4.66	4.46	0.21	0.24	0.24	0.25	<1
Lactobacillus brevis MRS1103	4.53	4.64	4.52	4.40	0.24	0.26	0.26	0.28	<1
Enterococcus sp. M171305	4.68	4.50	4.47	4.27	0.24	0.26	0.30	0.32	<1
Leuconostoc mesenteroides ssp *mesenteroides/dextranicum* M171306	4.65	4.54	4.54	4.48	0.23	0.26	0.26	0.27	15.51
Lactobacillus plantarum M171307	4.62	4.42	4.46	4.24	0.21	0.26	0.28	0.32	2.51
Lactococcus sp. M171310	4.57	4.49	4.52	4.44	0.22	0.27	0.25	0.28	22.19
Lactobacillus brevis M171313	4.70	4.56	4.51	4.52	0.21	0.26	0.25	0.27	17.74
Lactobacillus plantarum MRS1315b	4.63	4.61	4.55	4.34	0.17	0.24	0.25	0.30	<1
Lactobacillus plantarum MRS1317	4.50	4.48	4.56	4.34	0.26	0.24	0.26	0.30	3.81
Medium	4.69	4.56	4.54	4.41	0.22	0.25	0.26	0.31	

According to the results of the study, PAD values were found to be in the range of <1-22.19 µg tyrosine mL^{-1}. The highest proteolytic activity was determined in *Lactococcus* sp. M171310. Three strains were (*L. mesenteroides/dextranicum* M170408, *L. mesenteroides/dextranicum* M171306, and *L. brevis* M171313) showed moderate proteolytic activity values, and 15 strains showed weak proteolytic activity. The similarity of proteolytic activity values formed by *L. mesenteroides/dextranicum* M170408 and *L.*

mesenteroides/dextranicum M171306, and *L. plantarum* M171307 and *L. plantarum* MRS1317.

It is stated that *P. pentosaceus* and *L. helveticus* isolated from different foods are compatible with the values sought in starter cultures and some of *L. plantarum* isolates have high proteolytic activity (Ertekin and Çon, 2014). In a study, strains without proteolytic activity were used in Beyaz cheese production (Öner et al., 2006).

In the study, the disc diffusion method and E-test were used to determine the antibiotic susceptibilities of the strains. The resistance properties of chloramphenicol, tetracycline, penicillin, erythromycin, and vancomycin were examined (Table 3). As can be seen in Table 3, the strains formed an average of 26 mm zones in chloramphenicol and only *L. plantarum* M170803 strain showed resistance to chloramphenicol. As a result of chloramphenicol resistance test, it was determined that a 22-30 mm diameter zone was formed at non-resistant strains. Among these strains, the strains forming 22 mm zone diameter were *Lc. lactis* spp *lactis* MRS1003, *Enterococcus* sp. M171305, *Leu. mesenteroides/dextranicum* M171306, *L. plantarum* M171307, and *L. plantarum* MRS1317, while the strain forming 30 mm zone diameter was *L. plantarum* M170709. The zone diameters formed by the strains in tetracycline were in the range of 26-37 mm and on average 31 mm in diameter. *Leu. mesenteroides/dextranicum* M170702, and *L. plantarum* MRS1315b strains showed resistance to tetracycline. While the strains forming 26 mm zone diameter in tetracycline were *Streptococcus* sp. MRS1001, *L. brevis* MRS1103 and *Leu. mesenteroides/dextranicum* M171306, it was determined that the strain forming 37 mm zone diameter was *L. plantarum* MRS0501. While penicillin resistance average was 28 mm, it was determined that it was in the range of 22-36 zone diameters, and in erythromycin, it was in the range of 22 mm and 10-32 mm. It was determined that 6 strains showed resistance in vancomycin. Strains that resist vancomycin were *Leu. mesenteroides/dextranicum* M170408, *L. plantarum* MRS0501, *Leu. mesenteroides/dextranicum* M170702, *L. plantarum* MRS0703, *L. plantarum* M170709, and *L. brevis* MRS0801. While the resistance values against vancomycin were an average 22 mm zone diameter, this value formed a zone diameter in the range of 0-28 mm. The largest zone diameter was determined in strain *L. brevis* M171313. It has been shown that the lowest effective amount of chloramphenicol in strains ranged from 1.0-64 µg mL^{-1}, while tetracycline ranged from 0.125 µg mL^{-1} to 96 µg mL^{-1}, penicillin 0.094-1.5 µg mL^{-1}, erythromycin 0.064-1.0 µg mL^{-1}, and vancomycin ranging from 0.25 µg mL^{-1} to >256 µg mL^{-1}.

Artificial gastric juice resistance values are given in Table 4. According to the results obtained, no viability was detected at pH 2.0 after 3 hours of incubation. At pH 3.0, viability was determined between 89.49 and 111.79%. Accordingly, *L. plantarum* M170803 strain showed an increase of one log from 8.23 log to 9.20 log as a result of 3-hour incubation at pH 3.0 hence the highest viability is seen with it.

Table 3. Antibiotic resistance properties of cultures by zone diameters (mm) and E-test values (µg mL^{-1})

	Zone diameters					**E-test values (µg mL^{-1})**				
Culture	**Cl**	**TE**	**P**	**E**	**VA**	**Cl**	**TE**	**P**	**E**	**VA**
Leu. mesenteroides/dextranicum M170408	27	35	36	27	-	2.0	0.38	0.50	0.064	>256
L. plantarum MRS0501	28	37	31	10	-	6.0	0.75	1.0	0.094	>256
Leu. mesenteroides/dextranicum M170702	26	-	36	28	-	1.5	96	0.25	0.094	>256
L. plantarum MRS0703	26	36	22	32	-	1.5	0.5	1.5	0.064	>256
L. plantarum M170709	30	32	30	30	-	1.5	0.25	0.5	0.5	>256
L. brevis MRS0801	28	28	36	24	-	2.0	0.50	0.25	0.25	>256
L. plantarum M170803	-	28	28	12	22	64	0.125	0.125	0.25	0.38
L. plantarum M170806	26	32	30	12	24	2.0	0.19	0.19	0.38	0.38
Streptococcus sp. MRS1001	28	26	26	23	22	2.0	0.25	0.50	0.094	0.38
Lc. lactis spp *lactis* MRS1003	22	28	26	22	23	1.5	0.19	0.50	0.125	0.38
Streptococcus sp. M171103	26	36	34	28	25	2.0	0.19	0.19	0.064	0.38
L. brevis MRS1103	26	26	22	22	21	2.0	0.25	0.75	0.125	0.38
Enterococcus sp. M171305	22	32	24	11	23	2.0	0.19	0.125	0.064	0.25
Leu. mesenteroides/dextranicum M171306	22	26	30	22	20	2.0	0.19	0.19	0.25	0.38
L. plantarum M171307	22	34	22	10	22	2.0	0.19	0.75	1.0	0.38
Lactococcus sp. M171310	26	32	26	24	22	1.0	0.19	0.19	0.094	0.25

Table 3 (continued). Antibiotic resistance properties of cultures by zone diameters (mm) and E-test values (µg mL^{-1})

L. brevis M171313	28	34	28	28	28	3.0	0.25	0.19	0.094	0.25
L. plantarum MRS1315b	28	-	32	28	26	2.0	96	0.094	0.125	0.38
L. plantarum MRS1317	22	28	22	22	24	2.0	0.25	0.25	0.094	0.38
Medium	**26**	**31**	**28**	**22**	**23**					

In a study by Karasu (2006), the development of lactic starter cultures isolated from pickles and olives in pH 2.0 and pH 3.0 were investigated. Accordingly, it is stated that all the isolates obtained survive significantly at pH 3.0, whereas approximately 60% of the isolates are not alive at pH 2.0. The tolerance of 274 LABs to the gastrointestinal environment was investigated by incubating for 4 hours at 37 °C in artificial stomach juice (pH 3.0) containing 3 mg mL^{-1} pepsin. While 10% strain of the tested LABs was found to have a viability more than 50%, only a decrease in the viability rates of the same strains was reported at pH 2.5 (Hwanhlem et al., 2010). In a study by Mishra and Prasad (2005), it was reported that only 3 strains out of 7 tested LABs were resistant to pH 2.0 or 3.0. It is stated that probiotic bacteria may have decreased viability due to the pepsin enzyme or due to the combined effects of pepsin and high pH. On the other hand, milk proteins have a protective effect on bacteria, allowing the bacteria to survive in the acidic environment of the stomach. For this reason, it is generally recommended to use probiotic bacteria together with milk or meat. In another study, an environment similar to gastric juice conditions was tried to determine the ability of probiotics to reach the intestines from the highly acidic environment of the stomach, and *Lactobacillus* growth pH 2.0, pH 3.0, pH 4.0 values were observed in their environment. When the results were evaluated, it was observed that all strains showed weak growth in pH 2.0 and 3.0 in general. It was observed that *L. fermentum* BSP1, *L. brevis* BT1, KMP1, *L. helveticus* MNK3 strains showed the best development at pH 4.0 and other bacteria showed weak growth. With the increase of the acidity rate in the environment, the reproduction density of the bacteria decreased. Another feature of probiotic bacteria is that bacteria can also resist the small intestinal juice medium after gastric juice. Following the passage of the stomach, the small intestine is the second major barrier in the gastrointestinal tract. Although the pH of the small intestine is more suitable for the survival of bacteria, the presence of pancreatin and bile salts can have a negative effect. The pH of stomach juice is around 1.0. On the other hand, the pH of stomach juice reaches 3.0 and higher with the consumption of dairy products and other meals. For this reason, LABs were found to be able to maintain their viability at pH 3.0 and these bacteria gained importance in terms of probiotics (Dikbaş Yıldız, 2013). The number of plasmid DNA and the molecular sizes of these plasmids are given in Table 4. As can be seen in Table 4, it was determined that plasmid DNA was not found in 7 strains, whereas other strains' plasmid DNA numbers were between 1 and 5. Molecular sizes of the isolated plasmid DNA range between 2–16 kilobase (kb).

Table 4. Resistance to artificial gastric juice and plasmid numbers and sizes of strains

Culture	pH 3			pH 2			Plasmid Number	Plasmid Size (kb)
	0 hours (log mL^{-1})	3 hours (log mL^{-1})	Viability (%)	0 hours (log mL^{-1})	3 hours (log mL^{-1})	Viability (%)		
Leu. mesenteroides/dextranicum M170408	8.00	7.54	94.25	7.81	0	0	1	10
L. plantarum MRS0501	8.38	8.43	100.60	7.70	0	0	1	10
Leu. mesenteroides/dextranicum M170702	8.08	7.79	96.41	7.95	0	0	3	10, 12, 14
L.plantarum MRS0703	8.61	8.62	100.12	8.32	0	0	-	-
L.plantarum M170709	8.34	8.00	95.92	8.08	0	0	-	-
L. brevis MRS0801	8.15	8.00	98.16	7.71	0	0	1	10
L. plantarum M170803	8.23	9.20	111.79	8.04	0	0	1	10
L.plantarum M170806	8.18	7.32	89.49	7.95	0	0	1	10
Streptococcus sp. MRS1001	8.40	8.30	98.81	7.71	0	0	1	10
Lc. lactis ssp *lactis* MRS1003	8.38	8.20	97.85	7.91	0	0	4	2, 10, 12, 14
Streptococcus sp. M171103	8.28	7.85	94.81	7.46	0	0	5	2, 10, 12, 14, 16
L. brevis MRS1103	8.48	8.15	96.11	8.00	0	0	-	-

Table 4 (continued). Resistance to artificial gastric juice and plasmid numbers and sizes of strains

Enterococcus sp. M171305	8.53	8.52	99.88	8.04	0	0	-	-
Leu. mesenteroides/dextranicum M171306	8.40	8.26	98.33	7.92	0	0	-	-
L. plantarum M171307	8.32	7.85	94.35	7.85	0	0	5	2, 10, 12, 14, 16
Lactococcus sp. M171310	8.34	7.92	94.96	7.20	0	0	-	-
L. brevis M171313	8.30	8.00	96.39	7.94	0	0	5	2, 10, 12, 14, 16
L. plantarum MRS1315b	8.20	8.04	98.05	8.11	0	0	5	2, 10, 12, 14, 16
L. plantarum MRS1317	8.15	7.85	96.32	8.45	0	0	-	-

When all strains were examined, the highest plasmid size was determined to be 16 kb. In many different studies, no linear relationship has been determined between the medium in which bacteria are isolated and the number and size of plasmid DNA they contain (Rossi et al., 2008). Similarly, there are studies in which the number and size of plasmids contained in cultures belonging to the same species isolated from the same environment are different. In general, LAB differ in the number, size and function of the plasmid they contain. It was determined that LAB sizes were 0.87-250 kb and plasmid number was 0-16 (Sağlam and Karahan, 2017).

In conclusion, İn the present study, lactic acid bacteria isolates from cheeses were found to have potentially probiotic and technological properties. A total of 91 lactic acid bacteria indicated Gram-positive and catalase-negative properties, 19 of them *Lactobacillus plantarum*, *Leuconostoc mesenteroides* spp. *mesenteroides/dextranicum*, *Lactobacillus brevis*, *Streptococcus* spp., *Lactococcus lactis* spp *lactis*, *Enterococcus* sp., and *Lactococcus* sp. were identified. In this study, the probiotic properties of these nineteen lactic acid bacteria demonstrated.

Due to the determination of the resistance of these lactic acid bacteria to acidic environments and bile salts, it will be possible for them to pass through the stomach and growth in the intestinal microflora. It was determined that the majority of lactic acid bacteria, which were determined to have probiotic potential, were sensitive to the antibiotics used in the research. It was observed that the acid production properties of the strains were low so can be used as starter culture alone. In the study, it was determined that the lactic acid bacteria obtained have probiotic potential and can also be used as starters in cheese and other fermented products. Further research work is needed to evaluate the *in vivo* probiotic characteristics and technological properties of these potential lactic acid bacteria. Since the selected lactic acid bacteria have good acid-producing abilities, they can be considered as starter cultures in fermented products. However, due to the low proteolytic activities of these bacteria, it may be possible to use mixed starter cultures for ripening cheeses. Also this research will enable us to establish a collection of lactic bacteria with probiotic potential from local products. These results obtained in our study will contribute to future probiotic researches, and probiotic cultures with these characteristics can be used in different fields and applications.

ÖZET

Amaç: Bu çalışmanın amacı Kilis yöresinde çiğ sütten üretilen 15 adet Türk Beyaz taze peynirinden izole edilen laktik asit bakterilerinin (LAB) teknolojik özellikleri ve probiyotik potansiyellerini değerlendirmektir.

Yöntem ve Bulgular: 15 peynir örneğinden 287 koloni seçilmiş ve bunlardan 91'i Gram pozitif ve katalaz negatif olarak analiz edilmiştir. 91 koloninin 19 suşu pH 3.5 ve %0.3 safra tuzunda canlı kaldıkları için potansiyel probiyotik olarak kabul edilmiştir. Biyokimyasal testler ve API test kiti kullanılarak yapılan tanımlama sonucunda Lactobacillus sp. (11 suş), Leuconostoc sp. (3 suş), Streptococcus sp. (2 suş), Lactococcus sp. (2 suş) ve Enterococcus sp. (1 suş) belirlenmiştir. Asidifikasyon hızı bakımından 19 suşun yüksek; proteolitik olarak 15 suşun zayıf, 3'ünün orta ve 1'inin güçlü aktivite gösterdiği belirlenmiştir. Antibiyotik dirençlilik açısından bakıldığında, suşlardan 1'inin kloramfenikole, 2'sinin tetrasikline ve 6'sının vankomisine dirençli olduğu belirlenmiştir. Penisilin ve eritromisine dirençli suş tespit edilememiştir. Yapay mide özsuyu direnç testinde pH 2,0'da canlılık saptanmamasına rağmen, pH 3.0'da canlılık değerlerinin %89,49-111,79 arasında değiştiği tespit edilmiştir. Suşların plazmit profillerine göre 19 potansiyel probiyotik suşun 13'ünün 1-5 aralığında plazmit DNA'sına sahip olduğu, diğer suşların plazmit DNA'sına sahip olmadığı belirlendi. İzole edilen suşların plazmit DNA'sının moleküler boyutları 2-16 kb arasında değişmekteydi.

Genel Yorum: Araştırmada, Kilis'te üretilen Türk beyaz taze peynirlerden izole edilen LAB'ların probiyotik potansiyelleri olduğu ve çeşitli fermente gıdalarda starter olarak da kullanılabilecekleri belirlenmiştir.

Çalışmanın Önemi ve Etkisi: Elde edilen sonuçlara göre bazı suşların peynir üretiminde probiyotik starter kültür olarak, bazılarının ise peynirin olgunlaşmasında kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Laktik asit bakterileri, başlatıcı kültür, teknolojik özellikler, probiyotik özellikler, Türk beyaz taze peynir

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

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