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Discovery of Novel Probiotics That Can Support Gut Microbiota

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Abstract

Today's stressful living conditions, high consumption of refined food, unbalanced diet and irregular use of antibiotics adversely affect the human body and microbiota. In the study, it was aimed to isolate probiotic microorganisms from local fermented milk products (white cheese and tulum cheese) and to investigate some properties of these microorganisms in order to be used as nutritional support. Microorganisms were isolated by culture method. They were identified by carbohydrate fermentation and molecular tests. Acid-bile tolerance, exopolysaccharide production, antimicrobial activities and resistance profile against some antibiotics were investigated. Considering the probiotic properties investigated, it is predicted that 5 Lactobacillus spp. strains meet the desired criteria and can be used in the development of new products. In order to support the gastrointestinal microbiota affected by adverse conditions and to provide flora balance, it is important to supplement with superior probiotic products. It is very important to carry out further studies with the probiotic bacteria we obtained from our study and to include these bacteria in probiotic product ingredients in terms of microbiota support.

Keywords: *Gut microbiota, Probiotics*

I. INTRODUCTION

Probiotic studies began in the early 20th century by Ellie Metchnikoff with the findings that some food supplements positively support the gut microflora and inhibit the growth of some toxin-producing bacteria [1]. Probiotic microorganisms, known to have beneficial properties for human health, represent an important part of the gut microbiome that regulates various systems. Probiotic bacteria are included in food supplements and fermented foods as living microflora [2].

The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) Working Group define probiotics as "living microorganisms that, when administered in sufficient quantities, have beneficial effects on health on the host" [3]. It is known that probiotic microorganisms have health-regulating role such as strengthening the intestinal barrier, contributing to the diarrhea treatment process, antimicrobial activity, managing lactose intolerance, lowering serum cholesterol levels, supporting the immune system and reducing the risks of some cancer types [4, 5, 6, 7, 8]. In our age, the importance of healthy nutrition, changing living conditions and increasing education level have increased the search for new functional foods and consumption. One of the most preferred functional food industries is probiotics. Studies on this subject and the search for new products are increasing. Fermented milk and dairy products (yogurt, kefir, cheese, butter, etc.) produced with newly discovered probiotic microorganisms have become more popular [9,

10]. The identification and discovery of novel probiotics will be more beneficial in terms of health and use in the food industry. In our study, it was aimed to isolate new probiotic microorganisms from local fermented milk products and to determine some probiotic properties of them.

II. MATERIALS AND METHODS

II.1.Isolation

Local fermented milk products (5 white cheese and 3 tulum cheese) (5 g) were shaken well with 45 ml PBS solution and homogenized, then diluted, ranging from 10^{-1} to 10^{-6}. 0.1 ml of samples were plated on de Man, Rogosa and Sharpe (MRS) agar and incubated at 42±0.5 ºC for 48 hours (10% CO_2). The isolation of bacteria was determined by gram staining, catalase test and colony morphologies were purified by subsequent subcultures. After the pure cultures were grown in MRS broth at 42±0.5 ºC for 24 hours (10% CO_2), 0.1 ml culture were transferred into fresh medium and incubated at 42±0.5 ºC for 16-18 h (10% CO_2). The pure cultures were stored at −80°C for further examination.

II.2.Identification of Species

Biochemical identification: A total of 28 LAB strains were isolated from fermented milk samples. API 50CHL kit (Biomerieux, Marcy l'Etoile, France) was used according to the manufacturer's instructions based on bacterial carbohydrate fermentation. According to the color change, the results were evaluated as (+/-). Strains were determined according to their biochemical profiles with database (V5.1) and apiweb™ identification software.

Molecular identification: Firstly DNA extraction and then polymerase chain reaction (PCR) methods were used to identify the twenty-eight isolated LABs by molecular methods. It was developed in MRS broth for 16-18 hours at 42 ± 1 °C (10% CO_2) for DNA extraction. Genomic DNAs for 16S rRNA analysis from isolates were extracted using commercial DNA extraction kit (PureLinkGenomic DNA Kit, Invit-rogen K) as indicated in the user manual. The DNA obtained was collected in 75 µl washing solution and stored at -20 ºC. 16S rRNA gene regions were amplified by PCR. 16S rRNA universal primers 27F (5p-AGAGTTTGATCCTGGCTCAG-3 ') and 1492R (5p-TACGGTTACCTTGTTACGACTT-3') were used for PCR. For PCR reaction using thermal cycler (Bio-Rad T100, USA); at 94 ° C for 5 minutes, 94 ° C for 1 minute 40 cycles, at 42 ° C for 1 minute, at 72 ° C for 1 minute and final elongation at 72 ° C for 10 minutes. PZR products were run in 1% agarose gel and visualized under UV light. The PCR product was sequenced with an Applied Biosystems BigDye terminator loop sequencing version 2.0 kit (Applied BioSystems, Foster City, CA). The sequencing products were purified with Sephadex spin columns (Cold Spring Harbor Protocol, 2002, California) and resolved with an Applied Biosystems model 3130XL automated DNA sequencing system (Applied Biosystems). The results displayed by the Sequencing Analysis Software v5.3.1 were investigated with the NCBI blast tool to find percentages of identity with sequences contained in the databases.

II.3.Evaluation of acid and bile tolerance

In order to determine the resistance of bacteria in acidic conditions in the stomach, artificial gastric juice was prepared and their vitality was determined at certain times. Pre-study bacteria were activated twice, and then their density was adjusted to 0.6 by spectrophotometer (Biochrom, Bioscience, USA) (OD_{600} = ~ 0.600). The bacteria added at a rate of 2% to artificial gastric juices with different pH values (2.0; 2.5; 3.0; 4.0 and 6.8 (control). The viability rates of the control group were accepted as 100% and the viability rates in other pH conditions were calculated accordingly. Then they were incubated under microaerophilic conditions at 42±1 °C for 24 hours and absorbance were determined at 600 nm by spectrophotometer. Survival percentages of microorganisms in media with different acid concentration were calculated by the following formula;

$$\%\text{vitality} = (OD_{600\ (\text{sample})} \times 100) / OD_{600\ (\text{control})}$$

II.4.Evaluation of bile tolerance

The bile solutions were prepared with powdered bile extract (Oxbile, Sigma) and then these solutions were filter sterilized by 0.42 µm filter, finally bile solutions were added in MRS broth media to be a final concentration of 0.3%; 0.5% and 1.0%. MRS broth without bile was used as a control. The isolates were grown in MRS broth under microaerophilic conditions at 42 ± 1°C overnight and absorbance adjusted to OD_{600}=0.6 value, 2% of cultures were inoculated into bile-media and incubated at 42±1°C for 24 h. Bacterial growth was measured spectrophotometrically at 600 nm. All experiments were repeated twice. The survival ratio was calculated with the same formula of acid tolerance.

II.5.Exopolysaccharide (EPS) production

In the EPS production experiments, twice activated and absorbance adjusted (OD_{600} = ~0.600) cultures were used. Then, 1 ml of activated cultures was incubated in boiling water for 10 minutes and 170 µl of 85% TCA was added on cooled samples. The cultures were centrifuged at 13 000 rpm for 25 min. The supernatant was mixed with equal volume of ethanol and centrifugation at 13 000 rpm for 15 min. The pellet was dissolved in 1 ml of ultrapure ddH_2O. EPS production capacity was determined by modified penol-sulphuric acid method [11]. To 1 ml of sample, 0.5 ml 5% (w/v) phenol was added followed by 5 ml concentrated sulphuric acid. The mixture was incubated at room temperature for 10 min, then incubated at 30ºC for 20 minute and the absorbance was measured at 490 nm. The standards were prepared using glucose in the range of 0-100 mg/L. According to standard curve equation, the amounts of EPS produced by the samples were calculated as mg/ml.

II.6.Evaluation of antimicrobial effects

The antimicrobial effect of the isolates against test microorganisms was determined by disk diffusion method. Gram-positive *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), Gram negative *Pseudomonas aeroginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were used as test microorganisms and *Candida albicans* (ATCC 10231) strains as fungi. After these microorganisms were activated twice in the appropriate medium, their absorbance was adjusted as 0.600 at OD_{600}. 100 µl of the densities adjusted cultures were inoculated into Müller-Hinton Agar medium. 5 µl and 10 µl of isolates were dropped onto sterile discs on which filters were placed. It was incubated at 42 ° C for 24 hours under microaerophilic conditions. After incubation, inhibition zone diameters were measured as mm ± standard deviation.

II.7.Antibiotic susceptibility's testing

Antibiotic susceptibility was determined by disc diffusion method. Fifteen antibiotics discs used and results were evaluated according to The European Committee on Antimicrobial Susceptibility Testing. (Version 9.0, 2019. http://www.eucast.org.). The results (mean four readings) were expressed as sensitive (S), partially sensitive (I) and resistant (R). Used discs; AZM: Azithromycin, SAM: Ampicillin/Sulbactam, MUP: Mupirocin, AMP: Ampicillin, AMC: Amoxicillin/Clavulonic acid, CIP: Ciprofloxacin, AK: Amikacin, TE: Tetracycline, CN: Gentamicin, OX: Oxacillin, VA: Vancomycin: Erythromycin, S: Streptomycin, C: Chloramphenicol, P: Penicillin.

II.8.Statistically analysis

The statistical analyses were performed according to SPSS Inc. Software (22.0 version; SPSS Inc., Chicago, IL) program. Data are reported as means ± SD. Pearson's correlation was used for determine any significant differences between EPS production and different pHs/different bile concentrations. The critical significance level for the statistical tests was determined as 0.05.

III. RESULTS AND DISCUSSION

III.1. Identification and Isolates of Bacteria

Twenty-eight isolates showed the typical features of LAB (cocci, rod-shaped, Gram-positive, and catalase negative). The biochemical and molecular characterization of all isolates were determined. The strains were identified using API 50 CHL medium (Bio Mérieux La Bali Grottes, France). The biochemical and molecular data were given in Table 1. In this study we conducted, 28 *Lactobacillus* spp. strain was isolated and 4 different *Lactobacillus* spp. has been defined. From 5 white cheese samples, 18 *Lactobacillus* spp. were isolated and from 3 Tulum cheese samples, 10 *Lactobacillus* spp. were obtained. The distribution of the isolated strains by species was determined as 13 *L. delbrueckii* (46.4%), 7 *L. fermentum* (25%), 6 *L. helveticus* (17.9%) and 2 *L. plantarum* (10.7%). The strain codes, biochemical and molecular similarity rates of the isolates are shown in Table 1. Similarly, Erginkaya et al. (2018) *Lactobacillus* spp., *Streptococcus* spp., *Bifidobacterium* spp. and *Enterecoccus* spp. isolated from different some dairy products such as yoghurt, white cheese and cokelek [12].

TABLE 1. THE BIOCHEMICAL AND MOLECULAR IDENTIFICATION DATA OF STRAINS

Strains Code	Similarity (%)		Species		Strains Code	Similarity (%)		Species
	Biochemical	Molecular				Biochemical	Molecular	
El-A2	96	99	*L. delbrueckii*		El-A1	100	100	*L. fermentum*
El-A3	96	99			El-A4	100	100	
El-A5	98	100			El-A9	98	100	
El-A6	100	100			El-A10	100	100	
El-A7	98	100			El-A17	100	99	
El-A11	98	100			El-A18	100	100	
El-A12	100	100			El-A21	100	100	
El-A15	96	99			El-A8	100	100	*L. helveticus*
El-A16	96	99			El-A13	100	100	
El-A22	100	100			El-A23	96	100	
El-A25	100	100			El-A29	100	99	
El-A28	100	100			El-A37	100	100	
El-A31	100	100			El-A42	100	100	
El-A24	100	100	*L. plantarum*					
El-A34	100	100						

III.2. Acid and Bile Salt Tolerance

Both low pH resistance and bile tolerance are a very crucial criterion for probiotic microorganisms. Probiotic microorganisms must be able to withstand pH values close to gastric acidity (<3.0) until they reach the small intestine [13, 14, 15]. For this reason, it is very important to have high bile salt and acid tolerance to evaluate microorganisms as probiotics [16]. The conditions between pH 1.5 and 4.0 values were evaluated in studies [17, 18]. We also used pH 2.0, 2.5, 3.0, 4.0 and 6.8 (control) in our study. As seen in Table 2, as the pH value increases the cells stay alive and an increase in their number is observed. Generally, it is observed that all microorganisms maintain their vitality at pH 3.0 level. It was determined that some of the strains we obtained in our study could not survive in low pH conditions (especially below pH 3). However, the survival rate of 6 *L. delbruckii*, 4 *L. fermentum* and 2 *L.helveticus* strains was found to be sufficient to reach the intestines under low

pH (pH 2.0 and pH 2.5) conditions. It was determined that El-A4, El-A5, El-A10, El-A28, and El-A31 strains had higher viability than others (Table 2). In the study of Ahmed et al. (2019), survival rate of some lactobacillus species obtained from fermented products under pH 3 conditions was reported as 90% [19]. Another important criterion for probiotic microorganisms is tolerance to bile salts. Because the bacteria that enter the human digestive system encounter bile acids and salts in the intestines after passing through the acid environment of the stomach. In order for these bacteria to survive, their bile salt tolerance must be high [20]. Bile salts have many important functions such as supporting the digestive system or antimicrobial activity. However, they also have functions that can adversely affect bacteria. It has disadvantages such as intracellular acidification, cell wall damage and oxidative stress development. Therefore, the capacity of strains to tolerate bile is important for evaluating the probiotic properties of microorganisms [21]. In our study, microorganisms were grown in media containing 0.3%, 0.5%, and 1.0% (w/v) concentration of bile to investigate bile survivability. The fourteen strains had good resistance in media including 0.3% and 1.0% bile (Table 3). It was determined that the bile tolerance of El-A4, El-A5, El-A10, El-A28, and El-A31 strains with high acid resistance was higher than the other strains.

TABLE 2. ACID RESISTANCE DATA OF STRAINS

Species	Strains Code	Acid resistance				
		pH2.0	pH2.5	pH3.0	pH4.0	pH6.8
L. delbrueckii	El-A2	-	-	0.80±0.01	1.43±0.04	1.88±0.11
	El-A3	0.24±0.01	0.43±0.01	0.98±0.01	1.42±0.01	1.82±0.01
	El-A5	0.52±0.01	0.66±0.01	0.82±0.01	1.32±0.01	1.66±0.01
	El-A6	-	-	0.32±0.01	0.65±0.02	0.92±0.01
	El-A7	0.22±0.01	0.51±0.02	0.71±0.01	1.12±0.01	1.66±0.31
	El-A11	-	0.32±0.01	0.59±0.03	0.98±0.01	1.42±0.27
	El-A12	-	-	0.22±0.01	0.66±0.02	0.97±0.01
	El-A15	-	-	0.41±0.01	0.82±0.02	1.51±0.01
	El-A16	-	-	0.21±0.01	0.56±0.01	0.79±0.03
	El-A22	0.32±0.01	0.67±0.03	0.82±0.01	1.42±0.21	1.96±0.11
	El-A25	-	-	0.44±0.01	0.72±0.01	0.98±0.01
	El-A28	0.54±0.01	1.03±0.03	1.72±0.01	2.11±0.02	2.36±0.42
	El-A31	0.82±0.02	1.23±0.07	1.57±0.12	2.21±0.01	2.43±0.08
L. fermentum	El-A1	0.23±0.01	0.38±0.01	0.65±0.02	0.98±0.06	1.65±0.45
	El-A4	0.62±0.01	0.92±0.01	1.52±0.01	1.86±0.01	2.42±0.01
	El-A9	-	0.41±0.03	0.82±0.04	1.62±0.01	2.21±0.01
	El-A10	0.56±0.01	0.74±0.02	0.91±0.01	1.46±0.52	2.14±0.26
	El-A17	-	0.46±0.01	0.81±0.01	1.16±0.13	1.57±0.03
	El-A18	-	0.32±0.01	0.57±0.01	0.82±0.01	1.36±0.01
	El-A21	0.34±0.01	0.45±0.01	0.67±0.02	1.14±0.01	1.88±0.47
L. helveticus	El-A8	-	-	0.12±0.01	0.71±0.05	0.82±0.01
	El-A13	-	-	0.24±0.01	0.51±0.01	1.22±0.01
	El-A23	0.32±0.01	0.42±0.01	0.78±0.03	1.12±0.01	1.79±0.44
	El-A29	0.38±0.01	0.55±0.01	0.76±0.01	1.42±0.07	2.23±0.23
	El-A37	-	-	0.33±0.01	0.38±0.01	0.67±0.01
	El-A42	-	0.38±0.01	0.55±0.01	0.86±0.01	1.46±0.01
L. plantarum	El-A24	-	-	0.24±0.01	0.52±0.01	0.92±0.02
	El-A34	-	-	0.16±0.01	0.38±0.01	0.80±0.01

TABLE 3. BILE TOLERANCE DATA OF STRAINS

Species	Strains Code	Bile tolerance (%)			
		1.0	0.5	0.3	Control
L. delbrueckii	El-A2	0.12±0.01	0.68±0.02	1.10±0.01	1.43±0.04
	El-A3	0.44±0.01	0.73±0.01	1.28±0.01	1.42±0.01
	El-A5	0.72±0.01	0.86±0.01	0.92±0.01	1.32±0.01
	El-A6	-	-	0.12±0.01	0.65±0.02
	El-A7	0.31±0.01	0.68±0.02	0.82±0.01	1.12±0.01
	El-A11	0.13±0.01	0.37±0.01	0.48±0.03	0.98±0.01
	El-A12	-	0.23±0.01	0.41±0.01	0.66±0.02
	El-A15	-	-	0.21±0.01	0.82±0.02
	El-A16	-	-	0.10±0.01	0.56±0.01
	El-A22	0.33±0.01	0.57±0.03	0.76±0.07	1.42±0.21
	El-A25	-	-	0.28±0.01	0.72±0.01
	El-A28	0.86±0.03	1.52±0.13	1.63±0.13	2.11±0.02
	El-A31	0.97±0.08	1.73±0.17	1.97±0.21	2.21±0.01
L. fermentum	El-A1	0.13±0.01	0.68±0.01	0.85±0.04	0.98±0.06
	El-A4	0.71±0.02	1.21±0.01	1.62±0.04	1.86±0.01
	El-A9	-	0.11±0.01	0.32±0.04	1.62±0.01
	El-A10	0.75±0.04	0.96±0.02	1.10±0.01	1.46±0.52
	El-A17	-	0.16±0.01	0.27±0.01	1.16±0.13
	El-A18	-	0.11±0.01	0.32±0.01	0.82±0.01
	El-A21	0.62±0.01	0.85±0.04	0.92±0.03	1.14±0.01
L. helveticus	El-A8	-	0.48±0.02	0.52±0.01	0.71±0.05
	El-A13	-	-	0.11±0.01	0.51±0.01
	El-A23	0.17±0.01	0.38±0.01	0.51±0.03	1.12±0.01
	El-A29	0.29±0.01	0.75±0.01	0.84±0.01	1.42±0.07
	El-A37	-	-	0.13±0.01	0.38±0.01
	El-A42	-	0.27±0.01	0.34±0.01	0.86±0.01
L. plantarum	El-A24	-	0.14±0.01	0.47±0.03	0.52±0.01
	El-A34	-	-	0.09±0.01	0.38±0.01

III.3. Determination of Exopolysaccharide Production

Exopolysaccharides are an important structure that acts as a protective barrier for bacteria. EPS production amount is affected by many conditions such as heavy metal concentration, salt ions, pH, temperature, oxygen concentration or media composition [22]. Researches show that the amount of exopolysaccharide production of bacteria at genus and species level varies greatly. In fact, different production amounts are determined in different strains of the same species [10, 22, 23,]. In some studies, *L. fermentum* strains isolated from cheese have been reported to have EPS content varying between 100-600 mg/L [24, 25]. In studies conducted with *Lactobacillus delbrueckii* subsp *bulgaricus*, an EPS production capacity of 5-175 mg / L was shown [24]. In our study, it was found that the lactobacilli isolated have an EPS production between 31-321 mg / L (Table 4). The data we have obtained are similar to the data of many study groups and it has been determined that some species have high EPS production amounts.

TABLE 4. EPS PRODUCTION AMOUNTS OF STRAIN

Species	Strains Code	EPS (mg/L)	Species	Strains Code	EPS (mg/L)
L. delbrueckii	El-A2	36.00±2.0	*L. fermentum*	El-A1	39.26±1.1
	El-A3	154.45±11.5		El-A4	296.88±21.7
	El-A5	198.26±16.2		El-A9	48.24±2.3
	El-A6	42.63±3.4		El-A10	321.36±27.6
	El-A7	184.16±9.4		El-A17	133.67±14.2
	El-A11	63.18±5.1		El-A18	56.89±6.8
	El-A12	57.66±3.6		El-A21	187.28±14.6
	El-A15	168.82±16.6	*L. helveticus*	El-A8	53.60±2.5
	El-A16	171.00±13.8		El-A13	63.26±4.6
	El-A22	39.21±2.4		El-A23	56.45±3.7
	El-A25	38.04±2.8		El-A29	163.11±11.6
	El-A28	304.21±24.9		El-A37	68.68±4.2
	El-A31	289.78±22.2		El-A42	58.34±5.7
L. plantarum	El-A24	31.03±1.6			
	El-A34	53.22±5.8			

III.4. Antimicrobial Activity

In many studies, the ability of LAB to exert antimicrobial effects on many pathogenic bacteria in humans have been demonstrated [26]. *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) were used as pathogenic microorganisms in our study. However, no significant antimicrobial activity was observed in the lactobacilli strains isolated.

III.5. Antibiotic Susceptibility

Antibiotic resistance profile is important in probiotics, which are thought to be included in supplement products in our age when unconscious antibiotic use is common. In this study, it was observed that *Lactobacillus* strains are generally sensitive to antibiotics. All strains were found to be susceptible, especially ampicillin, tetracycline and erythromycin. However, it has been observed that they are more resistant to the commonly used antibiotics gentamicin, chloramphenicol and vancomycin (Table 5). Temmerman et al. (2003), antibiotic susceptibility of LAB isolated from various probiotic products was tested by disk diffusion method. Researchers found that the strains were susceptible to tetracycline and erythromycin [27]. In the study conducted by Arıcı et al. (2004) on 21 *Lactobacillus* strains with probiotic properties, it was stated that the strains were susceptible to tetracycline [28]. Gueimonde et al. (2013) showed that *Lactobacillus* species are mostly resistant to vancomycin, ciprofloxacin, erythromycin and tetracycline, especially *L. fermentum* strain is resistant to ampicillin, penicillin, chloramphenicol, erythromycin, tetracycline antibiotics, while it is sensitive to oxacillin, vancomycin, ciprophlaxin, and stdamlinxin [29]. In the study conducted by Sharma et al. (2017), it was reported that *L. delbrueckii* strains isolated from cheese are susceptible to ampicillin, chloramphenicol, clindamycin, erythromycin and tetracycline, but resistant to vancomycin, ciprophylaxin, gentamicin and streptomycin [30].

TABLE 5. *LACTOBACILLUS* SPP. SUSCEPTIBILITY RESULTS OF STRAINS TO DIFFERENT ANTIBIOTICS (MM)

Strains	Antibiotics														
	AZM	SAM	MUP	AMP	AMC	CIP	AK	TE	CN	OX	VA	E	S	C	P
El-A2	S	S	I	S	S	I	I	S	R	R	R	S	S	R	S
El-A3	S	S	R	S	S	I	I	S	S	R	R	S	S	S	S
El-A5	S	S	I	S	R	R	R	S	R	R	I	S	I	R	S
El-A6	S	S	R	S	S	S	S	S	R	R	S	S	I	R	S
El-A7	S	I	I	S	S	S	S	S	S	S	R	S	S	S	S
El-A11	R	I	R	S	S	S	S	S	I	S	R	R	R	R	S
El-A12	I	I	I	S	S	S	R	S	I	S	R	S	S	R	S
El-A15	R	I	I	S	S	S	S	S	R	S	R	R	S	I	S
El-A16	I	I	S	S	S	S	S	S	I	R	I	S	S	I	S
El-A22	S	R	S	S	S	S	R	S	I	I	R	R	S	I	S
El-A25	S	S	S	S	S	S	I	S	I	I	R	S	R	R	I
El-A28	R	S	R	R	I	R	R	S	I	I	R	S	R	I	I
El-A31	S	S	S	R	I	I	I	S	R	R	R	S	I	R	S
El-A1	S	S	S	S	I	I	I	S	S	S	R	S	R	S	S
El-A4	R	S	R	S	I	R	I	S	R	R	R	S	I	R	S
El-A9	S	S	S	S	S	R	S	S	R	R	I	S	I	R	S
El-A10	S	S	R	S	S	S	S	S	R	I	R	S	R	S	S
El-A17	R	S	R	S	S	S	S	S	I	S	R	S	S	I	I
El-A18	I	I	R	S	R	S	R	I	I	S	R	R	S	I	R
El-A21	I	I	R	S	R	I	S	I	R	S	S	S	S	I	R
El-A8	I	I	S	S	R	I	S	S	R	S	R	S	R	I	R
El-A13	I	I	S	S	S	I	S	S	R	S	S	S	I	R	R
El-A23	S	R	S	S	S	R	I	S	I	S	R	S	I	S	R
El-A29	S	I	S	S	S	S	I	S	R	R	R	S	R	R	S
El-A37	S	R	R	S	S	S	S	S	I	I	I	S	S	R	S
El-A42	S	S	I	S	S	S	R	S	R	I	I	S	S	R	S
El-A24	R	S	I	S	I	S	S	S	R	R	S	S	S	R	S
El-A34	R	S	I	S	I	R	S	S	R	R	R	S	S	R	S

Used discs; AZM: Azithromycin, SAM: Ampicillin/Sulbactam, MUP: Mupirocin, AMP: Ampicillin, AMC: Amoxicillin/Clavulonic acid, CIP: Ciprofloxacin, AK: Amikacin, TE: Tetracycline, CN: Gentamicin, OX: Oxacillin, VA: Vancomycin: Erythromycin, S: Streptomycin, C: Chloramphenicol, P: Penicillin.
S: sensitive, I partially sensitive, and R: resistant.

IV. CONCLUSION

Habits such as stressful living conditions of today's people, consumption of high amounts of refined foods, unbalanced nutrition and irregular antibiotic use negatively affect the human body and microbiota. In order to support the gastrointestinal microbiota affected by adverse conditions and to provide flora balance, it is important to supplement with superior probiotic products. It is very important to carry out further studies with the superior probiotic bacteria we obtained from our study and to include these bacteria in probiotic product ingredients in terms of microbiota support.

REFERENCES

[1] M. Govender, Y.K. Choonara, P. Kumar, L.C. du Toit, S. van Vuuren and S. Pillay, A review of the advancements in probiotic delivery: Conventional vs nonconventional formulations for intestinal flora supplementation. *The Association of Pharmaceutical Scientists Journal*, *15(1)*, 2014, 29-43.

[2] M.L. Marco, S. Pavan and M. Klerebezem, M, Towards understanding molecular modes of probiotic action. *Current Opinion in Biotechnology*. *17*, 2006, 204–210.

[3] Food and Agriculture Organization of the United Nations/World Health Organization FAO/WHO. *Guidelines for the Evaluation of Probiotics in Food*; Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food: London, Ontario, Canada. 2002.

[4] G.T. Macfarlane, and J.H. Cummings, Probiotics, infection and immunity. *Current Opinion in Infectious Diseases*. *15*, 2002, 501-506.

[5] J. Hawrelak, Probiotics: choosing the right one for your needs. *Australian Traditional Medicine Society*. *9*, 2003, 67-75.

[6] P. Hlivak, J. Odraska, M. Ferencik, L. Ebringer, E. Jahnova, and Z. Mikes, One-year application of probiotic strain *Enterococcus faecium* M-74 decreases serum cholesterol levels. *Bratisl. Lek. Listy*. *106*, 2005, 67-72.

[7] M. Mego, J. Majek, R. Koncekova, L. Ebringer, S. Ciernikova, P. Rauko, M. Kovac, J. Trupl, P. Slezak, and V. Zajac, Intramucosal bacteria in colon cancer and their elimination by probiotic strain *Enterococcus faecium* M-74 with organic selenium. *Folia Microbiology*. *50*, 2005, 443-447.

[8] M. Cammarota, M. De Rosa, A. Stellavato, M. Lamberti, I. Marzaioli, and M. Giuliano, In vitro evaluation of *Lactobacillus plantarum* DSMZ 12028 as a probiotic: emphasis on innate immunity. *International Journal of Food Microbiology*. *135*, 2009, 90-98.

[9] E.M. Comak-Gocer, F. Ergin, and A. Kucukcetin, Sindirim Sistemi Modellerinde Probiyotik Mikroorganizmaların Canlılığı. *Akademik Gıda*. *14*, 2016, 158-165.

[10] G.A. Avci, Selection of superior bifidobacteria in the presence of rotavirus. *Braz J Med Biol Res*, 10; 49(11), 2016, e5562.

[11] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Peters and F. Smith, Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 1956, 350-356.

[12] Z. Erginkaya, E.U. Turhan, and D. Tatlı, Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products. *Iranian Journal of Veterinary Research, Shiraz University*, *19(1)*, 2018. 53-56

[13] E. Tuomola, R. Crittenden, M. Playne, I. Erika and S. Salminen, *Am. J. Clin. Nutr*, *73*, 2001, 73, 393–39S.

[14] M. Bull, S. Plummer, J. Marchesi, and E. Mahenthiralingam, The life history of *Lactobacillus acidophilusas* a probiotic: a tale of revisionary taxonomy, misidentification and commercial success. *FEMS Microbiology Letters*. *349*, 2013, 77-87.

[15] D. Ren, C. Li, Y. Qin, R. Yin, S. Du, F. Ye, C. Liu, H. Liu, M. Wang, and Y. Li, In vitro evaluation of the probiotic and functional potential of Lactobacillus strains isolated from fermented food and human intestine. *Anaerobe*. *30*, 2014, 1–10.

[16] P.A. Kobierecka, A.K. Wyszyńska, T. Aleksandrzak-Piekarczyk, M. Kuczkowski, A. Tuzimek, W. Piotrowska, A. Gorecki, I. Adamska, A. Wieliczko, J. Bardowski, and E.K. Jagusztyn-Krinicka, In vitro characteristics of *Lactobacillus* spp. strains isolated from the chicken digestive tract and their role in the inhibition of Campylobacter colonization. *Microbiologyopen*. *6*, 2017, e512.

[17] R.P.K. Sahadeva, S.F. Leong, K.H. Chua, C.H. Tan, H.Y. Chan, E.V. Tong, S.Y.W. Wong and H.K. Chan, Survival of commercial probiotic strains to pH and bile. *International Food Research Journal*. *18*, 2011, 1515-1522.

[18] K.G. Deshpande, C.B. Dolas and N.S. Chavan, Investigation of tolerance of Lactobacillus casei to the presence of acids, bile salts and Deconjugation of bile salts. *International Journal of Current Microbiology and Applied Sciences. 3(7),* 2014, 600-6.

[19] Z. Ahmed, M.S. Vohra M.N. Khan, A. Ahmed, and T.K. Khan, Antimicrobial role of *Lactobacillus species* as potential probiotics against enteropathogenic bacteria in chickens. *Journal of Infectious Developing Countries. 13*, 2019, 130-136.

[20] G. Alp and B. Aslim, Relationship between the resistance to bile salts and low pH with exopolysaccharide (EPS) production of Bifidobacterium spp. isolated from infants feces and breast milk. Anaerobe 16 (2), 2010, 101-105

[21] C.O. Dunne, L. Mahony, L. Murphy, G. Thorton, D.O. Morrissey, S. Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly and B.O. Kiely, G.C. Sullivan, F. Shanahan, J.K. Collins, In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *American Journal of Clinical Nutrition, 73*, 2001, 386–392.

[22] H. Baser and Z. Yuksekdag, Exopolysaccharide (EPS) production of halophilic bacteria Eurasian Journal of Biological and Chemical Sciences. *Eurasian J Bio Chem Sci, 2(2)*, 2019, 84-89.

[23] D. Bergmaier, C.P. Champagne, and C. Lacroix, Exopolysaccharide production during batch cultures with free and immobilized *Lactobacillus rhamnosus* RW-9595M. *Journal of Applied Microbiology*. *95*, 2003, 1049-1057.

[24] A.P. Laws and V.M. Marshall, The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. *International Dairy Journal, 11*,2001, 709-721.

[25] C. Elisa, M.J. Ale Perezlindo, P. Burns, E. Tabacman, J.A. Reinheimer and A.G. Binetti, Exopolysaccharide from *Lactobacillus fermentum* Lf2 and its functional characterization as a yogurt additive. Journal of Dairy Research. *83,* 2016, 487–492.

[26] E. Martín R, Jiménez, M. Olivares, M.L. Marín, L. Fernández, J. Xaus and J.M. Rodríguez, Lactobacillus salivarius CECT 5713, a potential probiotic strain isolated from infant feces and breast milk of a mother-child pair. *Int J Food Microbiol, 15;112 (1),* 2006, 35-43.

[27] R. Temmerman, I. Scheirlinck, G. Huys and J. Swings. Culture-Independent Analysis of Probiotic Products by Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology*, 2003, p. 220–226

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

[29] M. Gueimonde, B. Sanchez, C.G. Gavilan and A. Margolles, Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology, 4,* 2013, 1-6.

[30] C. Sharma, S. Gulati, N. Thakur, B.P. Singh, S. Gupta, S. Kaur, S.K. Mishra, A.K. Puniya, J.P.S. Gill and H. Panwar, H. Antibiotic sensitivity pattern of indigenous *lactobacilli* isolated from curd and human milk samples. *Biotechnology. 7,* 2017, 53.