


## Determination of Lactic Acid Production and Antagonistic Activity against *Helicobacter pylori* of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* Strains<sup>[1]</sup>

Belma ASLIM \* Dilsad ONBASILI \*\*  Zehra Nur YUKSEKDAG \*\*\*

[1] This research has been supported by TUBITAK through project no: TBAG-2090 (101T129) and FEF Gazi University BAP project no: 05/2002-65

\* Gazi University, Molecular Biology Research Center, TR-06830 Gölbaşı, Ankara - TURKEY

\*\* Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology TR-38039 Kayseri - TURKEY

\*\*\* Gazi University, Faculty of Science and Arts, Department of Biology, TR-06500 Teknikokullar, Ankara - TURKEY

Makale Kodu (Article Code): KVFD-2011-4326

### Summary

In this study, lactic acid production was determined of a total of 32 strains of lactobacilli and streptococci. Different amounts of lactic acid were produced by strains studies; however, lactic acid levels were 13.4 to 18.6 mg/mL for *Lactobacillus delbrueckii* subsp. *bulgaricus* strains and 0.6 to 11.5 mg/mL for *Streptococcus thermophilus* strains. Antimicrobial activity was tested against *Helicobacter pylori* ATCC 43504. The some strains were able to inhibit *H. pylori*. Bacteriocin-like substances (BLS) producing strains were determined under conditions eliminating the effects of organic acids. As a result, it was observed that inhibitory activities of only ten strains were due to bacteriocin-like substances.

**Keywords:** *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, Lactic acid, Antimicrobial activity, *Helicobacter pylori*

## *L. delbrueckii* subsp. *bulgaricus* ve *S. thermophilus* Suşlarının *Helicobacter pylori*'ye Karşı Antogonistik Aktivitesi ve Laktik Asit Üretimlerinin Belirlenmesi

### Özet

Bu çalışmada, toplam 32 adet lactobacilli and streptococci suşlarının laktik asit üretimleri belirlenmiştir. Laktik asitin farklı miktarları, çalışmadaki suşlar tarafından üretilmiştir, fakat laktik asit düzeyleri *Lactobacillus delbrueckii* subsp. *bulgaricus* suşları için 13.4-18.6 mg/mL, *Streptococcus thermophilus* suşları için 0.6-11.5 mg/mL arasındadır. Antimikrobiyal aktivite, *Helicobacter pylori* ATCC 43504' ye karşı belirlenmiştir. Bazı suşlar *H. pylori*'yi inhibe etmiştir. Baktriyoisin benzeri maddeler üreten suşlar organik asitlerin etkilerini ortadan kaldıran koşullar altında belirlenmiştir. Sonuç olarak sadece on suşun inhibitor aktivitesinin bakteriyosin benzeri maddelerden dolayı olduğu görülmüştür.

**Anahtar sözcükler:** *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, Laktik asit, Antimikrobiyal aktivite, *Helicobacter pylori*

### INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria united by a constellation of physiologic, morphologic, and metabolic features. General properties of the group are that they are Gram-positive, non-spore-forming, anaerobic, microaerophilic, or facultative anaerobic rods or cocci, which ferment carbohydrates to produce

lactic acid as the major end product. Historically, LAB genera are composed of *Bifidobacterium*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, etc.<sup>1</sup>. Among them a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*,



#### İletişim (Correspondence)



+90 352 4380486



dilsad@erciyes.edu.tr

which collaborate synergistically into yoghurt, are widely used to as the major starter bacteria for the production of yoghurt <sup>2</sup>.

Probiotics, including LAB are known to inhibit the growth of a wide range of intestinal pathogens in humans and animals <sup>3</sup>. The inhibitory activities of LAB are due to its ability to produce different acids and metabolites: These are; lactic acid and acetic acid-like organic acids <sup>4,5</sup>, H<sub>2</sub>O<sub>2</sub> <sup>6,7</sup>, reuterin <sup>6</sup>, bacteriocin and bacteriocin-like substances <sup>8</sup> diacetyl and CO<sub>2</sub> <sup>9</sup>. Currently, studies regarding the antagonistic effect of certain lactic acid bacteria (LAB) strains, i.e., *L. acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. salivarius*, *L. bulgaricus* and *L. gasseri*, against *Helicobacter pylori* have been reported <sup>10,11</sup>. *H. pylori* is a spiral-shaped, Gram-negative rod that has developed sophisticated strategies to colonize epithelial cells lining the antrum of the stomach and to survive in acidic environments <sup>12,13</sup>.

Because LAB are acid-tolerant and able to persist in the stomach longer than other bacteria, some LAB preparations have been proved to be useful as adjuncts to the treatment and prevention of gastritis <sup>10</sup>.

The aim of the present study was to investigate the lactic acid production of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains from Turkish fermented dairy products. The other objective of this study was to determine the bacteriocin-like substance (BLS) producing ability of these strains and its effect against *H. pylori* *in vitro*.

## MATERIAL and METHODS

### Bacterial Strains and Media

Bacterial strains used in this study are listed in [Table 1](#). Five of the 20 *S. thermophilus* strains were obtained from

**Table 1.** Strains used in this study

**Tablo 1.** Bu çalışmada kullanılan suşlar

Bacterial Strains		Source
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	A12	This study, from yoghurt
	A13	This study, from yoghurt
	EB7	This study, from yoghurt
	F11	This study, from yoghurt
	F12	This study, from yoghurt
	G11	This study, from yoghurt
	G12	This study, from yoghurt
	H12	This study, from yoghurt
	M12	This study, from yoghurt
	W12	This study, from yoghurt
	B2	This study, from yoghurt
	B3	This study, from yoghurt
	<i>Streptococcus thermophilus</i>	7.7
7.8		Tunail <i>et al.</i> (14), from raw milk
13.5		Tunail <i>et al.</i> (14), from raw milk
13.6		Tunail <i>et al.</i> (14), from raw milk
26.5		Tunail <i>et al.</i> (14), from raw milk
A21		This study, from yoghurt
A22		This study, from yoghurt
AK21		This study, from yoghurt
ET5		This study, from yoghurt
ET11		This study, from yoghurt
H21		This study, from yoghurt
H22		This study, from yoghurt
M21		This study, from yoghurt
M22		This study, from yoghurt
N22		This study, from yoghurt
W21		This study, from yoghurt
W22		This study, from yoghurt
T8	This study, from yoghurt	
T12	This study, from yoghurt	
T13	This study, from yoghurt	

Tunail et al.<sup>14</sup> isolated from raw milk, and the remaining strains were isolated from traditional home made yoghurts. *L. delbrueckii* subsp. *bulgaricus* strains were inoculated in MRS broth (Oxoid), while *S. thermophilus* strains were inoculated in M17 broth (Oxoid). All of the strains were stored at -80°C in MRS/M17 broth with 10% glycerol, and regenerated twice before being used in the manipulations.

Initial characterization of isolates included colony and cell morphology, Gram staining and catalase test. Carbohydrate fermentation characteristics of Gram-positive and catalase-negative isolates were also determined by using API 50 CHL identification system. Results were recorded after 48 h at 42°C. Finally, the isolates were identified by 16S rDNA sequence<sup>15-17</sup>. The sequences obtained were searched against The Gen Bank DNA database using the blast function.

### Acid Production

Lactic acid production was determined spectrophotometrically according to Steinholt and Calbert<sup>18</sup>. Overnight cultures of strains were inoculated in MRS broth (for *L. delbrueckii* subsp. *bulgaricus*) and M17 broth (for *S. thermophilus*) and incubated at 42°C for 18 to 20 h (the exponential growth phase). Lactic acid was quantified using a lactic acid standard curve, performed with concentrations, ranging from 1 to 20 mg/mL.

### Inhibitory Effect By the Agar-Well Diffusion Method

*H. pylori* strain used in this study supplied an American Type Culture Collection reference strain (ATCC 43504). *H. pylori* strain was cultivated in Brain Heart Infusion (BHI) broth supplemented with 5% inactivated fetal horse serum at 37°C with shaking at 120 rpm in a CO<sub>2</sub> incubator with 10% CO<sub>2</sub>. The stock culture was kept at -80°C in peptone/glycerol medium.

The determination of the inhibitory effect of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains was carried out with an agar diffusion method<sup>19</sup>. Petri dishes with 20 mL of Brain Heart Infusion (BHI) agar supplemented with 5% inactivated fetal horse serum (Oxoid) were prepared. The *H. pylori* culture was adjusted to 2x10<sup>6</sup> cfu/mL with culture medium, and 100 µL of the *H. pylori* culture was inoculated in each petri dish. Once solidified, the dishes were stored for 2 h at 4°C. Four wells (Ø 6 millimeter) were made and the wells were filled with 100 µL culture filtrate. The inoculated plates were incubated for 24 h at 37°C in a CO<sub>2</sub> incubator with 10% CO<sub>2</sub>. The diameter of the inhibition zone was measured with calipers<sup>20,21</sup>.

### Preparation of Culture Filtrates

*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains were inoculated in MRS and M17 broth, respectively, at 42°C for 18 to 20 h. Culture broths were centrifuged

at 4.200 rpm for 15 min at 4°C. The clear supernatants obtained were used in the experimental trials as follows:

i. Supernatant fluids (SF) were filter sterilized by membrane filtration (0.2 µm membrane filters) and used in the assay.

ii. pH of supernatant fluids was adjusted to pH 6.5-7.0 with 1 N NaOH solution and heated with catalase at a concentration of 200 unit/mL. The neutralized and heated supernatant fluids (NHSF) were filter sterilized as in (i) and used in the assay<sup>22,23</sup>.

### Statistical Analysis

All experiments were performed in triplicate and the results are the arithmetic mean ± SD (standard deviation). Statistical analysis was performed on the data by SPSS 11.0 Bivariate Correlation Analysis (SPSS Inc., Chicago, Ill.) with statistical significance determined at P<0.05. The Pearson rank order correlation test was used for comparisons between lactic production amounts of the strains and antimicrobial activity of the strains.

## RESULTS

### Acid Production

In our study, *Lactobacillus delbrueckii* subsp. *bulgaricus* (12 strains) and *Streptococcus thermophilus* (20 strains) were isolated from raw milk, and traditional home made yoghurts. The acid produced by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains in MRS/M17 is exhibited in Table 2 and 3. *L. delbrueckii* subsp. *bulgaricus* B3 and *S. thermophilus* AK21 strains produced the highest amount (18.6 mg/mL, 11.5 mg/mL, respectively) and *L. delbrueckii* subsp. *bulgaricus* W12 and *S. thermophilus* 26.5 strains produced the lowest (13.4 mg/mL, 0.6 mg/mL) amount of lactic acid.

### Antimicrobial Activities of Strains

The strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were assayed for ability to produced inhibitory substances against the growth of *H. pylori*. The bacterial activity exhibited by cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are presented in Table 2 and 3. Supernatant fluids (SF) obtained from A12, F11, F12, G11, G12, M12, B3 strains of *L. delbrueckii* subsp. *bulgaricus* and 7.7, 13.6, AK21, H21, H22, M21, M22, W21, T12 strains of *S. thermophilus* exhibited varying degrees of inhibitory activity against strains of *H. pylori*. The highest antimicrobial activities of *L. delbrueckii* subsp. *bulgaricus* B3 strain and *S. thermophilus* AK21 were 15.4 mm and 12.0 mm, respectively, whereas the lowest antimicrobial activities of *L. delbrueckii* subsp. *bulgaricus* F11 strain and *S. thermophilus* 13.6 were 4.0 mm and 3.3 mm, respectively against *H. pylori*.

**Table 2.** Lactic acid production and inhibitory effect against *H. pylori* by supernatant fluid from cultures of *L. delbrueckii* subsp. *bulgaricus* strains.

**Tablo 2.** *L. delbrueckii* subsp. *Bulgaricus* suşlarının kültürlerden sıvı supernatant ile *H. pylori*'ye karşı inhibitör etkisi ve laktik asit üretimi

Strains		Lactic Acid (mg/mL)	Inhibitory Effect Against <i>H. pylori</i> (ATCC 43504)*	
			SF	NSF
<i>L. delbrueckii</i> <i>ssp. bulgaricus</i>	A12	16.0±0.4	10.4±0.5	-
	A13	15.0±0.5	-	-
	EB7	15.3±0.5	-	-
	F11	13.7±0.5	4.0±0.4	-
	F12	14.2±0.0	4.2±0.2	-
	G11	14.0±0.0	5.4±0.3	3.3±0.3
	G12	15.1±0.2	6.8±0.1	-
	H12	15.0±0.3	-	-
	M12	14.3±0.4	8.0±0.0	6.8±0.1
	W12	13.4±0.2	-	-
	B2	14.0±0.7	-	-
	B3	18.6±0.3	15.4±0.2	8.0±0.0

Values are the means ± standard deviations of triplicate measurements

\*Diameter of the inhibition zone (mm)

-; No inhibition zone; SF: Supernatant fluids; NSF: Neutralized supernatant fluids

**Table 3.** Lactic acid production and inhibitory effect against *H. pylori* by supernatant fluid from cultures of *S. thermophilus* strains

**Tablo 3.** *S. thermophilus* suşlarının kültürlerden sıvı supernatant ile *H. pylori*'ye karşı inhibitör etkisi ve laktik asit üretimi

Strains		Lactic Acid (mg/mL)	Inhibitory Effect Against <i>H. pylori</i> (ATCC 43504)*	
			SF	NSF
<i>S. thermophilus</i>	7.7	3.0±0.5	4.3±0.0	-
	7.8	2.7±1.5	-	-
	13.5	1.8±0.5	-	-
	13.6	4.0±1.0	3.3±0.2	1.7±0.2
	26.5	0.6±0.3	-	-
	A21	4.1±0.4	-	-
	A22	4.3±0.0	-	-
	AK21	11.5±0.4	12.0±0.0	8.0±0.0
	ET5	5.3±0.1	-	-
	ET11	5.0±1.5	-	-
	H21	3.4±1.0	5.5±0.2	4.1±0.5
	H22	5.2±0.0	4.9±0.1	1.4±0.0
	M21	5.0±0.0	6.1±0.0	5.0±0.2
	M22	4.3±1.5	8.0±0.0	6.0±0.0
	N22	5.3±0.3	-	-
	W21	11.4±0.5	5.5±0.2	3.3±0.0
	W22	3.2±0.7	-	-
	T8	6.5±0.3	-	-
	T12	5.7±0.5	3.6±0.5	-
T13	7.6±0.2	-	-	

Values are the means ± standard deviations of triplicate measurements

\*Diameter of the inhibition zone (mm)

-;No inhibition zone; SF: Supernatant fluids; NSF: Neutralized supernatant fluids

Among the cultures of all strains assayed, it was observed that neutralized supernatant fluids (NSF) of *L. delbrueckii* subsp. *bulgaricus* G11, M12, B3 (Table 2) and *S. thermophilus* 13.6, AK21, H21, H22, M21, M22, W21 (Table 3) exhibited inhibitory activity against *H. pylori*.

## DISCUSSION

Lactic acid bacteria (LAB) have long been used in the food industry as starter cultures for the manufacture of dairy products<sup>7</sup>. LAB are frequently said to have health-promoting effects in the human and animal intestinal tract. Their probiotic effects have also been reported that they produce antimicrobial substances such as lactic acid, H<sub>2</sub>O<sub>2</sub>, alcohols and other metabolites. These metabolites inhibit undesirable pathogens causing diarrhea or other diseases in the human intestine<sup>24</sup>.

Lactic acid, the major by-product of the fermentation, is a commercially valuable product with applications in the food, manufacturing and pharmaceutical industries<sup>25</sup>. The primary function of lactic cultures used to manufacture of yoghurt is to produce lactic acid required for the formation of the coagulum<sup>26</sup>. Lactic acid is the most essential metabolic product formed by the lactic-acid-producing streptococci<sup>27</sup>.

Different amounts of lactic acid were produced by strains studied; however, lactic acid levels were 13.4 to 18.6 mg/ml for *L. delbrueckii* subsp. *bulgaricus* (Table 2), 0.6 to 11.5 mg/mL for *S. thermophilus* (Table 3). Aroutcheva et al.<sup>28</sup> reported that the lactic acid concentration produced in *Lactobacillus* strains ranged between 0.68-2.52 mg/L after anaerobic incubation at 37°C for 24 h (BBC GasPak System). In this study our strains produced very high amount of lactic acid for *L. delbrueckii* subsp. *bulgaricus*. Yuksekdog et al.<sup>29</sup> found that the lactic acid levels were 2.3 to 9.9 mg/mL in *Streptococcus* strains. In this study, the same results were obtained for *S. thermophilus*.

The antagonistic activity by LAB might be a part of the fermentation process and the antimicrobial compound be a primary metabolic end-product such as lactic acid and acetic acid, diacetyl, hydrogen peroxide, fatty acids, aldehydes, bacteriocins and other compounds<sup>28</sup>. Organic acids, such as lactic acid and acetic acid, produced by probiotics play important roles in the antagonism of microflora in environments as well as in fermented foods. Lactic acid and acetic acid account for more than 90% of the acids produced. Other acids produced in small quantities include citric, hippuric, orotic, and uric acids<sup>30</sup>.

The antibacterial activity of supernatant fluids of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains against *H. pylori* ATCC 43504 was examined to determine the effect of organic acids and other components in the antagonism of the pathogen (Table 2 and 3). Supernatant fluids without neutralization (SF) of 16 strains of *L.*

*delbrueckii* subsp. *bulgaricus* and *S. thermophilus* showed inhibitory activity against *H. pylori*.

When the pH values of supernatant fluids were adjusted to pH 7.0 (NSF), only 10 cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* exhibited antibacterial activity in neutralized (Table 2 and 3).

Lorca et al.<sup>11</sup> reported that antibacterial activity of 17 strains of lactobacilli was tested against 10 strains of *H. pylori*. They found that the inhibition observed was related to the acid production and low pH attained. At the same time, Midolo et al.<sup>31</sup> reported that lactic, acetic and hydrochloric acids demonstrated an inhibition of *H. pylori* growth in a concentration-dependent manner, with lactic acid resulting in the highest inhibition. This suggested the presence of bacteriocin-like substances with inhibitory activity against *H. pylori*. In this study, the same results were obtained. *L. delbrueckii* subsp. *bulgaricus* B3 and *S. thermophilus* AK21 strains produced both high amount of lactic acid (18.6 mg/mL, 11.5 mg/mL, respectively) and showed high antimicrobial effect (15.4 mm, 12.0 mm, respectively) and bacteriocin-like substances (8 mm) against *H. pylori*. These results showed that a relationship exists between the lactic acid production and antimicrobial activity in some strains. This result is important for yoghurt, as a fermented probiotic product proved to be useful to control several diseases.

The relation between lactic acid production and antimicrobial activity was supported by statistical analyses. Our results showed that there is a positive correlation ( $r=0.698$  for lactobacilli strains,  $r=0.504$  for streptococci strains) between the lactic acid production quantity of the strains and the antimicrobial activities and their correlation is significant at the 0.05 level.

The most commonly probiotics used in clinical practice, which are also the most studied, include lactic acid-producing bacteria (*Lactobacillus* and *Streptococcus* spp.). As for other several gastrointestinal infections, there are valid data in the literature that demonstrate the ability of some specific strains of probiotics to downregulate the infective activity of *Helicobacter pylori*. The present study describes the lactic acid production and antimicrobial activity of lactic acid bacteria isolated from hand-made yoghurts. It was also observed that inhibitory activities of only ten strains originating from Turkish dairy products were due to bacteriocin-like substances. May the results piled and obtained in this study cast some light on the use of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* strains for further research as starters for high quality probiotic products and yoghurts.

## REFERENCES

1. Huang HY, Huang SY, Chen PY, King VAE, Lin YP, Tsen JH: Basic Characteristics of *Sporolactobacillus inulinus* BCRC 14647 for potential probiotic properties. *Curr Microb*, 54, 396-404, 2007.

- 2. Aslim B, Beyatli Y, Yuksekdog ZN:** Productions and monomer compositions of exopolysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from traditional home-made yoghurts and raw milk. *Int J Food Sci Tech*, 41, 973-979, 2006.
- 3. Kim P, Young JM, Young-Hyo C, Saehun K, Seong-Jae K, Yong-Ha P:** Probiotic properties of *Lactobacillus* and *Bifidobacterium* strains isolated from porcine gastrointestinal tract. *Appl Microb Biotechnol*, 74, 1103-1111, 2007.
- 4. Panesar PS, Kennedy JF, Knill CJ, Kosseva MR:** Applicability of pectate-entrapped *Lactobacillus casei* cells for L(+) lactic acid production from whey. *Appl Microb Biotechnol*, 74, 35-42, 2007.
- 5. Rojo-Bezares B, Sáenz Y, Zarazaga M, Torres C, Ruiz-Larrea F:** Antimicrobial activity of nisin against *Oenococcus oeni* and other wine bacteria. *Int J Food Microb*, 116, 32-36, 2007.
- 6. Juven BJ, Schived F, Lindner P:** Antagonistic compounds produced by a chicken intestinal strain of *Lactobacillus acidophilus*. *J Food Protec*, 55, 157-161, 1992.
- 7. Yuksekdog ZN, Beyatli Y, Aslim B:** Metabolic activities of *Lactobacillus* spp. strains isolated from kefir. *Nahrung/Food*, 48, 218-220, 2004.
- 8. Aslim B, Yuksekdog ZN, Sarikaya E, Beyatli Y:** Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. *LWT/Food Sci Tech*, 38, 691-694, 2005.
- 9. Weber H:** Dry sausage manufacture, the importance of protective cultures and their metabolic products. *Fleischwirtschaft*, 74, 278-282, 1994.
- 10. Tsai CC, Huang LF, Lin CC, Tsen HY:** Antagonistic activity against *Helicobacter pylori* infection *in vitro* by a strain of *Enterococcus faecium* TM39. *Int J Food Microb*, 96, 1-12, 2004.
- 11. Lorca GL, Wadström T, De Valdez GF, Ljungh A:** *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori* *in vitro*. *Curr Microb*, 42, 39-44, 2001.
- 12. Hamilton-Miller JMT:** The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. *Int J Antimicrob Agents*, 22, 360-366, 2003.
- 13. Takahashi T, Matsumoto T, Nakamura M, Matsui H, Tsuchimoto K, Yamada H:** L-Lactic acid secreted from gastric mucosal cells enhances growth of *Helicobacter pylori*. *Helicobacter*, 12, 532-540, 2007.
- 14. Tunail N, Ayhan K, Akçelik M, Durlu-Özkaya F, Doğan HB, Tükel Ç, Kaleli D, Acar E:** Yoğurt fabrikalarında faj probleminin çözümüne yönelik araştırmalar. *TÜBİTAK PROJE*. NO: TARP-2106, 162s, 2002.
- 15. Tilsala-Timisjarvi A, Alatossava T:** Enzymatic amplification of 16s rRNA gene sequences from dairy *Lactobacillus* species. *Milchwissenschaft*, 48, 123-125, 1993.
- 16. Ventura M, Casas IA, Morelli L, Callegari M:** Rapid amplified ribosomal DNA restriction analysis (ARDRA) identification of *Lactobacillus* spp. isolated from fecal and vaginal samples. *Syst Appl Microb*, 23, 504-509, 2000.
- 17. Roy D, Sirois S, Vincent D:** Molecular discrimination of *Lactobacilli* used as starter and probiotic cultures by amplified ribosomal DNA restriction analysis. *Curr Microb*, 42, 282-289, 2001.
- 18. Steinholt K, Calbert HE:** A rapid colorimetric method for the determination of lactic acid in milk and milk products. *Milchwissenschaft*, 15, 7-11, 1960.
- 19. Reinheimer JA, Demkow MR, Condioti MC:** Inhibition of coliform bacteria by lactic cultures. *Aust J Dairy Tech*, May, 5-9, 1990.
- 20. Attaie R, Whalen PJ, Shahani KM, Amer, MA:** Inhibition of growth of *Staphylococcus aureus* during production of acidophilus yoghurt. *J Food Protect*, 50, 224-228, 1987.
- 21. Okereke A, Montville TJ:** Bacteriocin inhibition of *Clostridium botulinum* spores by lactic acid bacteria. *J Food Protect*, 54, 349-353, 2000.
- 22. Tagg JR, Dajani AS, Wannamaker LW:** Bacteriocins of Gram-positive bacteria. *Bacteriol Rev*, 40, 722-756, 1976.
- 23. Zhu WM, Liu W, Wu DQI:** Isolation and characterization of a new bacteriocin from *Lactobacillus gasseri* KT7. *J Appl Microb*, 88, 877-886, 2000.
- 24. Temmermanan R, Pot B, Huys G, Swings J:** Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int J Food Microbiol* 81, 1-10, 2003.
- 25. Boonmee M, Leksawasdi N, Bridge W, Rogers PL:** Batch and continuous culture of *Lactococcus lactis* NZ133: experimental data and model development. *Biochem Eng J*, 14, 127-135, 2003.
- 26. Gursoy A, Durlu-Özkaya F, Yıldız F, Aslim B:** Set type yoghurt production by exopolysaccharide producing Turkish origin domestic strains of *Streptococcus thermophilus* (W22) and *Lactobacillus delbrueckii* ssp. *Bulgaricus* (B3). *Kafkas Univ Vet Fak Derg*, 16, 81-86, 2010.
- 27. Ozbaş ZY, Aytaç SA:** Behavior of *Yersinia enterocolitica* and *Aeromonas hydrophila* in skim milk during fermentation by various lactobacilli. *Z Lebensm Unters Forsch*, 202, 324-328, 1996.
- 28. Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S:** Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol*, 185, 375-379, 2001.
- 29. Yuksekdog ZN, Beyatli Y, Aslim B:** Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefir with natural product. *LWT/Food Sci Tech*, 37, 663-667, 2004b.
- 30. Kim TS, Hur JW, Yu M, Chenigh CI, Kim KN, Hwang JK, Pyun YR:** Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. *J Food Protect*, 66, 3-12, 2003.
- 31. Midola PD, Korman MG, Turdinge JD, Lambert JR:** *Helicobacter pylori* resistance to tetracycline. *Lancet*, 347, 1194-1195, 1996.