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Determination of Some Metalic Antimicrobial Activities and Plasmid and DNA Prophiles of *Lactobacillus* Strains Isolated from Fermented Caper Pickle

[1]Birol Özkalp, [1]Mustafa Onur Aladağ, [2]Zümrüt Ögel,
[3]M. Musa Özcan and [1]Battal Çelik

[1]Department of Medicinal Laboratory, College of Health Care,
University of Selçuk, 42031 Konya, Turkey
[2]Department of Food Engineering, Faculty of Agriculture,
University of Middle East Technical Universty, Ankara, Turkey
[3]Department of Food Engineering, Faculty of Engineering,
University of Selcuk, 42031 Konya, Turkey

Abstract: 19 caper (*Capparis spinosa* Desf. var. *canescens*) samples, which were obtained from the flower buds and then fermented in 10% NaCl of brine for 2 months at 30°C, were studied in this experiment. *Lactobacillus* spp. were isolated from the caper pickle samples using the classical methodologies. The isolated *Lactobacillus* spp. were identified to determine their acid and hydrogen peroxide production, general inhibitory effects, bacteriocin or bacteriocin-like substance effects and hydrogen sulfur production. The plasmid, DNA and protein profiles of identified *Lactobacillus* spp. were determined. As a consequence, 18, 6, 5 and 3 of 32 isolated *Lactobacillus* spp. were determined to be *L.plantarum, L.casei, L.fermentum* and *L.brevis*, respectively. Plasmid was not observed in the identified lactic acid bacteria. Their DNA magnitudes were determined between 1400 bp to 500 bp.

Key words: Caper · Fermentation · Lactobacillus · Plasmid · DNA

INTRODUCTION

Fermented capers are an important seasoning in the Mediterranean kitchen and are greatly appreciated for their flavour, appetite and digestive properties. The manufacture of many traditional foods relies on spontaneous fermentations and the safety of the LAB involved is largely unknown. The fermentation of capers is very popular in Mediterranean countries [1, 2, 3]. Lactic acid bacteria (LAB) naturally occur in foods and play a key role in the manufacture of fermented foods as well as in food preservation. They are also commensal bacteria in humans and animal microflora [4] and many isolates find specific applications as probiotics [5]. Studies on starter cultures used in fermented products are very limited in Turkey. Starter cultures are mostly used in meat products like faggots, frankfurters and in salami [6, 7, 8, 9, 10, 11]. There are some studies on starter cultures and *Lactobacillus* strains used in the fermented products like pickle and the like. These are mostly on soybean products in Far-Eastern countries. Besides, there are some plant fermented products all over the world [12]. It is also known that the metabolic products and antagonistic effects of bacteria are very prominent factors in choosing starter cultures. The antagonistic effects of lactic acid bacteria are attributed to their different biochemical characteristics. Lactic acid bacteria can produce organic acids such as lactic and acetic acid from carbohydrates. Most of the food sourced pathogens or non-pathogen contaminants are not resistant to this decrease in pH [9, 13, 14, 15, 16, 17]. Besides, lactic acid bacteria produce a lot of complex having an inhibitory effect. Among of them, there are H_2O_2, diasetiline, CO_2 and bacteriocins [9,14, 13, 15, 16, 17].

In the literature, no study has appeared to focus on the microorganisms, which produces capers pickle. Therefore, it was aimed to determine the dominant species of *Lactobacillus*, which are effective in the fermentation

Corresponding Author: Birol Özkalp, Department of Medicinal Laboratory, College of Health Care,
University of Selçuk, 42031 Konya Turkey

of caper pickles and to find out their metabolic and antimicrobial effect and to identify the plasmid profiles of the strains and also to check the accuracy of the identification by comparing the DNA profiles of reference strains to those of the isolated strains.

MATERIALS AND METHODS

Capers (*Capparis spinosa* Desf. var. *canescens*) buds were collected from Konya (Selcuklu) in July, 2006. After undesired substances were removed from the flower buds (x<8 mm<13 mm), they were classified. Then, the buds were fermented in hermetic covered glass bottles including brine with % 10 NaCl concentration at rate of ½;w/v at 30°C for two months. After the fermentation process, the brined samples were brought under aseptic conditions and analyzed. 19 samples were used in the experiment.

The Isolation of *Lactobacillus*: To isolate *Lactobacillus* bacteria from pickle, the 1 ml of samples was taken and then mixed using vortex in a tube including 9 ml of physiological water. Appropriate dilutions were prepared from each of sample and were cultivated on MRS Agar culture according to the scattering platter method and then the platter were incubated in the aerobic conditions at 35°C for 42 hours. After the colonies developed on the MRS Agar were analyzed, the bacteria colonies, which were determined to have *Lactobacillus* of odd, even or chain morphology, were richened by injecting them into 10% litmus milk and then incubating them at 35°C for 2 hours. Then, the cultures were put into eppendorf tubes with 2 ml capacity and stored at -20°C until they were used [18].

The Identification of Bacteria: In the identification of isolated bacteria, their morphological and physiological features were tested and some biochemical tests were also used. The assessment and identification of all isolates as a genus were carried out according to general identification methods [19, 8].

The Determination of Acid Production of Strains: Sterilized and skimmed 25 ml milk at 10% concentration from active cultures were injected to the culture environment on the petri dishes at the ratio of 2% and then incubated at 35°C for 42 hours. After incubation, the acid content produced by the strains was titrated with 0.1 N of NaOH and the acid amounts produced by strains were calculated as percentage. The relationship between acid production levels of strains and their inhibition zones were statistically determined using the correlation coefficient in SPSS software.

The Determination of Hydrogen Peroxide Level Produced by Strains: Hydrogen peroxide level was determined by Reinheimer *et al.* (1990) using a spectrophotometric technique[20]. The relationship between hydrogen peroxide production of strains and their inhibition zones was statistically determined as a correlation coefficient using SPSS software.

The Determination of General Inhibition Effects of Strains and Bacteriocins or Bacteriocinlike Substances: In this study, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 1187, *Klebsiella pneumoniae* NCTC 5049, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076 strains were used as the test bacteria.

The general inhibition and bacteriocins effects of strains on test bacteria were determined using agar-diffusion method. The *Lactobacillus* strains were injected onto MRS Broth culture containing 10 ml glucose at 2% of concentration and then were incubated for 48 hours at 35°C. To quench the inhibition effect of lactic acid bacteria while bacteriocins production was determined, they were incubated under the same conditions without oxygen. After the incubation, the petri dishes including the culture environment were centrifuged at 5 rpm and then the clear supernatant was filtered through a 0.2 μm membrane filter [21].

To determine the inhibition and bacteriocin effect of *Lactobacillus* strains, test bacteria were activated in Nutrient Broth to achieve the density of Mac Farland No: 0.5. 100 μm *Lactobaccilus* culture filtrates were filled into the small holes at the diameter of 0.8 cm in Nutrient Agar culture environment including the test bacteria and then incubated at 35°C for 48 hours and the diameters of formed zones were specified in mm units [21].

The Determination of Hydrogen Sulfur Level Produced by Strains: To activate in TSI (Triple Sugar Iron) Agar culture environment, the 0.5 ml *Lactobacillus* strains was pipetted and scattered on MRS Broth using scattering method. They were incubated at 35°C for 21 days. In the culture environment, the H_2S production was determined based on the darkening of the colonies [22].

Plasmid Isolation Method: The plasmid isolation was carried out as reported in the literature [23].

DNA Isolation Method: The DNA was isolated according the method modified by [24]. 1 kb plus DNA ladder (Life Technologies, Italy) was used as a marker.

Polymerase Chain Reaction (PCR): 5′-CTCATCATGTCGACCCGG-3′ (P1), 5′-TCACTGATTTTAACA-3′ (P2), 5′-GGGCGGTGTGTACAAGGC-3′ (P3) were used primer. By making use Eppendorf Personal Thermal Cycler, the reactions were carried out firstly at 94°C 1 cycle at 2 minutes, one minute at 94°C, one minute at 56°C and followed by the final 25 cycle for one minute at 72°C, at 56°C. The last extension was performed at 72°C for 10 minutes [25, 26].

RESULTS

18 of 32 strains isolated from the 19 pickle samples were *Lactobacillus plantarum* (56.2 g/100g), the 6 were *Lactobacillus casei* (18.8 g/100g), 5 were *Lactobacillus fermentum* (15.6 g/100g), 3 were *Lactobacillus brevis* (9.4 g/100g) (Table 1), which indicated that the dominant strain in the pickles was *Lactobacillus plantarum*. Average hydrogen peroxide production of the strains was 2,14 μg/ml. The highest hydrogen peroxide was produced by *Lactobacillus plantarum* B9 strain at the amount of 2.62 μg/ml. The hydrogen peroxide productions of the strains were presented in Table 2.

Average acid production of strains was 0.73% and the highest acid was produced by *Lactobacillus plantarum* B8. The acid production of strains is also given in Table 2.

Out of a total of 32 strains, 8 of *Lactobacillus plantarum*, 2 of *Lactobacillus casei*' , 3 of *Lactobacillus fermentum* and one of *Lactobacillus brevis* produced hydrogen sulfur as seen in Table 2. General inhibition zone diameter effect of strains on test microorganisms is also given in Table 2 and the inhibition effect zone

Table 1: The biochemical properties of the isolated strains

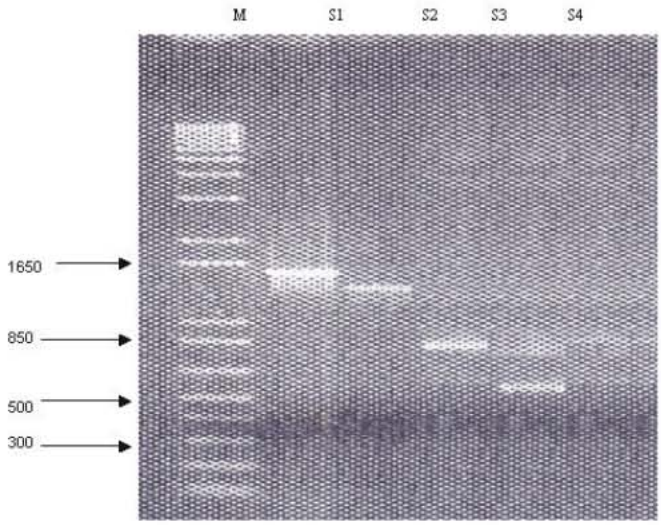
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Lactobacillus plantarum B1	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B2	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B3	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B4	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B5	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B6	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B7	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B8	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B9	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B10	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B11	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B12	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B13	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B14	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B15	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B16	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B17	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus plantarum B18	+	D	+	+	+	+	+	+	+	+	+	+	D	+	+	-	+	+	+	+	+	D
Lactobacillus casei MO1	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus casei MO2	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus casei MO3	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus casei MO4	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus casei MO5	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus casei MO6	+	-	+	+	+	+	+	+	D	+	+	+	+	-	-	-	+	+	+	+	+	-
Lactobacillus fermentum MB1	-	D	D	-	+	+	+	+	+	+	-	?	-	+	+	-	+	-	-	+	D	D
Lactobacillus fermentum MB2	-	D	D	-	+	+	+	+	+	+	-	?	-	+	+	-	+	-	-	+	D	D
Lactobacillus fermentum MB3	-	D	D	-	+	+	+	+	+	+	-	?	-	+	+	-	+	-	-	+	D	D
Lactobacillus fermentum MB4	-	D	D	-	+	+	+	+	+	+	-	?	-	+	+	-	+	-	-	+	D	D
Lactobacillus fermentum MB5	-	D	D	-	+	+	+	+	+	+	-	?	-	+	+	-	+	-	-	+	D	D
Lactobacillus brevis M1	-	+	-	D	+	D	+	+	D	+	-	-	-	+	D	-	+	-	-	D	-	D
Lactobacillus brevis M2	-	+	-	D	+	D	+	+	D	+	-	-	-	+	D	-	+	-	-	D	-	D
Lactobacillus brevis M3	-	+	-	D	+	D	+	+	D	+	-	-	-	+	D	-	+	-	-	D	-	D

1 = Amygdalin, 2 = Arabinose, 3 = Sellobiyose, 4 = Esculin, 5 = Fructose, 6 = Galactose, 7 = Glucose, 8 = Glukonate, 9 = Lactose, 10 = Maltose, 11 = Mannitol, 12 = Mannose, 13 = Melezitose, 14 = Melibiyose, 15= Raffinose, 16 = Rhamnose, 17 = Riboze, 18 = Salicin, 19 = Sorbitol, 20 = Sucrose, 21 = Trehalose, 22 = Xylose

Table 2: The General Inhibition effect (zone radius; mm) of the Isolated Strains on Test Bacteria which produces Hydrogen Peroxide (H_2O_2), Acid, Hydrogen Sulfur (H_2S)

	H_2O_2	%Acidity	H_2S	*B.cereus*	*S.aureus*	*S.enteritis*	*E.coli*	*K.pneumoniae*	*P.aureginosa*
Lactobacillus plantarum B1	1.88	0.61	+	7	Y	5	5	3	5
Lactobacillus plantarum B2	2.20	0.75	+	5	Y	6	5	2	9
Lactobacillus plantarum B3	1.90	0.69		5	Y	7	5	5	8
Lactobacillus plantarum B4	2.21	0.70		5	Y	8	6	6	8
Lactobacillus plantarum B5	2.24	0.80		3	Y	7	6	5	7
Lactobacillus plantarum B6	2.42	0.78	+	4	2	6	5	2	6
Lactobacillus plantarum B7	2.20	0.85		7	6	9	4	6	9
Lactobacillus plantarum B8	2.60	0.90	+	6	7	9	9	7	9
Lactobacillus plantarum B9	2.62	0.88	+	5	8	8	8	6	8
Lactobacillus plantarum B10	2.57	0.79		5	7	4	8	6	8
Lactobacillus plantarum B11	2.10	0.69	+	3	4	9	5	7	6
Lactobacillus plantarum B12	2.20	0.77		4	4	8	4	6	7
Lactobacillus plantarum B13	1.90	0.65	+	3	4	9	9	5	8
Lactobacillus plantarum B14	2.21	0.88		2	7	8	8	5	7
Lactobacillus plantarum B15	2.57	0.88		5	6	6	9	3	8
Lactobacillus plantarum B16	2.61	0.85	+	6	5	7	8	4	8
Lactobacillus plantarum B17	2.40	0.80		5	5	5	6	3	8
Lactobacillus plantarum B18	2.45	0.84		4	5	5	7	2	7
Lactobacillus casei MO1	1.80	0.70	+	6	3	8	5	5	8
Lactobacillus casei MO2	2.05	0.74		6	2	5	5	6	5
Lactobacillus casei MO3	1.10	0.67		5	3	4	5	6	4
Lactobacillus casei MO4	1.90	0.69	+	4	4.	5	5	5	3
Lactobacillus casei MO5	2.10	0.67		9	Y	6	5	4	4
Lactobacillus casei MO6	2.25	0.68		8	Y	6	7	9	5
Lactobacillus fermentum MB1	1.88	0.60		8	3	5	7	8	5
Lactobacillus fermentum MB2	1.95	0.59	+	5	4	4	6	8	5
Lactobacillus fermentum MB3	2.10	0.61		5	Y	9	6	5	5
Lactobacillus fermentum MB4	1.90	0.62	+	5	5	8	5	5	5
Lactobacillus fermentum MB5	2.00	0.60	+	5	3	8	4	5	7
Lactobacillus brevis M1	2.21	0.70		5	3	8	9	5	3
Lactobacillus brevis M2	2.10	0.65		3	4	5	8	5	4
Lactobacillus brevis M3	1.90	0.72	+	4	Y	4	8	5	5

Y: no inhibition



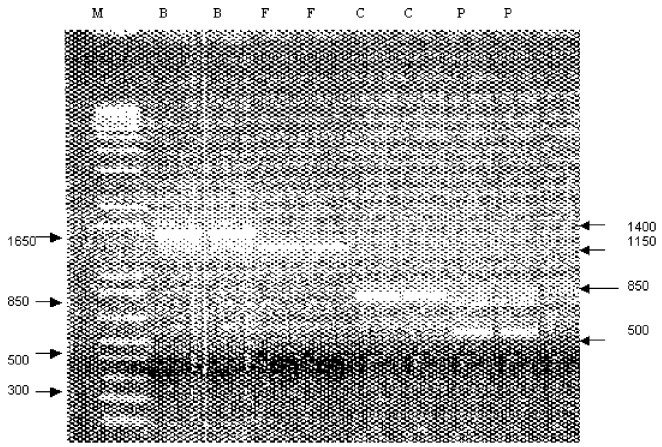
S1: *Lactobacillus brevis* ATCC 14869 (P1 ve P2 primers were used)
S2: *Lactobacillus fermentum* ATCC 14931 (P1 ve P2 primers were used)
S3: *Lactobacillus casei* ATCC 393 (P2 ve P3 primers were used)
S4: *Lactobacillus plantarum* ATCC 14917 (P2 ve P3 primers were used)

Fig. 1: The gel Images of DNA fragments of Control Strains

Table 3: The inhibition effect (zone radius; cm) of the bacteriocins and the similar substances of strains on test bacteria

	B.cereus	*S.aureus*	*S.enteritis*	*E.coli*	*K.pneumoniae*	*P.aureginosa*
Lactobacillus plantarum B1	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B2	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B3	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B4	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B5	1,1	Y	Y	Y	Y	Y
Lactobacillus plantarum B6	Y	Y	1.2	1.2	Y	Y
Lactobacillus plantarum B7	Y	Y	Y	1	Y	Y
Lactobacillus plantarum B8	1.6	Y	1.2	1.3	1.2	1.7
Lactobacillus plantarum B9	1.7	1.1	1.1	Y	Y	Y
Lactobacillus plantarum B10	1.1	Y	Y	Y	Y	Y
Lactobacillus plantarum B11	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B12	Y	Y	1.2	1.2	Y	Y
Lactobacillus plantarum B13	Y	Y	1.2	1.2	Y	Y
Lactobacillus plantarum B14	Y	Y	Y	Y	Y	Y
Lactobacillus plantarum B15	1.1	Y	1.1	1.1	1.2	1.1
Lactobacillus plantarum B16	1.2	1.1	Y	1.1	Y	1.1
Lactobacillus plantarum B17	Y	Y	1.1	Y	Y	Y
Lactobacillus plantarum B18	Y	Y	1	Y	Y	Y
Lactobacillus casei MO1	Y	Y	Y	Y	Y	Y
Lactobacillus casei MO2	1	Y	Y	Y	Y	Y
Lactobacillus casei MO3	Y	Y	Y	Y	Y	Y
Lactobacillus casei MO4	Y	Y	Y	Y	Y	Y
Lactobacillus casei MO5	Y	Y	1	1.1	Y	Y
Lactobacillus casei MO6	Y	Y	Y	1	Y	Y
Lactobacillus fermentum MB1	Y	Y	Y	Y	Y	Y
Lactobacillus fermentum MB2	Y	Y	Y	Y	Y	Y
Lactobacillus fermentum MB3	Y	Y	1.2	Y	1.1	Y
Lactobacillus fermentum MB4	Y	Y	Y	Y	Y	Y
Lactobacillus fermentum MB5	Y	Y	Y	1.2	Y	Y
Lactobacillus brevis M1	Y	Y	Y	1	Y	Y
Lactobacillus brevis M2	Y	Y	Y	Y	Y	Y
Lactobacillus brevis M3	Y	Y	Y	Y	Y	Y

Y: no inhibition



M: Marker
$B_{1,2}$: *Lactobacillus brevis* (P1 ve P2 primers were used)
$F_{1,2}$: *Lactobacillus fermentum* (P1 ve P2 primers were used)
$C_{1,2}$: *Lactobacillus casei* (P2 ve P3 primers were used)
$P_{1,2}$: *Lactobacillus plantarum* (P2 ve P3 primers were used)

Fig. 2: The gel Images of DNA fragments of Isolated Strains

Table 4: The correlation between the hydrogen peroxide and acid production of strains

	Asitlik (r)	H_2O_2 (r)
B cereus	-0.184	-0.030
S aureus	0.554**	0.408*
S enterit	0.159	0.202
E coli	0.354*	0.435*
K pneumon	-0.252	-0.204
P auregin	0.599**	0.499**

*P<0.05, **P<0.01

diameters of similar substances is given in Table 3. The relationships between the hydrogen peroxide and acid production and inhibition zones are calculated using SPSS software and they are given as correlation coefficient in Table 4. Birnhoim and Doly (1979) have reported that no plasmid DNA was observed as a result of plasmid isolation. PCR results revealed that the DNA band sizes of *Lactobacillus brevis* ATCC 14869, L*actobacillus fermentum* ATCC 14931, *Lactobacillus casei* ATCC 393 and *Lactobacillus plantarum* ATCC 14917 were 1400, 1150, 850 and 500 bp, respectively. The isolated DNA band sizes of the strains, namely, *Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus casei and Lactobacillus plantarum* were observed to be 1400, 1150, 750 and 50 bp respectively. These values were close to that of the control group (Fig. 1, 2).

DISCUSSION

It is known that Lactobacillus species are effective on the maturation of pickles. In the maturation of pickles through natural micro-flora, *L. beijerinck*, *L. plantarum*, *L. casei*, *L. brevis*, *L. buchneri* species are known to e effective [27]. In a study [12], *L. plantarum*, *L. pentasus*, *L. fermentum*, *L. brevis* developing in eggplant were isolated. They have determined that the dominant species was *L. plantarum*. In our study, *L. plantarum*, *L. casei*, *L. brevis*, *L. fermentum* developing in pickle samples were isolated and *L. plantarum* was found to be the dominant species with the percentage of 56.2%. there was a difference between this study and that of Susana *et al.*(2005) in respect of a strain, which could be attributed to different types of pickles studied in both research. Bacteria containing similar plasmids or IS-elements were commonly isolated from vegetables and other habitats [28]. Pulido *et al.*(2005) isolated to the *L. plantarum, L. brevis* and *L. fermentum* from fermented caper and established to the *L. plantarum* as dominant bacteria.

In this study, *L. plantarum* B8 was found to be the most acid producing strain with the percent of 0.90%, which was similar to the result of the study of Susana *et al.* (2000) who found that *L. plantarum* was the most acid producing strain. On the other hand, *L. fermentum* was determined to be the least acid producing species among the others. The acid production of the 13 *L. plantarum* strains isolated from sausages produced using starter culture was found to range between 1.7 and 0.7%. In our study, the amount of hydrogen peroxide was determined between 1,1 μg/ml to 2,62 μg/ml with an average of 2,14 μg/ml.

In their study, Toksoy *et al.*(1999) found that the average hydrogen peroxide production of *L. plantarum* strains was 2,14 μg/ml[30]. In another study, the hydrogen peroxide production of strains was determined to be 0.85 μg/ml. However, another study indicated that the hydrogen peroxide production amount was between 0.59 and 0.65 μg/ml. Susana *et al.* (2000) showed that the hydrogen peroxide production of *L. fermentum* was the highest. Researchers have noted that varying amounts of hydrogen peroxide production of strains resulted from the difference in their oxireductase activities. Table 3 where the general inhibition zones of the bacteria are shown shows a relationship between the inhibition zone and acid levels. It was noted that under the effect bacteriocins and similar substances, the 6 of *L. plantarum* strain inhibited the *Bacillus cereus* strain, 2 inhibited the *Staphylococcus aureus* strain, 8 inhibited *Salmonella enteridis,* 7 inhibited *Escherichia coli,* 2 inhibited *Klebsiella pneumoniae,* 3 inhibited *Pseudomonas aeuroginosa*. As for *L. casei,* 3 strains inhibited *B. cereus, E. coli* and *S. enteridis* and for *L. fermentum* 2 strains inhibited *S. enteridis*, *K. pneumoniae* and *E. coli*' and in *L. brevis* 1 strain inhibited *E. coli.*

In our study, 8 strains from *L. plantarum*, 2 strains from *L. casel*, 2 strains from *L. fementum* and 1 strain from *L. brevis* were used to obtain hydrogen sulfur. In his study Toksoy *et al.* (1999) observed that 9 of *L. plantarum* strains produced hydrogen sulfur at different levels. It was reported that some species of Lactobacillus produced hydrogen sulfur at low levels and under anaerobic conditions including low levels of carbohydrate. It was observed that Lactobacillus species did not produce hydrogen sulfur in studies which indicated that hydrogen sulfur production was dependent on sulfide reductase and disulfohydrease enzyme activities [31, 32]. Klare *et al.* (2007) found different to the resistances against to the different antibiotic of *Lactobacillus* from several resources human and

probiotic borne. Accoring to findings, our results showed similar with literature.

In this study, it was found out that the size of DNA fragments varied between 1400 bp and 500 bp and the size of the DNA fragments isolated from *Lactobacillus* strains was between 1350 bp and 540 bp. The DNA band size of the isolated strains and standard strain was very similar. In this study, the DNA band size of *L. fermentum* was determined to be 1100 bp. Patrict *et al.* (2003) found the DNA band size of the *L. fermentum* isolated from clinical samples to be 527 bp [34]. In this study, the DNA band size of *L. plantarum* was found to be 540 bp. Kim *et al.* (2000) found the DNA band size of the *L. plantarum* isolated from Kimchi to be 419 bp [35]. Spano *et al.* (2002) determined the DNA band size of the *L. plantarum* isolated from red wine to be 318 bp [36].

Griffa *et al.* (2000) determined 288 bp DNA fragments in the *L. helveticus* strains isolated from cheese [37]. Andrighetto *et al.* (1998) found 1400 bp DNA fragments *L. delbruckii* 1100 bp, *L. helveticus* ve *L. acidophilus* isolated from dairy products [38]. Kimoto *et al.*(2004) determined 600 bp DNA fragments from *L. lactic* strains obtained from plant materials. The DNA fragment sizes of the strains isolated in this study were similar to those of *Lactobacillus* species isolated in similar studies [39].

The findings of this study indicated that it was appropriate to determine its metabolic properties.

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