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Research Article

Prevention of the Growth of *Salmonella* spp. and *Listeria* spp. in Tahini by Using Antagonistic Microorganisms

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ABSTRACT
Since tahini is consumed raw, pathogenic bacteria that may come from sesame threaten food safety. In this study, it was aimed to provide biocontrol of *Salmonella* spp. and *Listeria* spp. contamination in tahini. For this purpose, 10 different tahini samples were obtained from Kahramanmaraş market and enumeration of *Salmonella* spp., *Listeria* spp., total mesophilic aerobic bacteria (TMAB), yeast-mold, *Staphylococcus* spp. and coliform bacteria were made. Antagonistic effects of *Lactiplantibacillus plantarum* and *Companilactobacillus alimentarius* strains against *Salmonella* Typhimurium and *Listeria monocytogenes* strains were determined. The numbers of all microorganisms inoculated on the 7, 14, 21 and 28th days, including the first day, were determined from the stored samples. *Salmonella enterica* serovar Typhimurium was found in one sample. The mean TMAB and yeast-mold counts of the samples were determined as 3.50 and 3.39 log cfu/g, respectively. *L. plantarum* strains showed stronger antagonistic effects than *C. alimentarius* strains against pathogens in the medium.
Keywords: Tahini, *Salmonella*, *Listeria*, antagonist bacteria, *L. plantarum*, biocontrol.

Antagonistik Mikroorganizmalar Kullanılarak Tahinde *Salmonella* spp. ve *Listeria* spp. Gelişiminin Önlenmesi

ÖZ
Tahin çiğ tüketildiği için, susamdan gelebilecek olan patojen bakteriler gıda güvenilirliğini tehdit etmektedir. Bu çalışmada tahinde *Salmonella* spp. ve *Listeria* spp. kontaminasyonunun biyokontrolünün sağlanması amaçlanmıştır. Bu amaçla Kahramanmaraş piyasasından 10 adet farklı tahin örneği temin edilerek *Salmonella* spp., *Listeria* spp., total mezofilik aerobik bakteri (TMAB), maya-küf, *Staphylococcus* spp. ve koliform bakteri sayımları yapılmıştır. *Lactiplantibacillus plantarum* ve *Companilactobacillus alimentarius* suşlarının *Salmonella* Typhimurium ve *Listeria monocytogenes* suşlarına karşı antagonistik etkileri belirlenmiştir. Depolanan örneklerden 1. gün dahil 7., 14., 21. ve 28. günlerde inoküle edilen tüm mikroorganizmaların sayıları belirlenmiştir. Bir örnekte *Salmonella enterica* serovar Typhimurium bulunmuştur. Örneklerin ortalama TMAB ve maya-küf sayıları sırasıyla 3.50 ve 3.39 log kob/g olarak belirlenmiştir. *L. plantarum* suşları, besiyerindeki patojenlere karşı *C. alimentarius* suşlarına göre daha güçlü antagonistik etki göstermiştir.
Anahtar Kelimeler: Tahin, *Salmonella*, *Listeria*, antagonist bakteri, *L. plantarum*, biyokontrol.

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Prevention of the Growth of *Salmonella* spp. and *Listeria* spp. in Tahini by Using Antagonistic Microorganisms

Introduction

Sesame is known as the first oilseed plant cultivated in the world and has been consumed for more than 5000 years (Batu and Elyıldırım, 2009). Therefore, it has a wide variety of uses. Although sesame production is not at very high levels in our country, it is frequently used as raw material or semi-finished product in many different sectors. For this reason, Turkey is among the countries that import the most sesame seeds in the world (Anonymous, 2021). In addition to being a valuable source of oil, it is also used in bakery products, confectionery, desserts and sauces due to its properties and taste in terms of human health (Çiftçi et al., 2008). The oil obtained from sesame is used as a raw material in the food industry, as well as in the cosmetics, pharmaceutical, insecticide and paint industries. One of the most important usage areas in the food industry is tahini production (Güven et al., 2007).

Tahini is the product obtained by separating the shells of sesame seeds (*Sesamum indicum* L.) in accordance with the technique and crushing them in a mill after drying and roasting in the oven (Anonymous, 2015a). Tahini, which is one of our traditional foods that is consumed with pleasure in many parts of the world, especially in the Middle East countries, and in Turkey, is known as an ideal food due to its high nutritional value and cheapness (Artık and Ceyhun, 2010; Var et al., 2007). In our country, it is consumed excessively by mixing with sugar, honey or molasses; it is also used in the production of dishes and desserts in local cuisines (Özcan, 1993). But the most used product is the tahini halva (Batu and Elyıldırım, 2009).

Contamination of tahini with pathogenic microorganisms is a particularly important because it is usually consumed without any additional heat treatment (Torlak et al., 2013). It has been reported that a total of 175 tahini-induced salmonellosis cases occurred in Australia, New Zealand, Norway, USA, Sweden and Canada between 2001-2014 (Al-Nabulsi et al., 2014).

Sesame seeds can be contaminated with *Salmonella* spp. and other microorganisms during growth, storage or processing. It has also been reported that *Listeria* spp. contamination occurs in industrially produced tahini in various countries (Anonymous, 2008; Ly et al., 2019). Microbial contamination can occur from pre-harvest sources or post-harvest sources including harvesting equipment, transport containers, insects, dust, rinse water, ice, transport vehicles, processing equipment, and working staff (Olaimat and Holley, 2012). Although there is a heat treatment such as roasting within the production stages, it is reported that *Salmonella* spp. contamination is more likely to occur after the roasting process and to occur due to unfavourable hygiene conditions during grinding, slicing, packaging or transportation (Al-Nabulsi et al., 2020; Brockmann et al., 2004).

In this study, it was aimed to provide biocontrol of *Salmonella* spp. and *Listeria* spp. contamination in tahini. For this purpose, 10 different tahini samples were obtained from the Kahramanmaraş market and the presence of related pathogens in these samples was investigated. In addition, TMAB, yeast-mold, *Staphylococcus* spp. and coliform bacteria enumeration were made in tahini samples.

Materials and Methods

Materials

Tahini samples used in the study were obtained from Kahramanmaraş market. For this purpose, 10 different tahini samples, 6 of which are from national brands and 4 from local producers, were brought to the laboratory unopened, and after verifying whether *Salmonella* spp and *Listeria* spp. were present in these samples, they were kept in the refrigerator at +4°C until the analyses were performed. In addition, sesame samples were obtained from the same 4 local producers.

L. plantarum and *C. alimentarius* strains used as antagonists were obtained from the culture collection of Erzurum Atatürk University Faculty of Agriculture Department of Food Engineering. *S.* Typhimurium ATCC 14028 and *L. monocytogenes* ATCC 7644 strains used in antagonist activity tests were obtained from the culture collection of Çukurova University Faculty of Agriculture Department of Food Engineering.

Methods

pH determination

For pH measurement in tahini samples, 10 g of tahini was thoroughly homogenized in 15 mL of distilled water and pH values were determined using a combined electrode pH meter (Thermo Scientific) (Hooi et al., 2004). pH measurements were carried out to determine whether the acidity changed during storage in the LAB added samples.

Microbiological enumeration

For each microbiological analysis, 10 g of tahini sample was homogenized with 90 ml of sterile 0.85% NaCl solution under aseptic conditions and dilutions were prepared. All analyses were performed in duplicate (Anonymous, 2014).

Total mesophilic aerobic bacteria counting was carried out (TMAB) at 37°C on plate count agar (Merck) medium (Anonymous, 2014), yeast and mold counting was carried out (Anonymous, 2012) at 25°C on potato dextrose agar (Merck) medium, antagonistic LAB counting was carried out at 30-32°C on de Man Rogosa and Sharpe (MRS) agar (Merck) medium (Anonymous, 2015b), coagulase positive S. aureus counting was carried out at 37°C on Baird Parker agar (Oxoid) medium with egg yolk telluride emulsion (5%) (Tallent et al., 2001) coliform bacteria counting was carried out at 37°C on Violet Red Bile Agar (Merck) medium (Anonymous, 2010) for tahini samples. To count the yeasts and molds, incubation was performed for 3-5 days, and 24-48 h for all other microbial counting. To provide anaerobic conditions for LAB counting, Anaerocult (Merck Millipore) brand kits were placed in the anaerobic jar by soaking the kits with appropriate amount of pure water.

Detection of *Salmonella* spp.

The method proposed by Wallace et al. (2020) was modified and used for the detection of *Salmonella* spp. in tahini samples obtained from the market and subsequently inoculated. For this purpose, the preserved samples were weighed 25 g under aseptic conditions and transferred into 225 mL sterile buffered peptone water (0.1% peptone) for pre-enrichment and mixed homogeneously with a magnetic stirrer. After 18 hours of incubation at 37°C, the selective enrichment stage was initiated. At this stage, 100 µL of the pre-enrichment culture was inoculated into 10 mL of sterile Selenite Cystine Enrichment Broth (Merck) medium and incubated at 37°C for 24 hours. At the end of the incubation, a loop was taken from each tube with selective enrichment to perform colony isolation and inoculated into *Salmonella* Shigella Agar (SSA) medium using the streak plate method. After 24 hours of incubation at 37°C, typical *Salmonella* spp. colonies were determined and Triple Sugar Iron (TSI) Agar test was started.

Active cultures of typical colonies were inserted into the bottom part of Triple Sugar Iron Agar medium prepared horizontally with a needle loop and then applied to the surface. After 24 hours of incubation at 37°C, the results were evaluated as *Salmonella* positive or negative. Typical colonies with yellow at the bottom, black at the middle, red at the surface and gas slits were evaluated as probable *Salmonella* strains. Then, genetic identification of typical colonies of *Salmonella* spp. was made by 16S rRNA PCR method.

Detection of *Listeria* spp.

The method suggested by Hitchins et al., (2017) was modified and used for determining the *Listeria* spp. in tahini samples obtained from the market and subsequently inoculated. Each tahini sample was weighed 10 g, transferred to 90 ml sterile *Listeria* Enrichment Broth (Merck) medium, mixed well, and incubated at 37°C for 24 hours. 0.1 mL of this selective enrichment medium was taken and inoculated on *Listeria* Selective Agar (Merck) plates by spread plate method, and the cultivated petri plates were incubated at 37°C for 2 days. As a result of the incubation, enumeration was made by considering the green-black coloured colonies with black zones. After the enumeration, 5-10 colonies were selected from the agar plates and inoculated on Nutrient Agar (Merck) medium by streak plate method. After 24 hours of incubation at 37°C, Gram staining, motility at 22°C and catalase tests were performed and typical *Listeria* colonies were determined according to the results obtained. Then, genetic identification of these isolates with 23S rRNA PCR method was performed.

Genetically Identification

To isolate DNA from the obtained bacterial isolates, 100 µL of bacterial suspensions kept at -20°C in sterile distilled water were taken and mixed with 500 µL sterile distilled water in 1.5 mL Eppendorf tubes. The suspension was centrifuged at 5000 x g for 3 minutes and washed 3 times with phosphate buffer. 0.5 mL of 6 M urea and 0.1 mL of 10% SDS (Sodium Dodecyl Sulphate) were added to the bacterial suspension obtained by washing and incubated at 37°C for 20 minutes. After this mixture was kept at 95°C for 5 minutes, it was centrifuged at 8000 x g for 10 minutes at 25°C. The supernatant obtained after centrifugation was discarded, 0.1 mL of 0.2 N NaOH was added to the remaining part (pellet) and incubated at 37°C for 10 minutes. Then, after centrifugation at 3000 x g for 3 minutes at 25°C to separate non-nucleic acid cell fragments, the supernatant was discarded again. Then, 2.5 times the volume of pure ethyl alcohol was added to purify the DNA and incubated at -20°C for 2 hours. After this time, the mixture was centrifuged at 13200 x g for 15 minutes at 4°C and washed with 70% ethanol. After the obtained DNA pellet was dried at room temperature, it was resuspended in 20 µL of Tris-EDTA (TE) buffer and made ready for PCR (Singh and Ramesh, 2009).

After DNA isolations, 16R (5'-GGAACTTACCCGACAAGG-3') and 16F (5'-AATACGTTCCCGGGCCTTG-3') universal primers were used for PCR identification of 16S rRNA and 23S rRNA. In this aim, a total of 50 µl of PCR mix was prepared using 25 µL of PCR master mix (Qiagen), 18 µL of nuclease-free water, 4 µL of template DNA, 1.5 µL of 16R universal primer and 1.5 µL of 16F universal primer. The first denaturation was done at 95°C for 5 minutes. The PCR protocol was performed at 94°C for 30 seconds (first denaturation), at 51°C for 45 seconds (annealing), and at 72°C for 45 seconds (extension). After the 35th cycle of the protocol, the final extension was performed at 72°C for 10 minutes. 12 µL of the mixture subjected to the PCR process was taken, loaded into a 2% agarose gel, and run at 35 V for 6 hours. At the end of the run, the gel was visualised under UV light and the isolate of appropriate base pair length was sequenced. DNA sequencing of PCR products was carried out by using the ABI 3130 XL genetic analyzer (Applied Biosystems, Foster City, CA). The obtained DNA sequences were evaluated using the American National Center for Biotechnology Information (NCBI) database and the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm.

Tests for Antagonistic Activity

The agar spot test method was used to measure the antimicrobial activities of *L. plantarum* and *C. alimentarius* strains, which are preferred as antagonistic LAB. For this purpose, appropriate strains of microorganisms were first inoculated into 10 mL of sterile MRS Broth (Merck) medium and incubated at 32°C for 24 hours. Then, one loop was taken from these 24-hour cultures and 2 spots were planted on MRS Agar medium and incubated in anaerobic environment at 32°C for 24-48 hours. Before 24-hour cultures of *S.* Typhimurium and *L. monocytogenes* strains, which are preferred as pathogens for agar spot test, 0.1 mL of TSB (Tryptone Soy Broth-Merck) medium containing 7 mL of semi-solid agar is inoculated and MRS Agar (Merck), which has been cultivated in 2 spots previously was poured slowly onto the medium. After waiting for solidification, it was incubated at 37°C for 24 hours. At the end of the incubation period, the inhibition zone diameters around the LAB colonies were measured (Belicová et al., 2013).

Inoculation of Antagonist and Pathogen Strains in Tahini Samples and Storage

1 mL of 24-hour cultures of appropriate strains from both antagonist microorganisms and pathogens were taken and inoculated into 100 mL Nutrient Broth (Merck) medium in 300 mL Erlenmeyer flask. Then it was incubated for 24 hours at 37°C in a shaking incubator set at 180 rpm. After incubation, the concentrated cells obtained by centrifugation at 2500 x g and 4°C for 10 minutes were washed 2-3 times with 0.85% NaCl. Concentrated cell pellets were suspended in 1 mL of sterile 0.85% NaCl, and the number of viable organisms per millilitre was determined by inoculating into Nutrient Agar (Merck) medium (Bosse et al., 2016).

Stock solutions were diluted according to the determined number and each strain was

inoculated into tahini samples at the level of 6 log cfu/g. For the purpose of inoculation, tahini sample, which was bought from the market and in which no pathogen was detected, was preferred. Inoculation groups composed as: Group 1 (PL + SL), Group 2 (AL + SL), Group 3 (PL+ AL+ SL), Group 4 (PL), Group 5 (AL), Group 6 (SL), Group 7 (PL + LI), Group 8 (AL + LI), Group 9 (PL + AL + LI), Group 10 (LI), Group 11 (PL + AL + SL + LI).

The meanings of the abbreviations used here are as follows, PL; *L. plantarum*, AL; *C. alimentarius*, SL; *S.* Typhimurium and LI; *L. monocytogenes*. In addition, LAB strains were inoculated separately and together in a tahini sample that was found to be contaminated with *Salmonella* spp. Enumeration of all bacteria inoculated were performed for each group on the 7, 14, 21 and 28th days, including the first day.

Statistical Calculations

Statistical analyses of the data obtained during the study were carried out with the SPSS for Windows Release ver. 2000 package program. The averages of the significant sources of variation were calculated by the Duncan Multiple Comparison Test. The correlations between different variations and parameters used in the study were examined. In addition, the Independent-T test to examine the relationship between sesame samples and the tahini samples produced from them, and to examine the changes observed during storage, a comparison was made according to the experimental design of divided parcels in random parcels (Efe et al., 2000).

Results and Discussion

Microbiological Enumeration

The microbiological enumeration results of tahini and sesame samples obtained from the market were given in Table 1.

As a result of microbiological enumeration done in tahini samples, it was seen that the samples generally have a low microbial load. This is because tahini has both low water activity and high oil content. According to the TMAB counting results performed in the samples, the lowest value was obtained from sample 5 with 2.03 log cfu/g, and the highest value was obtained from sample 3 with 5.02 log cfu/g. The mean TMAB count of all tahini samples was calculated as 3.50 log cfu/g.

Among the yeast-mold enumeration results of tahini samples, the lowest value was observed with 2.34 log cfu/g for sample 8, and the highest value was observed for sample 3 with 4.79 log cfu/g. The average yeast-mold count of the samples was determined as 3.39 log cfu/g. In the mold count analysis, only 2 of the samples showed mold colonies at countable intervals. Coliform bacteria were found in only one of the tahini samples.

The difference between the mean TMAB count of the samples and the difference between the mean number of yeast and mold were found to be statistically very significant ($p<0.01$). According to the Duncan's multiple comparison test results of TMAB means, 10 different groups were formed, and 7 different groups were formed in terms of yeast-mold means. In this case, it was understood that the tahini on the market do not show any similarity in terms of microbial load.

Similarly, in a study performed by Khachfe et al., (2018) in which they investigated the microbiological quality of tahini samples, significant differences were found between the microbial loads of the samples. In that study, TMAB count results were found in the range of 2-5.6 log cfu/g, and yeast-mold counting results were found in the range of 2-6 log cfu/g. When the means of the results are compared with obtained in our study, it was seen that both TMAB and yeast-mold numbers are higher than in our study. In addition, in the same study, coliform bacteria were found in approximately 90% of the samples. In another study carried out by Ayaz and Al-Sogair, (1986), it was determined that the TMAB numbers of tahini samples were in the range of 1-4.5 log cfu/g, and the yeast-mold counts were in the range of 1-2 log cfu/g. In the same study, coliform bacteria were found in 20% of the samples. The TMAB results obtained in our study were like these results, and the yeast-mold counts were found to be higher.

Table 1. Microbiological enumeration results of tahini and sesame samples

	Sample No	TMAB log cfu/g	Yeast log cfu/g	Mold log cfu/g	*Stahpylococcus* spp. log cfu/g	Coliforms log cfu/g
National Market Tahini	1	3.62±0.02	<2	<2	3.68±0.03	2.01±0.08
	2	2.31±0.01	2.91±0.04	<2	<2	<1
	3	5.02±0.01	4.79±0.03	<2	<2	<1
	4	3.23±0.03	3.32±0.04	<2	<2	<1
	5	2.03±0.03	<2	<2	<2	<1
	6	4.36±0.01	3.26±0.03	3.26±0.03	3.46±0.04	<1
Local Market Tahini	7	2.61±0.02	2.89±0.03	<2	<2	<1
	8	2.70±0.01	2.34±0.02	2.34±0.02	<2	<1
	9	4.53±0.02	3.18±0.03	<2	<2	<1
	10	4.61±0.02	4.47±0.04	<2	2.03±0.02	<1
Minimum		**2.03±0.03**	**2.34±0.02**	-	-	-
Maximum		**5.02±0.01**	**4.79±0.03**	-	-	-
Mean		**3.50±0.15**	**3.39±0.27**	-	-	-
Local Sesame Samples	S7	3.18±0.05	5.11±0.03	<2	<2	<1
	S8	2.49±0.02	<2	<2	<2	<1
	S9	2.94±0.03	4.95±0.02	<2	<2	<1
	S10	2.03±0.03	2.60±0.04	<2	<2	<1
Minimum		**2.03±0.03**	**2.60±0.04**	-	-	-
Maximum		**3.18±0.05**	**5.11±0.03**	-	-	-
Mean		**2.66±0.44**	**4.22±1.14**	-	-	-

As a result of analyses carried out to determine the presence of coagulase positive S. aureus in tahini samples, typical colonies were found in three samples. Accordingly, among the coagulase, catalase, DNAse and thermonuclease positive colonies, 1-A, 6-A and 6-B coded colonies were found to have methicillin resistance. Therefore, it was thought that the toxins that these strains can produce may be risky in terms of food safety.

In similar studies, it was reported that S. aureus colonies were found in tahini samples (Ayaz and Al-Sogair, 1986; Khachfe et al., 2018; Olaimat et al., 2017). Although S. aureus can usually be isolated from the environment, its natural source is humans and animals. In humans, it is mostly isolated from the mucous layer of the nasal and throat cavity. In addition, it is found on the skin, face, hands, stool, inflamed wounds, boils, and pimples. For these reasons, it is highly probable that the S. aureus strains found in the tahini samples were transmitted by the staff who is responsible from the production (Castro et al., 2016). Staphylococci cause various inflammatory infectious diseases and cause septicaemia, arthritis, meningitis, endocarditis, dermatitis, and joint rheumatism in humans (Kloos and Bannerman, 1994).

As a result of TMAB enumeration performed on sesame samples, the lowest value was found in the sample S10 with 2.03 log cfu/g, and the highest value was found in the sample S7 with 3.18 log cfu/g. The mean TMAB number of the samples was calculated as 2.66 log cfu/g. As a result of yeast-mold enumeration, the lowest value was obtained from the sample S10 with 2.60 log cfu/g, and the highest value was obtained from the sample S7 with 5.11 log cfu/g. The average yeast-mold counts of the samples were determined as 4.22 log cfu/g. Yeast growth was not observed in one sample.

The Independent-T test was performed to compare the microbiological enumeration results of tahini obtained from local producers and the sesame samples they were produced from (Table 2). As a result of the analyses, the differences between the mean TMAB numbers of the sesame samples and the tahini samples made from them were found to be statistically very significant ($p<0.01$). The differences between the averages of yeast and mold numbers were found to be statistically insignificant ($p>0.05$). In this case, it was thought that the bacterial load in tahini which is obtained from local producers' changes during production and packaging and the yeast-mold load does not change.

Table 2. Independent-T test results for the comparison of microbial loads of sesame seeds and tahini produced from them

	Tahini		Sesame		Significance Level (p<0.05)
	Mean	Std. Deviation	Mean	Std. Deviation	
TMAB	3.61	1.00	2.66	0.46	0.007**
Yeast-mold	3.22	0.82	3.16	2.17	0.934ns

** 1% significance level, ns: statistically insignificant.

In addition, it is noteworthy that there was no mold in the sesame sample S8, but there was mold in the tahini produced from it, and there was no Staphylococcus spp. in the sesame sample S10, and it was found in the tahini sample 10. Considering all these, it was thought that the local tahini producers in Kahramanmaraş have problems in hygiene and sanitation conditions.

Presence of *Salmonella* spp. and *Listeria* spp. in Tahini Samples

As a result of the screenings for the presence of *Salmonella* spp. and *Listeria* spp. in the samples by cultural methods, typical *Salmonella* colonies were found only in sample 1. No typical *Listeria* spp. colonies were found in any of the samples. Isolations were made to identify the typical colonies of *Salmonella* spp. by 16S PCR method. Then, DNA isolation was made, and template DNAs were made ready for PCR. After the PCR analyses, the amplified DNAs were run on an electrophoresis gel and then visualized under UV light (Figure 1).

The gene sequences obtained as a result of the sequence analyses were determined by using the BLAST algorithm (BLAST, 2021). Accordingly, it was determined that the suspect isolate was *Salmonella enterica* serovar Typhimurium. *Salmonella* spp. has been found in many previous studies in tahini. In a study carried out by Khachfe et al. (2018), different *Salmonella* strains were detected in approximately 30% of tahini samples obtained from the Lebanese market. In a study by de Jong et al. (2001), it was reported that tahini halva contaminated with *Salmonella* Typhimurium DT104 in Sweden caused the disease in 27 people in 2001. Also in 2001, two outbreaks were reported, with 18 and 14 cases reported in Norway and Australia, respectively (Aavitsland et al., 2001; O'Grady et al., 2002).

In addition, a multi-stage epidemic (16 cases with one hospitalization and one death) was reported in the USA in 2013 related to the consumption of hummus prepared with tahini contaminated with *Salmonella mbandaka* and *S. montevideo* (Olaimat et al., 2018). In 2015, tahini contaminated with *Salmonella* was recalled from the market 9 times in Canada. In May 2018, tahini products contaminated with *Salmonella* spp. were detected and recalled in the USA market (Al-Nabulsi et al., 2020).

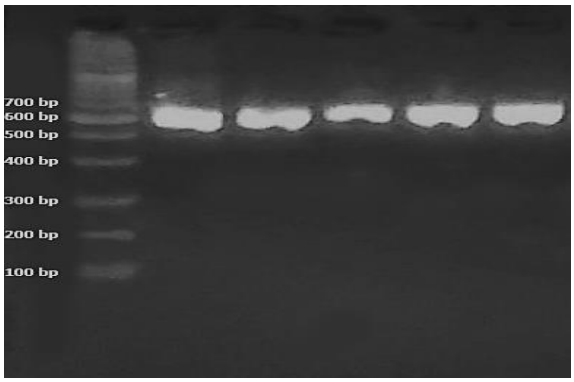

Figure 1. Images of DNA amplified by 16S PCR method under UV light.

In vitro Antagonistic Activity of the LAB Strains

In the antagonist activity tests, antimicrobial activities of *L. plantarum* and *C. alimetarius* strains against *S.* Typhimurium and *L. monocytogenes* reference strains were determined. For this purpose, the agar spot method was used, and the inhibition zones were measured.

Accordingly, *L. plantarum* strains showed the highest antimicrobial activity (27 mm) against *L. monocytogenes*. This was followed by *S.* Typhimurium (24 mm). In a study performed by Mashak (2016), it was reported that *L. plantarum* strains have strong antimicrobial activity against

the same pathogens. Similarly, Kamiloğlu et al. (2019) found that *L. plantarum* strains isolated from sausage have different levels of antimicrobial activity against pathogens such as S. aureus, *L. monocytogenes* and B. cereus in their study. In another study by Liu et al. (2019), it was determined that the use of *L. plantarum* strains as a biocontrol agent had a lethal effect on *Salmonella* serovars. In a similar study, some bacteriocin was isolated from *L. plantarum* ZJ316 strain and its antimicrobial effect on different *Salmonella* strains was investigated. As a result of the analyses carried out, it was reported that the isolated L-phenyl lactic acid has an antimicrobial effect on *Salmonella enterica* subsp. *enterica* ATCC 1402 strain (Zhou et al., 2020).

C. alimentarius strains showed the highest antimicrobial activity (17 mm) against *L. monocytogenes* strains, followed by *S.* Typhimurium (11 mm). Hu et al. (2017) found that the bacteriocin named lactosine produced by *C. alimentarius* strains has an antimicrobial effect on pathogenic bacteria. Mourad and Bettache (2018) determined that *C. alimentarius* strains have antimicrobial effects on E. coli and B. cereus in a study in which they characterized different lactic acid bacteria. In another study performed by Klingberg et al. (2005), *C. alimentarius* strains isolated from Scandinavian fermented sausages were found to have antimicrobial activity against *S.* Typhimurium, E. coli, *L. monocytogenes* and Yersinia enterocolitica strains.

When the agar spot test results were evaluated in general, it was determined that *L. plantarum* strains had a higher inhibitory effect on target pathogens than *C. alimentarius* strains. However, in a study investigating the antimicrobial activities of *Lactobacillus* strains, it was determined that although *L. plantarum* and *C. alimentarius* strains had an inhibitory effect on B. cereus and E. coli, they had not antimicrobial effect against *S.* Typhimurium and *Listeria* strains (Gandevia et al., 2017). Therefore, although not all strains belonging to *L. plantarum* and *C. alimentarius* species preferred to be used in that study did not have antagonistic effects on target pathogen groups, it was understood that the strains used in our study have an inhibitory effect.

Microbiological and pH Changes During Storage in Tahini Samples

Before storage, each microorganism was inoculated to the sample groups at the level of 6 log cfu/g. Afterwards, the numbers of all microorganisms were determined weekly, and their changes were observed. In order to reveal the time-dependent changes in the mean number of microorganisms in the samples during the storage period, the divided parcels in random parcels experimental design were used. The changes in the number of LAB and pH changes in the samples were shown in Table 3.

The fact that the LAB numbers in the groups were very close to 6 log cfu/g on the first day indicates that the inoculation was done at the desired level. The differences between the average LAB counts of each of the tahini groups at the end of the storage period were found to be statistically significant ($p<0.01$).

It was observed that the colony numbers of the groups, to which the same strains were added, were the same at the end of the period. At the end of storage, when the number of bacteria in the group with only *L. plantarum* strain was compared with that of the groups with pathogens, it was concluded that the number of *L. plantarum* was not affected by the presence of other microorganisms. However, it was seen that the number of *C. alimentarius* is negatively affected by the presence of pathogenic microorganisms. At the end of the 4-week storage, both antagonist LAB strains were found to be more than 4 log cfu/g in tahini samples and preserved their viability. In this way, it was thought that they continue to produce antimicrobial effective metabolites and inhibit the development of target pathogens at different levels.

Table 3. The changes of LAB numbers inoculated into tahini samples during storage

	Storage Time (week)					
Groups	**First day**	**1**	**2**	**3**	**4**	**Mean**
			LAB			
AL	5.85	4.32	5.18	5.44	5.36	**5.23 D**
PL	5.74	5.38	5.78	5.86	5.80	**5.71 A**
AL+SL	5.80	3.51	3.30	3.80	3.76	**4.03 F**
AL+LI	5.82	4.03	3.30	3.56	3.59	**4.06 F**
PL+LI	5.78	4.52	5.07	4.82	4.96	**5.03 E**
PL+SL	5.85	5.46	5.65	5.61	5.59	**5.63 B**
PL+AL+LI	5.87	5.48	4.91	5.06	4.98	**5.26 D**
PL+AL+SL	5.87	5.56	5.41	5.76	5.65	**5.65 B**
PL+AL+SL+LI	5.90	5.92	5.60	4.92	4.89	**5.45 C**
Mean	**4.77 A**	**4.02 C**	**4.02 C**	**4.08 B**	**4.05 B**	***4.19***
			pH			
LI	6.39	6.40	6.40	6.39	6.33	**6.38 A**
SL	6.38	6.40	6.39	6.43	6.38	**6.39 A**
AL	6.37	6.39	6.40	6.41	6.39	**6.39 A**
PL	6.39	6.38	6.41	6.38	6.39	**6.39 A**
AL+SL	6.40	6.44	6.40	6.42	6.36	**6.40 A**
AL+LI	6.37	6.39	6.41	6.38	6.34	**6.38 A**
PL+LI	6.37	6.40	6.43	6.37	6.35	**6.38 A**
PL+SL	6.37	6.43	6.40	6.39	6.36	**6.39 A**
PL+AL+LI	6.38	6.43	6.44	6.40	6.37	**6.40 A**
PL+AL+SL	6.39	6.40	6.42	6.38	6.35	**6.39 A**
PL+AL+SL+LI	6.39	6.40	6.40	4.40	6.41	**6.00 B**
Mean	**6.38 AB**	**6.40 A**	**6.41 A**	**6.21 C**	**6.37 B**	***6.36***
Significance Level (p<0.05)		**Group = 0.000****		**Time= 0.000****		

** %1 significance level. Means indicated by different letters are statistically different from each other.

The differences between the mean weekly LAB counts of all inoculation groups were found to be statistically significant ($p<0.01$). Three different groups were formed in terms of the average of the 4-week LAB count, including the first day. LAB counts decreased in the 1st week but did not change significantly during storage afterwards.

To determine whether the LAB strains added to the samples produce lactic acid and cause souring in the taste of the samples, the pH changes of the groups were also followed during the storage. In terms of the average pH values of the samples at the end of the storage period, only 2 groups were formed. In terms of weekly pH values, 3 different groups were formed ($p<0.01$). Almost no change was observed in the pH levels of the inoculation groups during storage. A significant decrease was observed only in the group with all bacteria. Therefore, it could be concluded that the use of these LAB strains as biocontrol agents in tahini and similar foods will not cause a sour taste.

Changes in *Salmonella* spp. and *Listeria* spp. numbers observed in tahini groups during storage were given in Table 4. The fact that the *Salmonella* spp. numbers in the groups were close to 6 log cfu/g on the first day indicates that the inoculation was done at the desired level. The differences between the average *Salmonella* spp. count at the end of the storage period and the weekly mean numbers were found to be statistically significant ($p<0.01$). In terms of the number of bacteria at the end of storage, 5 different groups were formed. In terms of weekly *Salmonella* spp. number means, 3 different groups were formed. *Salmonella* spp. contents of tahini samples decreased until the 2nd week, but there was no significant change after that.

At the end of the storage period, the fact that the level of *Salmonella* spp. in the group in which the antagonist LAB strain was not added was higher than in the other groups was an indication that the LAB strains have inhibitory effect. *L. plantarum* strain, which reduced *Salmonella* spp. level by 34.2%, was the most effective antagonist LAB strain.

Table 4. Changes of the number of *Salmonella* spp. during storage in tahini samples.

Storage Time (week)						
***Salmonella* spp.**						
Groups	**First day**	**1**	**2**	**3**	**4**	**Mean**
SL	6.34	4.98	5.02	5.01	5.06	**5.28 A**
AL+SL	6.29	4.94	4.44	4.38	4.40	**4.89 B**
PL+SL	6.32	4.92	4.19	4.17	4.15	**4.75 D**
PL+AL+SL	6.26	4.78	4.45	4.46	4.41	**4.87 C**
PL+AL+SL+LI	6.28	4.78	4.15	4.13	4.11	**4.69 E**
Mean	**6.30 A**	**4.88 B**	**4.45 C**	**4.43 C**	**4.43 C**	***4.90***
***Listeria* spp.**						
LI	5.88	5.41	5.05	5.03	4.89	**5.25 A**
AL+LI	5.90	5.16	4.49	4.00	3.91	**4.69 B**
PL+LI	5.93	4.59	3.18	2.74	0.00	**3.29 D**
PL+AL+LI	5.99	5.05	4.23	3.53	2.17	**4.19 C**
PL+AL+SL+LI	5.92	5.49	2.86	0.00	0.00	**2.86 E**
Mean	**5.92 A**	**5.14 B**	**3.96 C**	**3.06 D**	**2.19 E**	***1.84***
Significance Level (p<0.05)		**Group = 0.000****		**Time= 0.000****		

** %1 significance level. Means indicated by different letters are statistically different from each other.

Antagonist LAB strains were inoculated separately and in a mixed form to sample 4 in which *S. enterica* serovar Typhimurium was detected, and storage was carried out. The changes in the number of *Salmonella* spp. in sample 4 during storage were shown in Figure 2. Accordingly, *Salmonella* spp. completely inhibited in the 3rd week in both groups with *L. plantarum* strain. In the group with only *C. alimentarius* strain, it was observed that *Salmonella* spp. was inhibited only at the end of the 4th week. Therefore, *L. plantarum* was the most successful antagonist bacterium in terms of the group in which *S. enterica* serovar Typhimurium was detected.

The fact that the numbers of *Listeria* spp. in the groups were close to 6 log cfu/g on the first day indicates that the inoculation was done at the desired level. Five different groups were formed in terms of both the number of microorganisms at the end of the storage period and the weekly number of microorganisms ($p<0.01$). The numbers of *Listeria* spp. decreased regularly during storage. In the group to which only *L. plantarum* strain was inoculated, *Listeria* spp. was inhibited at the end of the 4th week, and in the group to which both antagonists and pathogens were inoculated, *Listeria* spp. growth was not observed at the end of the 3rd week.

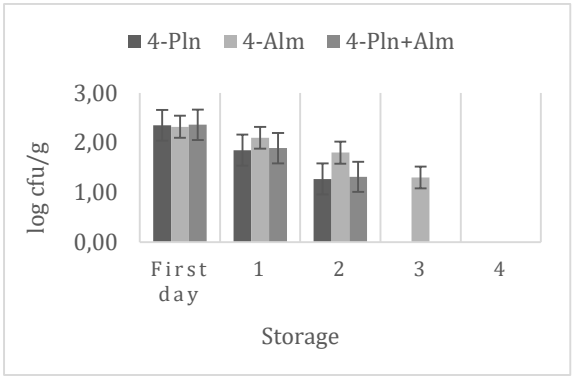

Figure 2. Changes of the number of *Salmonella* spp. observed in sample 4 during storage.

The presence of *Listeria* spp. at the end of the 4th week in the group with both antagonists and pathogenic microorganisms was an indication that the *L. plantarum* strain alone has a stronger antimicrobial effect. In the group with only *C. alimentarius* strain, the level of *Listeria* spp. decreased by 33.7% at the end of the storage period. In terms of *Listeria* spp., the most effective antagonist LAB was *L. plantarum*.

Conclusion

Since tahini is a highly consumed product both in Turkey and in the Middle East, and it is generally consumed raw, it is likely to be the cause of any food-borne illness. Therefore, it should be a priority for production to prevent pathogen contamination of tahini at any stage. However, even if pathogenic microorganisms are contaminated with the product for any reason, they must be inhibited and their toxins, if any, must be neutralized. The findings obtained in our study will shed light on biocontrol studies that can be done in tahini or tahini-like products in the future. In addition, the potential to isolate antimicrobial metabolites from the antagonist bacteria and to use only these metabolites as antimicrobial agents in foods for pathogen biocontrol are among the studies that can be done. Thus, the risk of antagonist microorganisms changing the taste and aroma of the product will be eliminated.

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