

## Selection of potential autochthonous starter cultures from shalgam, a traditional Turkish lactic acid-fermented beverage

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**Abstract:** The present study was done to select the potential autochthonous lactic acid bacteria (LAB) for the production of shalgam, which is a traditional Turkish lactic acid-fermented beverage. Eighteen LAB belonging to the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Leuconostoc* isolated previously from shalgam samples produced in the university laboratory and by small- and large-scale producers in industry were used. Pasteurized black carrot juice was inoculated individually with these selected LAB strains and fermented for 10 days. The strains of *Lb. plantarum* gave the highest numbers during fermentations with a range of 9.40–9.16 log cfu mL<sup>-1</sup>. *Lb. plantarum*<sup>bx</sup> produced the highest total acidity, 22.86 g L<sup>-1</sup> as lactic acid, followed by *Lb. paracasei* subsp. *paracasei* 2, *Lb. plantarum*<sup>cx</sup>, *Lb. plantarum*<sup>ax</sup>, and *Lb. fermentum*, with a range of 20.45–22 g L<sup>-1</sup> as lactic acid. Only *Lb. delbrueckii* subsp. *delbrueckii* and *Lb. fermentum* grew at 45 °C, but none of the LAB species grew with 18% NaCl and at pH 9.6. During the sensory analysis, the most preferred sample was that obtained by *Lb. plantarum*<sup>bx</sup>, followed by *Lb. fermentum* and *Lb. paracasei* subsp. *paracasei* 2. These findings indicate that *Lb. plantarum*<sup>bx</sup>, *Lb. fermentum*, and *Lb. paracasei* subsp. *paracasei* 2 have potential as starter cultures for the production of shalgam.

**Key words:** Autochthonous culture, lactic acid bacteria, lactic acid fermentation, naturally fermented beverage, shalgam (şalgam), starter culture

### 1. Introduction

Lactic acid fermentation (LAF) is one of the most practical and widely applied empirical methods for preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables such as cucumbers, cabbages, and olives (Tamang et al. 2005; Di Cagno et al. 2008).

Shalgam, a traditional Turkish lactic acid-fermented beverage, is obtained mainly by LAF of black carrot (*Daucus carota* L.), bulgur bran, rock salt, turnip (*Brassica rapa* L.), and sourdough, in which lactic acid bacteria (LAB) play an essential role. There are 2 main processing methods for commercial production: the traditional method and the direct method (Erten et al. 2008; Tanguler and Erten 2012a). The traditional method consists of 2 distinct steps: first fermentation and second fermentation (Canbas and Deryaoglu 1993; Erten et al. 2008; Tanguler and Erten 2012a). First fermentation is also called sourdough fermentation. It is carried out for the enrichment of LAB and yeasts. Bulgur flour (3%), rock salt (0.2%), sourdough (0.2%), and adequate table water are mixed and kneaded for the formation of dough. The dough is left for the

first fermentation at ambient temperature for 3–5 days. The fermented mixture of bulgur flour and sourdough is extracted with adequate water 3 to 5 times (Erten et al. 2008; Tanguler and Erten 2012a). The second fermentation is also known as the main or carrot fermentation. The extracts obtained from the first fermentation are combined to perform the second fermentation with the roots of black carrot, rock salt (1%–2%), and, if available, sliced turnip (1%–2%) in a tank. Adequate drinkable water is added to fill the tank. The black carrot is washed if necessary. It is sorted to remove all the damaged and defective carrots. The carrot is then cut into pieces of 3–9 cm in length. The second fermentation is naturally carried out for 3 to 10 days at ambient temperature, which can vary from 10 °C to 35 °C (Canbas and Deryaoglu 1993; Erten et al. 2008).

In the direct production method, dough fermentation is not carried out. The chopped black carrots, salt, bakers' yeast or sourdough, sliced turnip (if available), and adequate water are transferred to a tank and allowed to ferment at ambient temperature (Tanguler and Erten 2012b). During fermentation, it is mainly LAB that give

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shalgam its characteristic flavor by producing lactic acid, ethanol, acetic acid, and other organic compounds (Canbas and Deryaoglu 1993; Tanguler and Erten 2012a).

Shalgam is highly popular in the Çukurova region, and its consumption is currently increasing in other parts of Turkey (Mersin, Hatay, Kahramanmaraş, İstanbul, Ankara, and İzmir) (Tanguler and Erten 2012a). Lately, shalgam is also sold in markets in some European cities. The process of shalgam manufacture in commercial plants is carried out under nonsterile conditions. The quality of shalgam is closely related to the microbial ecology, mainly the LAB, of fermentation. Despite this fundamental contribution of microorganisms, shalgam microbiology is complex, and to date there is only limited information about the identification of LAB and microbiological quality (Erginkaya and Hammes 1992; Arici 2004; Tanguler and Erten 2012a, 2012b). LAB have a long history of importance in food technology (Stiles and Holzapfel 1997). The isolation and characterization of novel strains from uninvestigated samples could have the 2-fold advantage of revealing taxonomic characteristics (Ortu et al. 2007).

Traditional foods have persisted over centuries, and the uses of starter cultures improve the technological quality of the products (Karovičová et al. 1999; Zamfir et al. 2006). In the present day, the need for safe products with standard and desirable technological properties has resulted in the use of starter cultures for the production of fermented products. The most encouraging bacteria for starters are those which are isolated from the autochthonous microbiota of traditional products, because these microorganisms are well adapted in the environment and are capable of dominating the microbiota of the products. Autochthonous starter cultures are important to achieve the desired fermentation parameters specific for the product type (Drosinos et al. 2005). The use of selected autochthonous LAB starters may guarantee the prolonged shelf-life of fermented products, which also maintains agreeable nutritional and sensory properties (Rodríguez et al. 2009; Di Cagno et al. 2011).

Unfortunately, no autochthonous LAB starter cultures are commercially available, nor are any used in the traditional production of shalgam. Therefore, the end product exhibits wide variation in terms of chemical, microbiological, and sensory characteristics. The use of starter culture helps to standardize the fermentation by controlling the microbiota. To our knowledge, no previous work deals with the selection of indigenous LAB as starter culture. All published reports dealing with shalgam have considered composition, especially anthocyanin contents, and very few studies have identified some species of LAB that develop during shalgam production (Erginkaya and Hammes 1992; Arici 2004). However, these studies were qualitative in contribution, and quantitative research

describing the development of individual LAB species during fermentation is lacking. Therefore, in previous studies, we examined the quantitative development of individual LAB species during fermentation of shalgams produced in the university laboratory and by different industrial producers (Tanguler and Erten 2012a, 2012b).

The objective of the present work was to select the most suitable strains as starter culture for shalgam production.

## 2. Materials and methods

Black carrot was kindly provided by the İçenbilir Hacı'nın Şalgamı Company (Adana, Turkey). LAB were isolated from shalgam samples produced in the laboratory and by different producers in various areas of Adana. Commercial samples of shalgam were directly obtained from 5 different producers in various areas of Adana. Samples A, B, D, F, and G were produced using the traditional method. Samples C and E, however, were obtained by the direct production method. The days when the LAB used in the experiments were isolated and the producers are given in Table 1.

### 2.1. Identification of lactic acid bacteria

In previous studies, a total of 447 isolates were identified by comparing the morphological, physiological, and biochemical characteristics of the strains. Isolates were tested for Gram reaction, catalase formation, cell morphology, CO<sub>2</sub> production from glucose, hydrolysis of arginine, nitrate reduction, acetoin formation, and MR-VP test. They were also tested for their ability to grow at 10 °C and 45 °C and at pH 4.4 and 9.6, and for their tolerance to 6.5% and 18% salt. Subsequently, API 50 CH strips and API CHL medium (bioMérieux, France) were used to study the carbohydrate assimilation ability of the test organism. APILAB PLUS database identification software (bioMérieux) was used to interpret the results (Harrigan and McCance 1990; Tamminen et al. 2004; Randazzo et al. 2005).

### 2.2. Preparation of carrot juice and enumeration of LAB

Carrot juice (CJ) was obtained in accordance to the methods of Demir et al. (2006). The washed fresh black carrots were blended with a juice extractor (F172 Felix Juicy). The CJ was pasteurized at 85 °C for 5 min, and then the carrot mash was cooled to 25 °C. The initial pH of the CJ was 6.1. All fermentations were performed in duplicate. For inoculation, strains were plated onto MRS agar and incubated for 48 h at 25 °C in jars made anaerobic with GasPaks (Anaerocult® A, Merck AG, Darmstadt, Germany) to obtain single cultures. Two colonies for each strain were inoculated into 80 mL of pasteurized CJ in a 100-mL sterile conical flask for 48 h at 25 °C with orbital shaking at 160 rev min<sup>-1</sup>. From these flasks, 5% cultures were inoculated in 500-mL sterile conical flasks containing 400 mL of pasteurized CJ and 2% rock salt. Fermentations

**Table 1.** Days when LAB were isolated.

Strain	Source*	Day**
<i>Lb. plantarum</i> <sup>ax</sup>	Large-scale producer (F)	8
<i>Lb. plantarum</i> <sup>bx</sup>	University laboratory (from extract) (G)	-
<i>Lb. plantarum</i> <sup>cx</sup>	University laboratory (from main fermentation) (G)	10
<i>Lb. brevis</i> 1	Small-scale producer (C)	12
<i>Lb. brevis</i> 2	Small-scale producer (A)	17
<i>Lb. brevis</i> 3	University laboratory (from main fermentation) (G)	11
<i>Lb. paracasei</i> subsp. <i>paracasei</i> 1	University laboratory (from main fermentation) (G)	11
<i>Lb. paracasei</i> subsp. <i>paracasei</i> 2	Small-scale producer (D)	5
<i>Lb. buchneri</i>	Small-scale producer (B)	3
<i>Lb. pentosus</i>	Small-scale producer (C)	12
<i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i>	University laboratory (from dough fermentation) (G)	2
<i>Lb. fermentum</i>	Large-scale producer (F)	7
<i>Lc. lactis</i> subsp. <i>lactis</i>	University laboratory (from main fermentation) (G)	9
<i>Leu. mesenteroides</i> subsp. <i>mesenteroides</i>	Small-scale producer (D)	1
<i>Leu. mesenteroides</i> subsp. <i>mesenteroides/dextranicum</i>	University laboratory (from main fermentation) (G)	0
<i>Leu. mesenteroides</i> subsp. <i>cremoris</i>	Small-scale producer (E)	0
<i>Pediococcus</i> sp.	Small-scale producer (E)	7
<i>P. pentosaceus</i>	University laboratory (from dough fermentation) (G)	2

\*: The source of shalgam from which the LAB were isolated, \*\*: the day on which the LAB were isolated. *Lb.*: *Lactobacillus*, *Lc.*: *Lactococcus*, *Leu.*: *Leuconostoc*, *P.*: *Pediococcus*.

were carried out in a fermentation room at 25 °C for 10 days. To enumerate LAB during fermentation, samples were taken, serially diluted with sterile physiological saline (0.85% w v<sup>-1</sup>), and then spread-inoculated onto plates. Plates were incubated for 48 h at 30 °C (Harrigan and McCance 1990; Halkman, 2005).

### 2.3. Determination of pH and total acidity

pH was determined using a pH meter (Inolab WTW, Weilheim, Germany). Total acidity was measured by titrating throughout the fermentation of CJ up to pH 8.1 with 0.1 N NaOH using a digital pH meter and was expressed as grams of lactic acid L<sup>-1</sup> (Zorba et al. 2003; Rozada-Sánchez et al. 2008). Total acidity and pH measurements were performed in duplicate.

### 2.4. Sensory analysis

Sensory evaluation was performed with a ranking test (Barillere and Benard 1986) and a taste panel consisting of 13 individuals under appropriate conditions. The panelists were staff of the Department of Food Engineering, and they ranged in age from 35 to 64 years. Bran bread

and water were given to the panelists to neutralize taste between the sample evaluations. Samples were numbered and served in mixed order, and they were ranked from the most preferred to the least preferred by each panelist.

### 2.5. Statistical analysis

For statistical analysis of sensory evaluation, data were analyzed with the Kruskal–Wallis test (Roessler et al. 1978; Barillere and Benard 1986).

## 3. Results

### 3.1. Selection of starter cultures

A successful LAF of fermented products depends on the inoculation of proper starter cultures, which generally belong to the LAB group. However, as is known, there are no autochthonous starter cultures available that are specifically designed for the fermentation of shalgam. For the selection of starter cultures, in addition to our shalgam produced in the laboratory in triplicate, 2 shalgams were produced commercially by large- and small-scale industrial producers. From dough fermentations, extracts, and carrot

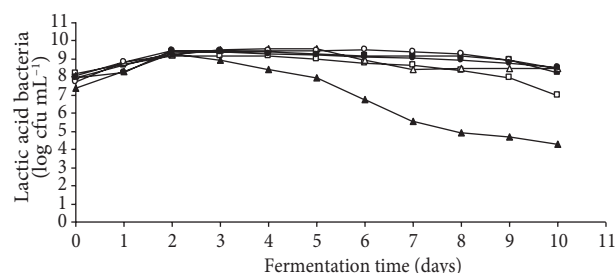
fermentations, 312 LAB were isolated (results not given). In addition, 135 strains in shalgam samples obtained from different plants were isolated. After identification of LAB, 18 autochthonous subspecies were preselected through 447 strains as possible starter cultures for shalgam production in the present study. These autochthonous subspecies are *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. brevis* (*Lb. brevis* 1, *Lb. brevis* 2, *Lb. brevis* 3), *Lb. paracasei* subsp. *paracasei* (*Lb. paracasei* subsp. *paracasei* 1, *Lb. paracasei* subsp. *paracasei* 2), *P. pentosaceus*, *Pediococcus* sp., *Lc. lactis* subsp. *lactis*, *Leu. mesenteroides* subsp. *mesenteroides*, *Leu. mesenteroides* subsp. *mesenteroides/dextranicum*, *Leu. mesenteroides* subsp. *cremoris*, *Lb. fermentum*, *Lb. pentosus*, *Lb. buchneri*, and *Lb. plantarum*. In this study, 3 *Lb. plantarum* species were used. These species were isolated from end fermentation produced by commercial large scale producers in industry (<sup>ax</sup>), from extract (<sup>bx</sup>), and from end fermentation carried out in our laboratory (<sup>cx</sup>).

### 3.2. Growth of LAB species during carrot juice fermentation

CJ had 1.64 g L<sup>-1</sup> total acidity (as lactic acid), a pH of 6.1, and 55.5 g L<sup>-1</sup> total sugar. Figures 1–3 show the growth of LAB during the CJ fermentation, which was carried out for 10 days in the laboratory because carrot fermentation of shalgam lasted for 10 days. Total viable LAB counts ranged from 6.56 log cfu mL<sup>-1</sup> (*Leu. mesenteroides* subsp. *cremoris*) to 8.29 log cfu mL<sup>-1</sup> (*Lb. paracasei* subsp. *paracasei* 2) at the beginning of fermentations of CJ.

*Lb. plantarum*<sup>ax</sup> showed maximum populations of 9.16 log cfu mL<sup>-1</sup> on day 3, but *Lb. plantarum*<sup>bx</sup> and *Lb. plantarum*<sup>cx</sup> showed populations of 9.40–9.58 log cfu mL<sup>-1</sup> on day 4. The highest number was obtained with *Lb. plantarum*<sup>cx</sup> at 8.46 log cfu mL<sup>-1</sup> at the end of fermentation, and the lowest count was obtained with *Lb. plantarum*<sup>ax</sup> (6.98 log cfu mL<sup>-1</sup>) (Figure 1).

Viable *Lb. brevis* counts ranged from 7.37 log cfu mL<sup>-1</sup> (*Lb. brevis* 1) to 7.97 log cfu mL<sup>-1</sup> (*Lb. brevis* 3) at

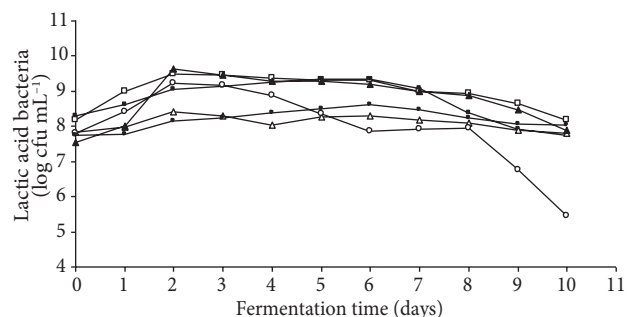


**Figure 1.** The growth of lactic acid bacteria during CJ fermentation. *Lb. plantarum*<sup>ax</sup>, <sup>bx</sup> and <sup>cx</sup>; species were isolated from end fermentation produced by commercial large-scale industrial producers, from extract, and from end fermentation carried out in our laboratory, respectively. (–□–) *Lb. plantarum*<sup>ax</sup>; (–■–) *Lb. plantarum*<sup>bx</sup>; (–△–) *Lb. plantarum*<sup>cx</sup>; (–▲–) *Lb. brevis* 1; (–○–) *Lb. brevis* 2; (–●–) *Lb. brevis* 3.

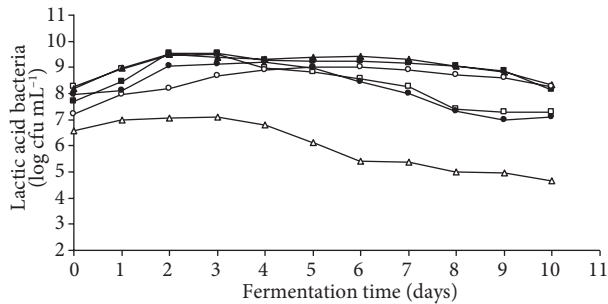
the beginning of fermentations of CJ. *Lb. brevis* 1 and *Lb. brevis* 3 counts reached the maximum numbers, 9.30–9.48 log cfu mL<sup>-1</sup>, on day 2, whereas *Lb. brevis* 2 reached the highest number, 9.49 log cfu mL<sup>-1</sup>, on day 6. However, after their slow initial proliferation, they decreased until the end of fermentation. The last counts of *Lb. brevis* species were determined to be in the range of 4.28–8.53 log cfu mL<sup>-1</sup> at the end (Figure 1).

*Lb. fermentum* counts increased regularly, reaching a maximum count of 9.64 log cfu mL<sup>-1</sup> on the second day of fermentation. Subsequently its number went down slightly towards the end of fermentation, with a count of 7.88 log cfu mL<sup>-1</sup> at the end. On the other hand, while the maximum number of *Lb. paracasei* subsp. *paracasei* 1 obtained on day 2 was 9.49 log cfu mL<sup>-1</sup>, the count of *Lb. paracasei* subsp. *paracasei* 2 was found to be 9.34 log cfu mL<sup>-1</sup> on day 5. Their numbers subsequently declined until the end of fermentation, reaching 8.18 and 7.76 log cfu mL<sup>-1</sup>, respectively (Figure 2). *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. buchneri*, and *Lb. pentosus* ranged from 7.75 to 7.83 log cfu mL<sup>-1</sup> at the beginning of CJ fermentations. Their counts reached maximum numbers of 8.41 log cfu mL<sup>-1</sup> (on day 2), 9.24 log cfu mL<sup>-1</sup> (on day 2), and 8.61 log cfu mL<sup>-1</sup> (on day 6), respectively. From then on, the numbers reduced to 7.80–5.45 and 8.03 log cfu mL<sup>-1</sup> at the end of fermentation.

*Leu. mesenteroides* counts ranged between 6.56 log cfu mL<sup>-1</sup> and 8.25 log cfu mL<sup>-1</sup> at the beginning of fermentation. After *Leu. mesenteroides* subsp. *cremoris* (7.11 log cfu mL<sup>-1</sup>), *Leu. mesenteroides* subsp. *mesenteroides* (9.51 log cfu mL<sup>-1</sup>), and *Leu. mesenteroides* subsp. *mesenteroides/dextranicum* (9.55 log cfu mL<sup>-1</sup>) reached a maximum, the numbers slightly declined, to 4.66 log cfu mL<sup>-1</sup>, 7.30 log cfu mL<sup>-1</sup>, and 8.15 log cfu mL<sup>-1</sup> at the end, respectively. After the commencement of CJ fermentation, *Lc. lactis* subsp. *lactis* started to increase, and its maximum number was determined to be 9.19 log cfu mL<sup>-1</sup> on day 4. From



**Figure 2.** The growth of lactic acid bacteria during CJ fermentation. (–□–) *Lb. paracasei* subsp. *paracasei* 1; (–■–) *Lb. paracasei* subsp. *paracasei* 2; (–△–) *Lb. delbrueckii* subsp. *delbrueckii*; (–▲–) *Lb. fermentum*; (–○–) *Lb. buchneri*; (–●–) *Lb. pentosus*.



**Figure 3.** The growth of lactic acid bacteria during CJ fermentation. (–□–) *Leu. mesenteroides* subsp. *mesenteroides*; (–■–) *Leu. mesenteroides* subsp. *mesenteroides/dextranicum*; (–△–) *Leu. mesenteroides* subsp. *cremoris*; (–▲–) *P. pentosaceus*; (–○–) *Pediococcus* sp.; (–●–) *Lc. lactis* subsp. *lactis*.

then on, no stationary phase occurred, and consequently it was found to be  $7.10 \log \text{cfu mL}^{-1}$  by day 10.

*Pediococcus* sp. and *P. pentosaceus* achieved maximum counts of  $9.01$ – $9.48 \log \text{cfu mL}^{-1}$  on day 6 and day 2, from initial counts of  $7.23$ – $8.18 \log \text{cfu mL}^{-1}$ , respectively. At the end of CJ fermentation, their counts were  $8.26$  and  $8.34 \log \text{cfu mL}^{-1}$ , respectively.

### 3.3. Growth of selected LAB species at different pH levels, temperatures, and NaCl concentrations

All LAB species selected grew at pH 4.4 (except *Lb. buchneri* and *Lc. lactis* subsp. *lactis*) and at  $10^\circ\text{C}$  (except *Lb. paracasei* subsp. *paracasei* 1, *Lb. paracasei* subsp. *paracasei* 2, and *Leu. mesenteroides* subsp. *cremoris*). Only *Lb. delbrueckii* subsp. *delbrueckii* and *Lb. fermentum* grew at  $45^\circ\text{C}$ , and none of the LAB species grew at pH 9.6. Twelve species of LAB selected were able to tolerate the tested NaCl concentration of 6.5%, whereas 6 species were sensitive. As expected, high salt concentration had a negative influence on the growth of the species, and none of the species could grow with 18% NaCl. It is indicated that 18% NaCl concentration has an inhibitory effect on selected LAB species (Table 2). The applied NaCl concentrations (6.5% and 18%) are the levels most often used in the processing of fermented vegetables (Carr et al. 2002; Karasu et al. 2010).

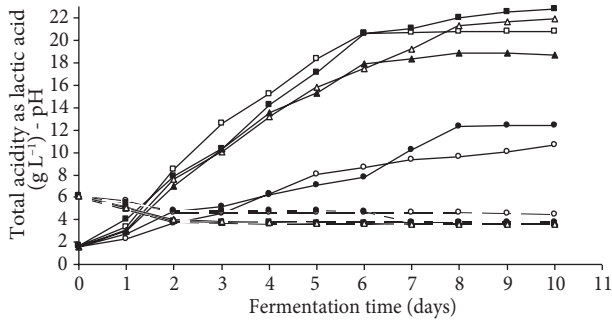
### 3.4. Total acidity and pH during carrot juice fermentation

Figures 4, 5, and 6 show the development of total acidity and pH during CJ fermentation by 18 subspecies of bacteria. The concentration of total acidity expressed as lactic acid

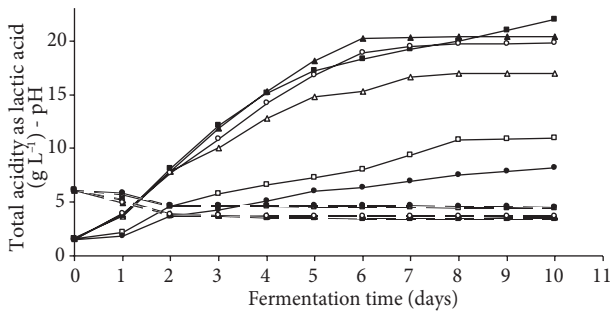
**Table 2.** Growth of selected LAB species at different pH levels, temperatures, and NaCl concentrations.

	Temperature		pH		NaCl	
	$10^\circ\text{C}$	$45^\circ\text{C}$	4.4	9.6	6.5%	18%
<i>Lb. plantarum</i> <sup>ax</sup>	+	-	+	-	+	-
<i>Lb. plantarum</i> <sup>bx</sup>	+	-	+	-	+	-
<i>Lb. plantarum</i> <sup>cx</sup>	+	-	+	-	+	-
<i>Lb. brevis</i> 1	+	-	+	-	+	-
<i>Lb. brevis</i> 2	+	-	+	-	+	-
<i>Lb. brevis</i> 3	+	-	+	-	+	-
<i>Lb. paracasei</i> subsp. <i>paracasei</i> 1	-	-	+	-	-*	-
<i>Lb. paracasei</i> subsp. <i>paracasei</i> 2	-*	-	+	-	-	-
<i>Lb. buchneri</i>	+	-	-	-	-	-
<i>Lb. pentosus</i>	+	-	+	-	+	-
<i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i>	+	+	+	-	-	-
<i>Lb. fermentum</i>	+	+	+	-	+	-
<i>Lc. lactis</i> subsp. <i>lactis</i>	+	-	-	-	-	-
<i>Leu. mesenteroides</i> subsp. <i>mesenteroides</i>	+	-	+	-	-	-
<i>Leu. mesenteroides</i> subsp. <i>mesenteroides/dextranicum</i>	+	-	+	-	+	-
<i>Leu. mesenteroides</i> subsp. <i>cremoris</i>	-	-	+	-	+	-
<i>Pediococcus</i> sp.	-*	-	+	-	+	-
<i>P. pentosaceus</i>	+	-	+	-	+	-

-: Negative result; -: one of the assays is positive; +: positive result. *Lb.*: *Lactobacillus*, *Lc.*: *Lactococcus*, *Leu.*: *Leuconostoc*, *P.*: *Pediococcus*.

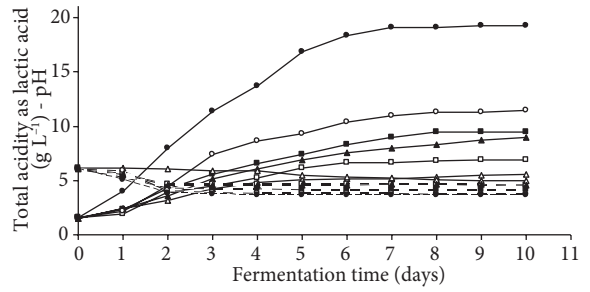


**Figure 4.** Variation of total acidity and pH during CJ fermentation by various LAB. Total acidity of LAB: (—□—) *Lb. plantarum*<sup>ax</sup>; (—■—) *Lb. plantarum*<sup>bx</sup>; (—Δ—) *Lb. plantarum*<sup>cx</sup>; (—Δ—) *Lb. brevis* 1; (—▲—) *Lb. brevis* 2; (—○—) *Lb. brevis* 3. pH of LAB: (····□····) *Lb. plantarum*<sup>ax</sup>; (····■····) *Lb. plantarum*<sup>bx</sup>; (····Δ····) *Lb. plantarum*<sup>cx</sup>; (····▲····) *Lb. brevis* 1; (····○····) *Lb. brevis* 2; (····●····) *Lb. brevis* 3.



**Figure 5.** Variation of total acidity and pH during CJ fermentation by various LAB. Total acidity of LAB: (—□—) *Lb. paracasei* subsp. *paracasei* 1; (—■—) *Lb. paracasei* subsp. *paracasei* 2; (—Δ—) *Lb. delbrueckii* subsp. *delbrueckii*; (—▲—) *Lb. fermentum*; (—○—) *Lb. buchneri*; (—●—) *Lb. pentosus*. pH of LAB: (····□····) *Lb. paracasei* subsp. *paracasei* 1; (····■····) *Lb. paracasei* subsp. *paracasei* 2; (····Δ····) *Lb. delbrueckii* subsp. *delbrueckii*; (····▲····) *Lb. fermentum*; (····○····) *Lb. buchneri*; (····●····) *Lb. pentosus*.

at the beginning of fermentation was approximately 1.57 g L<sup>-1</sup> in pasteurized CJ. Total acidity increased during the fermentation and the concentrations of total acidity were found to be 5.55–22.86 g L<sup>-1</sup> at the completion of fermentations. The 5 highest total acidity values were obtained by *Lb. plantarum*<sup>bx</sup> (22.86 g L<sup>-1</sup>), *Lb. paracasei*



**Figure 6.** Variation of total acidity and pH during CJ fermentation by various LAB. Total acidity of LAB: (—□—) *Leu. mesenteroides* subsp. *mesenteroides*; (—■—) *Leu. mesenteroides* subsp. *mesenteroides/dextranicum*; (—Δ—) *Leu. mesenteroides* subsp. *cremoris*; (—▲—) *P. pentosaceus*; (—○—) *Pediococcus* sp.; (—●—) *Lc. lactis* subsp. *lactis*. pH of LAB: (····□····) *Leu. mesenteroides* subsp. *mesenteroides*; (····■····) *Leu. mesenteroides* subsp. *mesenteroides/dextranicum*; (····Δ····) *Leu. mesenteroides* subsp. *cremoris*; (····▲····) *P. pentosaceus*; (····○····) *Pediococcus* sp.; (····●····) *Lc. lactis* subsp. *lactis*.

subsp. *paracasei* 2 (22.0 g L<sup>-1</sup>), *Lb. plantarum*<sup>cx</sup> (21.94 g L<sup>-1</sup>), *Lb. plantarum*<sup>ax</sup> (20.84 g L<sup>-1</sup>), and *Lb. fermentum* (20.45 g L<sup>-1</sup>). However, the lowest concentrations were determined in *Leu. mesenteroides* subsp. *cremoris* (5.55 g L<sup>-1</sup>), *Leu. mesenteroides* subsp. *mesenteroides* (6.92 g L<sup>-1</sup>), *Lb. pentosus* (8.17 g L<sup>-1</sup>), *P. pentosaceus* (8.93 g L<sup>-1</sup>), and *Leu. mesenteroides* subsp. *mesenteroides/dextranicum* (9.48 g L<sup>-1</sup>). It was also noted that there were large differences in total acidity among the used strains isolated from shalgam (Figures 4–6). In the present study, pH levels ranged from 6.02 to 6.16 at day 0.

### 3.5. Sensory analysis

For sensory analysis, 9 fermented CJs containing the highest total acidity from 18 samples were chosen. Thirteen panelists evaluated the overall acceptability of fermented CJs according to acidity, taste, and odor. The results are presented in Table 3.

*Lb. plantarum*<sup>bx</sup> had the highest acceptance ranking. On the other hand, slightly higher scores than those for *Lb. plantarum*<sup>bx</sup> were given to *Lb. fermentum* and *Lb. paracasei* subsp. *paracasei* 2 by panelists. CJ fermentations of *Lc. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *Delbrueckii*,

**Table 3.** Sensory analysis of CJ fermented by LAB species.

	Species*								
	2	8	9	3	1	7	6	4	5
The difference between points of classification (P < 0.01)**	39 <sup>d</sup>	40 <sup>d</sup>	41 <sup>d</sup>	47 <sup>c</sup>	52 <sup>c</sup>	88 <sup>b</sup>	90 <sup>b</sup>	93 <sup>b</sup>	95 <sup>a</sup>

\*Species: 1: *Lb. plantarum*<sup>ax</sup>, 2: *Lb. plantarum*<sup>bx</sup>, 3: *Lb. plantarum*<sup>cx</sup>, 4: *Lc. lactis* subsp. *lactis*, 5: *Lb. brevis* 1, 6: *Lb. delbrueckii* subsp. *delbrueckii*, 7: *Lb. buchneri*, 8: *Lb. fermentum*, 9: *Lb. paracasei* subsp. *paracasei* 2. \*\*Different superscripts (a–d) indicate statistically significant differences at 1%.

and *Lb. buchneri* had the lowest marks in the sensory tests. Statistical analysis of scores revealed that there was a significant effect of species ( $P < 0.01$ ).

#### 4. Discussion

To date, there have been few reports about shalgam. However, there is no report on the selection of the most suitable strains isolated from the environment as an indigenous starter culture for shalgam production. In this study, the suitability of LAB isolated and identified from shalgam fermentations was examined.

Lactobacilli play an important role in the fermentations of several foods, such as vegetables, meats, and dairy products (Caplice and Fitzgerald 1999; Bernerdau et al. 2008). Lactic acid-fermented cucumber and olive fermentations are dominated and terminated primarily by *Lb. plantarum* (Harris 1998). *Lb. plantarum* is a facultatively heterofermentative lactobacillus strain (Hammes and Vogel 1995; Stiles and Holzappel 1997; Axelsson 1998). Autochthonous LAB such as *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. curvatus*, *Lb. brevis*, *Lb. rossiae*, *Lb. pentosus*, *P. pentosaceus*, and *Leu. mesenteroides* have been previously isolated from raw fruits and vegetables (Muyanja et al. 2003; Yan et al. 2008; Vitali et al. 2012).

In order to achieve the appropriate technological and sensory properties of fermented vegetable juices, it is necessary to select strains that have the desirable characteristics. The selection of microorganisms as starter was based mainly on the rate and total production of acids and the change of pH (Karovičová et al. 1999; Karasu et al. 2010). Total acidity and sensory characteristics in shalgam are usually important criteria in the selection of strains to be used as a starter culture. In order to select the best LAB isolate, we incorporated data into the technological parameters. The starter LAB were determined on the basis of selection criteria reported by Buckenuskes (1993) and Holzappel (2002).

These criteria were fast growth; fast production of lactic acid; high acidification rate; the ability to grow at different temperatures, salt concentrations, and pH values; and being catalase-positive. In our study, according to the these results mentioned above and also to sensory analysis, *Lb. plantarum*<sup>bx</sup>, *Lb. fermentum*, and *Lb. paracasei* subsp. *paracasei* 2 could be used as starters in the manufacture of shalgam with sensorial qualities preferred by panelists. *Lb. plantarum*, *Lb. paracasei*, and *Lb. fermentum* are also used as starter culture in the production of fermented vegetables such as pickles, sorghum, sourdough, Chinese steamed buns, koumiss, Mongolian fermented dairy product, stinky tofu, and fermented meat products (Leroy and De Vuyst 2004; Wu et al. 2009; Correia et al. 2010; Liu et al. 2011).

Özdemir-Alper and Acar (1996) investigated the fermentation effects of RSKK 1062 *Lb. plantarum*, 706-N(3-1)319 *Lb. casei*, *Lb. brevis*, Northern Regional Research Laboratories (NRRLB)-763 *Lb. delbrueckii*, and NRRLB-4449 *Lb. xylosum* in CJ. They stated that RSKK 1062 *Lb. plantarum* was the main *Lactobacillus* species for the LAF of CJ.

Kostinek et al. (2007) studied the characterization and biochemical properties of predominant LAB from fermenting cassava for selection as starter cultures, and they selected 16 strains (*Lb. fermentum* BFE 6620 and BFE 6625; *Weissella paramesenteroides* BFE 7601 and 7608; *Leu. mesenteroides* subsp. *mesenteroides* BFE 7668; *Lb. plantarum* BFE 6688, BFE 6710, BFE 6711, BFE 6713, BFE 6739, BFE 6793, BFE 7685, and BFE 7687; and *Lb. pentosus* BFE 6748, BFE 7596, and BFE 7589) from the 32 strains as starter cultures.

Muyanja et al. (2003) stated that *Lb. brevis* was more frequently isolated than other species in household bushera, a traditional Ugandan fermented beverage. *Lb. fermentum*, *Lb. paracasei* subsp. *paracasei*, and *Lb. plantarum* predominated the fermentation of bushera in the later stages.

Although many LAB starter cultures are used in dairy, meat, and baked good fermentations, only a few cultures have been used for vegetable fermentations. *Lb. plantarum* is probably the most advantageous of the more commonly used bacteria for the conversion of sugars to lactic acid because it not only utilizes the sugars with high conversion rates, but also utilizes other compounds such as pectin in plant products. For this reason, it is the most frequently used species in the fermentation of cucumbers, cabbages, and olives (Demir et al. 2006; Di Cagno et al. 2008). Similar to our findings, *Lb. plantarum* has been suggested as a starter culture for the production of fermented vegetable juices by Schobinger (1988), Gökmen and Acar (1992), Karovičová et al. (1999), Özdemir-Alper and Acar (1996), Demir et al. (2006), and Di Cagno et al. (2008). At the same time, numerous reports indicate that *Lb. fermentum* and *Lb. paracasei* exhibit superior performance in lactic acid-fermented cereal and vegetable products when they are used as starters (Steinkraus 1996; Holzappel 2002).

In the current study, new information is presented on the selection of the most suitable strains as starter cultures for shalgam production. Eighteen autochthonous species were preselected from 447 strains as possible starter cultures for shalgam production. Fermentations were conducted in pasteurized CJ and lasted for 10 days. The highest numbers obtained during all fermentations were higher than 9 log cfu mL<sup>-1</sup>, except for fermentations with *Lb. delbrueckii* subsp. *delbrueckii*, *Leu. mesenteroides* subsp. *cremoris*, and *Lb. pentosus*. At the end of fermentation, the 5 bacteria yielding the highest total acidity were *Lb.*

*plantarum*<sup>bx</sup> (22.86 g L<sup>-1</sup>), *Lb. paracasei* subsp. *paracasei* 2 (22.0 g L<sup>-1</sup>), *Lb. plantarum*<sup>cx</sup> (21.94 g L<sup>-1</sup>), *Lb. plantarum*<sup>ax</sup> (20.84 g L<sup>-1</sup>), and *Lb. fermentum* (20.45 g L<sup>-1</sup>). Additionally, *Lb. plantarum*<sup>bx</sup>, *Lb. fermentum*, and *Lb. paracasei* subsp. *paracasei* 2 were ranked the highest by panelists with respect to sensory analyses.

According to the data obtained in the present study, *Lb. plantarum*<sup>bx</sup>, *Lb. fermentum*, and *Lb. paracasei* subsp. *paracasei* 2 showed the best potential for use as lactic starter cultures for shalgam production. The selection of autochthonous LAB as starter cultures in shalgam fermentation is important for the standardization of the

quality of shalgam. However, further investigations, such as studies of their use as starter cultures in the production of shalgam, are needed.

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