Isolation, identification and determination of technological properties of the halophilic lactic acid bacteria isolated from table olives

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Abstract Isolation, identification and technological properties of halophilic lactic acid bacteria (HLAB) from olive samples which were collected from different places in Turkey were examined in the present work. According to the genetic analysis of the isolates, it was determined that 42 (%57.53) *Lactobacillus plantarum*, 20 (%27.39) *Lactobacillus acidipiscis*, 7 (%9.58) *Enterococcus faecium*, 2 (%2.73) *Lactobacillus alimentarius*, 1 (%1.36) *Lactobacillus farciminis*, 1 (%1.36) *Lactobacillus namurensis*. *L. plantarum*, *L. alimentarius*, *L. farciminis* and *L. namurensis* were able to tolerate 8% NaCl. Twenty-seven isolates showed lipolytic activity between 0.2 and 1.09 U ml⁻¹. Thirty isolates had pectolytic activity as 3.24–5.29 U ml⁻¹. A total of twelve *L. acidipiscis*, *L. alimentarius*, *L. farciminis* and *L. plantarum* strains showed positive decarboxylase activity by decarboxylating tyrosine. Results indicated that *L. plantarum* Z64A, Z64B, Z66A, Z83B, Z100A and *L. acidipiscis* Z112D showed high salt resistance, no biogenic amine production, low pectinolytic and low lipolytic activity. HLAB are dominant bacteria in the fermented foods containing high levels of salt. There are no many studies about the presence of HLAB in table olives and their technological properties. The present work showed that HLAB isolates of *L. plantarum*, *L. acidipiscis* and *E. faecium* were the predominant species found in table olives collected from the western

part of Turkey. The selected strains which have high salt resistance, low pectinolytic and lipolytic activity are potentially good candidates as starter culture source in olive fermentations.

Keywords Olive · Halophilic lactic acid bacteria · NaCl

Introduction

Lactic acid bacteria (LAB) are a group of important microorganisms that can produce lactic acid during homo or heterofermentative metabolism. In fermented foods, the acidification and enzymatic activities of LAB are important for taste, texture and inhibition of food spoilage bacteria (Morales et al. 2011). Halophilic lactic acid bacteria (HLAB) required NaCl for growth and can tolerate a high NaCl concentration. HLAB is the most prevailing bacteria in the fermented foods containing high level of salt such as fish products. These microorganisms are important in the food industry for the production of flavor and aroma components, and organic acids, and capability of to being a starter culture (Zajc et al. 2014). The non-halophilic LAB can grow best in media containing less than 1% (w/v) total salts; slight halophiles can grow best in media containing 1–3% (w/v) salt and moderate halophiles can grow best in media containing 3–15% (w/v) salt. The bacteria which can grow best in media containing 9–23% (w/v) called borderline halophiles and the bacteria which can grow best in media containing 15–32% (w/v) salt called extreme halophiles (Marquez et al. 1986).

Olives are one of the most important agricultural products produced in Turkey and preservation of the olives with salt is an ancient method used for so many years especially in the Mediterranean countries. During the production of

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fermented olives pH, salt concentration and bacterial flora are important for the final product quality (Özay and Borcakli 1995). Salt is one of the most important ingredients used in pickles and canned food. Adding salt to foods prevent the growth of undesirable microorganisms and contribute to the end product quality. Plants, animals and microorganisms have been used as pectinolytic enzyme sources. Microorganisms are not influenced by climatic and seasonal factors and can be subjected to genetic and environmental manipulations to increase yield. For this reason, different types of microorganisms have been exploited for the production of pectinase (Torimiro and Okonji 2013). Interest in the production of microbial lipases for a wide range of applications in food sector, wastewater treatment and cosmetics has increased in recent years (Jaeger et al. 1999). Low lipolytic activity is an important advantage for the starter HLAB for breaking of the milk fat during maturation to induce aroma production without giving a rancid flavor in the product (Morales et al. 2011). One of the most important features that should be considered for the using LAB in the food industry is non-capability of producing biogenic amines. Biogenic amines are produced largely in protein-rich foods and fermented foods. When significant levels of biogenic amines usually found in food is considered to be a serious health hazard to people.

The present work was undertaken to isolate and identify HLAB from olive samples which were collected from different locations in Turkey and examine their some technological properties.

Materials and methods

Isolation of HLAB from table olives

One hundred and fifty black or green olive samples were collected from direct manufacturers or local open markets from Burdur, Isparta, Antalya, Eskişehir and Izmir provinces of Turkey. The samples were brought to the laboratory in sterile conditions with sterile sampling bags or sterile capped tubes. HLAB were isolated on MRS agar containing 7% NaCl by spread plating (Morales et al. 2011). After 2 days incubation at 32 °C, the colonies were purified in the same media and ninety-four colonies with different morphological characteristics were randomly selected. The strains were stored in MRS broth with 50% (v/v) aqueous glycerol at – 20 °C.

Identification of selected HLAB

DNA extraction

A four ml of overnight culture grown in the MRS broth containing 7% NaCl was used for the genotyping characterization. DNA was isolated by PureLink Genomic DNA Kit (Invitrogen K) according to the manufacturer and stored at – 20 °C until use (Udomsil et al. 2010).

PCR amplification for 16S rRNA analysis

PCR amplification was carried out in 50 µL volumes containing 5 µL of bacterial DNA and 45 µL of amplification cocktail, which contained the following components: 10 mM each primer, 0.2 mM (each) dNTPs, 5 µL of GeneAmp PCR buffer (Fermentas) and 1.2 U of Taq DNA polymerase (Fermentas). Primers (5'-AGAGTTT-GATCCTGGCTCAG-3') and (5'-ACGGCTACCTTGT-TACGACTT-3') (Weisburg et al. 1991) were used as the PCR primers. The PCR amplification was performed by using Biorad T100 (Applied Biosystems, Foster City, CA, USA). Samples were incubated for 5 min at 94 °C to denature the target DNA and went through 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min. The samples were then incubated at 72 °C for 10 min for a final extension and were maintained at 4 °C until they were tested. PCR products were separated on 1% agarose gel electrophoresis, staining with gel stain (SYBR Safe DNA gel stain, Invitrogen, USA) and visualized by UV light. An amplicon of 1800–2000 bp was expected (Sudagidan et al. 2005; Udomsil et al. 2010).

Restriction fragment length polymorphism (RFLP) analysis

The PCR products were digested with *AluI* and *MboI* restriction enzymes (RE) and the fragment sizes were analyzed by 2% (w/v) agarose gel electrophoresis. A 100 bp DNA ladder (GibcoBRL, Barcelona, Spain) was used as a size marker (Sudagidan et al. 2005; Udomsil et al. 2010). Restriction patterns were analyzed using Bio-ID ++ software (Vilber Lourmat) with a 12% homology coefficient. The similarity between the strains was determined automatically by specifying the formula of Nei and Li (1979). Strain clustering was performed using the unweighted pair group method with arithmetic means (Vilber Lourmat).

DNA sequencing was performed in an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) in BM Laboratory Systems. The sequences obtained were then evaluated in GenBank using BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast>) and deposited in the EMBL data library.

Determination of bacterial growth in different NaCl concentrations

Salt tolerance test was performed in MRS broth containing 7%, 8%, 9% and 10% (w/v) NaCl at 13 °C and 28 °C. The growth of microorganisms was evaluated by optical density (OD_{590}) and pH measurement was performed using a pH meter (Hanna Instruments/pH211, USA) at the 24 and 48 h of incubation (Morales et al. 2011).

Quantitative and qualitative assessment of lipolytic activity

Qualitative measurement by Tween-80 hydrolysis was carried out on medium with peptone and Tween-80. The lipolytic activity was evaluated by measuring the diameter of the clear zones surrounding the lipolytic colonies in the medium (Gupta et al. 2011). Lipolytic activity was recorded as negative if no zone was observed around the agar. Quantitative measurements were conducted by tributyrin substrate according to Morales et al. (2011).

Quantitative and qualitative assessment of pectinolytic activity

The colonies were spotted on Hankins's agar medium (Hi-Media) for determining the pectinolytic activity and the plates were incubated at 32 °C for 48 h. After incubation iodine solution was overlaid on these plates and kept for 30 min. Clear zones were observed around the colonies were evaluated as positive (Patil et al. 2012). Quantitative measurement of pectolytic activity was carried out by DNS method.

Determination decarboxylase activity of the isolates

0.5 McFarland standards were used as a reference to adjust the turbidity of bacteria. The cultures were inoculated into MRS medium supplemented with 2% each of the amino acids histidine, tyrosine and lysine. After 32 °C for 7 days of incubation, presents of zones around the colonies was accepted as an indicator of biogenic amine formation, if no color change was observed colonies were considered as negative (Joosten and Northolt 1989).

Results

Isolation and identification of HLAB from table olives

Isolated 73 different Gram positive and catalase negative thin or thick rod or cocci shaped bacteria were selected for further experiments. Total number of HLAB were ≤ 10 in

six olive samples and about $< 10^2$ CFU g^{-1} in fifteen olive samples. The HLAB count determined in five, sixteen, forty-one and sixty-one olive samples was about 10^2 , 10^3 , 10^4 and 10^5 CFU g^{-1} , respectively. While six samples had about 10^6 CFU g^{-1} , the highest colony formation observed was 2.94×10^6 CFU g^{-1} .

Even though 56 of the HLAB were distinguished, 17 could not be distinguished by the band pattern of RFLP analysis. According to the results, bacteria were divided into 15 different groups with the restriction endonuclease (RE) *AluI* and 14 different groups with *MboI* (Table 1). According to sequence analysis of the isolates; it was found that the most common LAB species in olive samples were *Lactobacillus plantarum* (42/73) and *Lactobacillus acidipiscis* (20/73). Two strains of *Lactobacillus alimentarius*, 1 strain of each *Lactobacillus farciminis* and *Lactobacillus namurensis* and 7 strains of *Enterococcus faecium* were also identified (Table 2). Indigestion of *L. acidipiscis* with *AluI*, 5 patterns were observed and 5 patterns were also observed with *MboI* (Fig. 1). PCR amplified products were between 250 and 1200 bp for *AluI* and 100 and 600 bp for *MboI*. A nine and six patterns in *L. plantarum* were observed for *AluI* (250–1000 bp) and *MboI* (100–3000 bp), respectively. By using *AluI*, three RFLP bands (800, 400, 250 bp) were obtained for *E. faecium* Z137A and Z3A. Three fragments that were approximately 900, 300 and 200 bp long were observed for *MboI* RE. In contrast, the DNA of 16S rRNA genes for *E. faecium* Z4B, Z4C, Z6A, Z8A and Z8B strains were digested with neither *AluI* nor *MboI*. The RFLP profiles for 2 strains of *L. alimentarius* were observed for *AluI* (between 1200 and 250 bp) and *MboI* (between 500 and 100 bp). *L. namurensis* Z112C contained four restriction sites for *AluI* and two restriction sites for *MboI*.

Determination of bacterial growth in different NaCl concentrations

Bacterial growth and pH values of bacteria at different NaCl concentrations were shown in Table 3. The results of the present study indicated that all 73 strains except *L. acidipiscis* Z27A, *L. plantarum* Z48A, Z59A and Z100A successfully tolerate 7% NaCl at 28 °C. For *L. plantarum* strains at 13 °C in 7% NaCl the lowest and highest pH values were 3.80 and 5.55, respectively. For *L. plantarum* strains at 28 °C in 7% NaCl, the lowest and highest pH values were 3.68 and 5.48, respectively. Fourteen *L. plantarum* and seven *E. faecium* strains showed good viability in 7 and 8% NaCl concentration at 28 °C. *L. plantarum* Z31A, Z64A, Z107C, Z111A and Z115A were also able to tolerate 7, 8 and 9% NaCl at 28 °C. *L. plantarum* Z64A was also able to tolerate 10% NaCl at 28 °C. *L. plantarum* Z9A, Z20A and *L. acidipiscis* Z112D showed

Table 1 List of identified bacteria and sizes of the restriction fragment with *AluI* and *MboI* visible on gels (bp)

Species	Strains	PCR-RLFP patterns	
		AluI	MboI
<i>L. acidipiscis</i>	Z1B, Z1C, Z1D, Z2A, Z2B, Z2C, Z27A, Z46A	1200, 900, 650, 450, 250	500, 300, 100
<i>L. acidipiscis</i>	Z6A, Z43A, Z47A, Z73A	1200, 900, 650, 450, 250	600, 500, 300, 100
<i>L. acidipiscis</i>	Z49A	1200, 900, 650, 450, 250	2000, 150
<i>L. acidipiscis</i>	Z30C	1200, 900, 700, 450, 250	500, 300, 100
<i>L. acidipiscis</i>	Z70A	1000, 900, 650, 450, 250	600, 400, 300, 100
<i>L. acidipiscis</i>	Z85B	1200, 900, 650, 450, 250	600, 400, 300, 100
<i>L. acidipiscis</i>	Z112A, Z112D	900, 600, 400, 250	600, 500, 300, 200
<i>L. acidipiscis</i>	Z118A, Z128A	1200, 900, 650, 400, 250	500, 300, 100
<i>L. plantarum</i>	Z9A	900, 600, 250	–
<i>L. plantarum</i>	Z30A, Z30B, Z31A, Z41A, Z64A, Z64B, Z66A, Z78B	1200, 900, 700, 450, 250	2000, 100
<i>L. plantarum</i>	Z78A, Z98A	900, 700, 450, 250	3000, 150
<i>L. plantarum</i>	Z100A, Z107A, Z107C	900, 600, 450, 250	3000, 200
<i>L. plantarum</i>	Z114A, Z114B, Z115A, Z120A, Z126A, 134A	900, 600, 400, 250	3000, 200
<i>L. plantarum</i>	Z59A	900, 600, 400, 250	2000, 100
<i>L. plantarum</i>	Z53A	900, 600, 400, 250	2000, 500
<i>L. plantarum</i>	Z81A, Z99A, Z103A	1000, 900, 600, 400, 250	3000, 2000
<i>L. plantarum</i>	Z80B, Z81B, Z134B	900, 610, 400, 250	3000, 2000
<i>L. plantarum</i>	Z107B, Z111A, Z121A	600, 450, 250	3000, 2000
<i>L. alimentarius</i>	Z112B	610, 450, 300	500, 450, 300
<i>L. alimentarius</i>	Z42A	1200, 800, 600, 450, 250	500, 350, 100
<i>L. namurensis</i>	Z112C	800, 600, 450, 250	3000, 200
<i>E. faecium</i>	Z137A, Z3A	800, 400, 250	900, 300, 200

the best viability in 10% NaCl concentration at 13 °C. In these conditions, the weakest viability was observed in *L. alimentarius* Z42A, *L. acidipiscis* Z46A, *L. plantarum* Z48A and Z64B. Absorbances at OD₅₉₀ were below 0.4 in 10% NaCl concentration at 13 °C for all bacteria.

Qualitative and quantitative assessment of lipolytic activity

The isolates were primarily screened for their lipolytic activity on agar plates. Six of 73 strains tested showed a positive result on agar medium. While 66 strains showed a negative result, 2 strains showed slight zones. All strains were also tested for lipolytic activity by spectrophotometric assay (Table 4). There was no lipolytic activity measured by two methods in 57 strains.

While *L. plantarum* Z78A and Z80B showed slight zones in agar medium, lipolytic activity was not determined in the spectrophotometric assay. *L. plantarum* Z78A also showed slight zone and lipolytic activity was measured as 0.27 U ml⁻¹. The highest activity was determined in *L. acidipiscis* Z128A as 1.09 U ml⁻¹. The minimum activity was observed in *L. plantarum* Z126A and Z134A

as 0.21 U ml⁻¹. Even though zones were not observed for 19 strains in agar medium, lipolytic activity was determined by spectrophotometric assay for these strains. Lipolytic activity of 27 strains was measured between 0.21 and 1.09 U ml⁻¹. Zones were not observed in 7 *E. faecium* strains but *E. faecium* Z4C, Z8A and Z137A showed lipolytic activity ranged from 0.38 to 0.76 U ml⁻¹.

Qualitative and quantitative assessment of pectinolytic activity

The pectinolytic activities of the strains were shown in Table 5. Seven (*L. acidipiscis* Z1B, Z49A, *L. alimentarius* Z42A, *L. plantarum* Z39A, Z41A, Z53A, Z55A) of the seventy-three strains showed zones in Hi-Media. All of the strains showed pectolytic activity in DNS method. Two *L. plantarum* strains (Z64A and Z107A) and *E. faecium* Z8B showed 2–3 U ml⁻¹ pectolytic activity. Total of 35 strains (48%) showed 3–4 U ml⁻¹, 26 strains (36%) showed 4–5 U ml⁻¹ and 8 strains (11%) showed > 5 U ml⁻¹ pectolytic activity. The highest pectolytic activity was observed in *L. acidipiscis* Z1B as 5.29.

Table 2 Distribution of bacteria identified in the 16S rRNA sequence analysis of LAB isolates' DNA

Bacteria	Accession number	% Identity	Bacteria	Accession number	% Identity
<i>L. plantarum</i> Z30B	MG051221	100	<i>L. plantarum</i> Z107B	MG052647	100
<i>L. plantarum</i> Z31A	MG051220	100	<i>L. plantarum</i> Z107A	MG052648	100
<i>L. plantarum</i> Z30A	MG051222	100	<i>L. plantarum</i> Z115A	MG052649	100
<i>L. plantarum</i> Z31B	MG051264	100	<i>L. plantarum</i> Z126A	MG052651	100
<i>L. plantarum</i> Z39A	MG051265	100	<i>L. plantarum</i> Z134B	MG052652	100
<i>L. plantarum</i> Z41A	MG051275	100	<i>L. acidipiscis</i> Z1B	MG050097	99
<i>L. plantarum</i> Z59A	MG051276	100	<i>L. acidipiscis</i> Z1C	MG722010	99
<i>L. plantarum</i> Z64A	MG051277	100	<i>L. acidipiscis</i> Z2C	MG050098	99
<i>L. plantarum</i> Z64B	MG051278	100	<i>L. acidipiscis</i> Z6A	MG050107	99
<i>L. plantarum</i> Z66A	MG051293	99	<i>L. acidipiscis</i> Z70A	MG050108	100
<i>L. plantarum</i> Z78A	MG051294	100	<i>L. acidipiscis</i> Z112D	MG050113	100
<i>L. plantarum</i> Z78B	MG051325	100	<i>L. acidipiscis</i> Z85B	MG050114	100
<i>L. plantarum</i> Z81A	MG051327	100	<i>L. acidipiscis</i> Z118A	MG050116	99
<i>L. plantarum</i> Z98A	MG051328	100	<i>L. acidipiscis</i> Z30C	MG050117	100
<i>L. plantarum</i> Z99A	MG051329	100	<i>L. acidipiscis</i> Z128A	MG050118	100
<i>L. plantarum</i> Z100A	MG051330	100	<i>L. acidipiscis</i> Z47A	MG050119	100
<i>L. plantarum</i> Z107C	MG051331	100	<i>L. acidipiscis</i> Z43A	MG050120	100
<i>L. plantarum</i> Z114A	MG051333	100	<i>L. acidipiscis</i> Z2A	MG050122	99
<i>L. plantarum</i> Z111A	MG051334	99	<i>L. acidipiscis</i> Z1D	MG050123	100
<i>L. plantarum</i> Z120A	MG051342	100	<i>L. acidipiscis</i> Z2B	MG050124	99
<i>L. plantarum</i> Z114B	MG051343	100	<i>L. acidipiscis</i> Z27A	MG051203	93
<i>L. plantarum</i> Z13A	MG051347	98	<i>L. acidipiscis</i> Z46A	MG051204	100
<i>L. plantarum</i> Z134A	MG051348	99	<i>L. acidipiscis</i> Z112A	MG051206	100
<i>L. plantarum</i> Z20A	MG051349	98	<i>L. acidipiscis</i> Z49A	MG051208	92
<i>L. plantarum</i> Z17A	MG051352	95	<i>L. acidipiscis</i> Z73A	MG051209	100
<i>L. plantarum</i> Z80C	MG051614	100	<i>L. alimentarius</i> Z42A	MG049744	98
<i>L. plantarum</i> Z83B	MG051615	100	<i>L. alimentarius</i> Z112B	MG031161	100
<i>L. plantarum</i> Z44B	MG051617	99	<i>L. farciminis</i> Z4A	MG031164	99
<i>L. plantarum</i> Z53A	MG051696	98	<i>L. namurensis</i> Z112C	MG031165	100
<i>L. plantarum</i> Z85A	MG051697	99	<i>E. faecium</i> Z6A	MG030751	100
<i>L. plantarum</i> Z23A	MG051699	97	<i>E. faecium</i> Z3A	MG030752	100
<i>L. plantarum</i> Z9A	MG051711	100	<i>E. faecium</i> Z8A	MG030808	96
<i>L. plantarum</i> Z81B	MG052591	100	<i>E. faecium</i> Z8B	MG031157	96
<i>L. plantarum</i> Z48A	MG052592	98	<i>E. faecium</i> Z4B	MG031159	98
<i>L. plantarum</i> Z80B	MG052593	100	<i>E. faecium</i> Z137A	MG031160	99
<i>L. plantarum</i> Z98B	MG052595	100	<i>E. faecium</i> Z4C	MG031171	100
<i>L. plantarum</i> Z103A	MG052646	100			

Decarboxylase activity of the isolates

Ability of seventy-three isolates to cleave histidine, lysine and tyrosine amino acids was investigated. Microorganisms did not show decarboxylase activity in medium containing histidine and lysine amino acids and no color change was observed in the medium. A total of eleven strains out of seventy-three bacteria decarboxylated only tyrosine. 7 strains of *L. plantarum* (Z9A, Z13A, Z31B,

Z44B, Z48A, Z53A and Z59A), 3 strains of *L. acidipiscis* (Z46A, Z47A, Z49A) and 1 strain of *L. alimentarius* (Z42A) showed positive decarboxylase activity by decarboxylating tyrosine.

Fig. 1 Restriction fragment length polymorphism (RFLP) gels of bacteria DNA encoding for the small subunit ribosomal RNA. The PCR-amplified product was digested with the restriction enzymes AluI (a) and MboI (b). Lines from left to right are of: (M Marker, 1 *L. acidipiscis* Z1B, 2 *L. acidipiscis* Z1C, 3 *L. acidipiscis* Z1D, 4 *L. acidipiscis* Z2A, 5 *L. acidipiscis* Z2B, 6 *L. acidipiscis* Z2C, 7 *L.*

plantarum Z9A, 8 *S. hominis* Z12A, 9 *L. plantarum* Z30A, 10 *L. plantarum* Z30B, 11 *L. acidipiscis* Z30C, 12 *L. plantarum* Z31A, 13 *L. plantarum* Z41A, 14 *L. alimentarius* Z42A, 15 *L. acidipiscis* Z43A, 16 *L. acidipiscis* Z46A, 17 *L. acidipiscis* Z47A, 18 *L. plantarum* Z64A, M Marker)

Table 3 The lowest and the highest growth and pH value at different NaCl concentrations

	Strains	13 °C (OD ₅₉₀)	pH	Strains	28 °C (OD ₅₉₀)	pH
The lowest value at 7% NaCl concentration	<i>L. namurensis</i> Z112C	0.08	5.57	<i>L. plantarum</i> Z100A	0.24	4.13
The highest value at 7% NaCl concentration	<i>L. plantarum</i> Z80B	0.77	5.39	<i>E. faecium</i> Z8B	2.15	3.75
The lowest value at 8% NaCl concentration	<i>L. plantarum</i> Z83B	0.08	5.42	<i>L. plantarum</i> Z23A	0.21	5.46
The highest value at 8% NaCl concentration	<i>L. acidipiscis</i> Z6A	1.10	5.39	<i>L. plantarum</i> Z31A	1.96	3.68
The lowest value at 9% NaCl concentration	<i>L. acidipiscis</i> Z30C	0.10	5.43	<i>L. plantarum</i> Z66A	0.12	5.41
The highest value at 9% NaCl concentration	<i>L. acidipiscis</i> Z73A	0.38	5.50	<i>L. plantarum</i> Z115A	1.87	3.95
The lowest value 10% NaCl concentration	<i>L. plantarum</i> Z48A	0.08	5.47	<i>L. plantarum</i> Z64B	0.10	5.43
The highest value 10% NaCl concentration	<i>L. acidipiscis</i> Z112D	0.38	5.50	<i>L. plantarum</i> Z64A	2.00	5.32

Table 4 Qualitative and quantitative lipolytic activity of the bacteria

Strains	Qualitative measurement (mm)	Quantitative measurement (U/ml)	Strains	Qualitative measurement (mm)	Quantitative measurement (U/ml)
<i>L. acidipiscis</i> Z2A	8	0.70	<i>L. plantarum</i> Z98A	–	0.54
<i>L. acidipiscis</i> Z2B	–	0.40	<i>L. plantarum</i> Z98B	–	0.27
<i>L. acidipiscis</i> Z6A	4	0.51	<i>L. plantarum</i> Z103A	–	0.32
<i>L. acidipiscis</i> Z49A	3	0.37	<i>L. plantarum</i> Z107A	–	0.49
<i>L. acidipiscis</i> Z70A	–	0.60	<i>L. namurensis</i> Z112C	–	0.32
<i>L. acidipiscis</i> Z73A	–	0.32	<i>L. plantarum</i> Z114A	–	0.32
<i>L. acidipiscis</i> Z85B	–	0.80	<i>L. plantarum</i> Z114B	–	0.76
<i>L. acidipiscis</i> Z128A	–	1.09	<i>L. plantarum</i> Z115A	–	0.60
<i>L. plantarum</i> Z17A	8	0.50	<i>L. plantarum</i> Z126A	–	0.21
<i>L. plantarum</i> Z20A	6	0.87	<i>L. plantarum</i> Z134A	–	0.21
<i>L. plantarum</i> Z23A	10	0.60	<i>E. faecium</i> Z137A	–	0.40
<i>L. plantarum</i> Z78A	Slightly zone	0.27	<i>E. faecium</i> Z4C	–	0.38
<i>L. plantarum</i> Z80B	Slightly zone	0.38	<i>E. faecium</i> Z8A	–	0.76
<i>L. plantarum</i> Z81A	–	0.43			

Discussion

In this study, 65 black and 85 green olive samples were used as isolation material and the total numbers of HLAB detected in MRS with 7% NaCl were between $< 10^2$ and 10^6 CFU g⁻¹. Number of HLAB was in the range of 4.0×10^1 – 2.94×10^6 CFU ml⁻¹ in black olive samples

and 4.00×10^2 – 1.80×10^6 CFU ml⁻¹ in green olive samples. Mourad et al. (2004) reported that in eleven olive samples, mean values of microbial counts ranged from 3.0×10^4 to 6.0×10^6 CFU ml⁻¹ in MRS agar and 1.1×10^4 to 2.0×10^5 CFU ml⁻¹ in M17 agar. Six naturally fermented (Greek-style) table olives were studied by Tofalo et al. (2012), after the fermentation process,

Table 5 Qualitative and quantitative pectolytic activity of the bacteria

Bacteria	Qualitative measurement (mm)	Quantitative measurement (U/ml)	Bacteria	Qualitative measurement (mm)	Quantitative measurement (U/ml)
<i>L. acidipiscis</i> Z1B	7	5.29	<i>L. plantarum</i> Z30A	–	4.43
<i>L. acidipiscis</i> Z1C	–	4.82	<i>L. plantarum</i> Z30B	–	4.25
<i>L. acidipiscis</i> Z1D	–	4.63	<i>L. plantarum</i> Z31A	–	5.04
<i>L. acidipiscis</i> Z2A	–	4.54	<i>L. plantarum</i> Z31B	–	5.00
<i>L. acidipiscis</i> Z2C	–	5.10	<i>L. plantarum</i> Z39A	6	5.15
<i>L. acidipiscis</i> Z6A	–	5.00	<i>L. plantarum</i> Z41A	6	4.18
<i>L. acidipiscis</i> Z27A	–	4.22	<i>L. plantarum</i> Z53A	8	5.14
<i>L. acidipiscis</i> Z30C	–	4.42	<i>L. plantarum</i> Z80C	–	4.45
<i>L. acidipiscis</i> Z49A	8	3.24	<i>L. plantarum</i> Z98A	–	4.35
<i>L. alimentarius</i> Z42A	4	3.96	<i>L. plantarum</i> Z98B	–	4.33
<i>L. plantarum</i> Z9A	–	4.83	<i>L. plantarum</i> Z120A	–	4.85
<i>L. plantarum</i> Z13A	–	4.05	<i>E. faecium</i> Z4b	–	4.63
<i>L. plantarum</i> Z17A	–	5.03	<i>E. faecium</i> Z4c	–	4.82
<i>L. plantarum</i> Z20A	–	4.61	<i>E. faecium</i> Z8a	–	4.57
<i>L. plantarum</i> Z23A	–	4.07	<i>E. faecium</i> Z8b	–	4.79

mesophilic lactobacilli counted were between 6.25 and 7.84 log CFU ml⁻¹. Argyri et al. (2014) determined the initial mean populations of LAB as 4.81 ± 0.43 log CFU ml⁻¹ and 3.70 ± 1.05 log CFU g⁻¹ in the brines and olives, respectively. The results of present study are within the expected range of LAB and yeasts reported previously for fermented black or green olives with or without covering brines (Dimou et al. 2013). Hurtado et al. (2012) described the factors influencing the development of fermentation and LAB as temperature, the amount of NaCl, pH, the polyphenol content and the availability of nutrients.

To distinguish seventy-three LAB strains from each other, two RE, *AluI* and *MboI*, were used. However, these enzymes did not distinguish the *L. plantarum*, *L. acidipiscis*, *L. alimentarius*, *L. farciminis*, *L. namurensis* and *E. faecium* strains. Satomi et al. (1997) successfully used *AluI* and *MboI* for the differentiation of *Tetragenococcus halophilus* and *Tetragenococcus muritatus*. Udomsil et al. (2010) also used *AluI* and *MboI* for comparing the patterns

of fragments obtained from 7 isolates which were similar to *T. halophilus* ATCC 33315. Many researchers found that the main HLAB are *Tetragenococcus* and *Pediococcus* species in highly salted common fermented products such as typical soybean and fish sauces (Uchida et al. 2014). However, in our study, these species were not identified. Harris (1998) determined *L. plantarum* only during natural olive fermentation. Chamkha et al. (2008) determined LAB, especially the genus *Lactobacillus*, *L. plantarum* and *L. collinoides* in the Tunisian olive fermentation brine. Hurtado et al. (2012) also mentioned that *L. plantarum* and *L. pentosus* were the predominant species isolated in table olives. The microbiota of olives varies somewhat from cultivar to cultivar and the type of selected olive processing. In many cases, table olive fermentations occur spontaneously without adding any starter culture (Heperkan 2013). The presence of *L. plantarum* is an important factor for the organoleptic characteristics of the final products and for this reason these species are mostly

preferred as a starter culture for production of fermented vegetables (Fleming et al. 1985). Mourad et al. (2004) has defined 32 bacteria isolated from 11 samples of traditionally fermented green olives as *Lactococcus lactis* (14), *L. plantarum* (11), *E. spp.* (7). In our study, 21 *L. plantarum*, 2 *L. acidipiscis*, 5 *E. faecium* and 1 *L. farciminis* were found among 29 green olive samples, whereas 18 *L. acidipiscis*, 21 *L. plantarum*, 2 *E. faecium*, 2 *L. alimentarius* and 1 *L. namurensis* were identified among 44 black olive samples. Abriouel et al. (2010) detected HLAB such as *L. pentosus* and *L. plantarum* in brine during Aloreña green olive fermentation.

In this present study, *L. acidipiscis* and *E. faecium* showed good viability at 7% NaCl concentration. *L. plantarum*, *L. alimentarius*, *L. farciminis* and *L. namurensis* were also able to tolerate 8% NaCl at 28 °C. Marsilio and Lanza (1998) reported that microorganisms could grow well in 6–8% NaCl concentrations while the microbial growth could be suppressed in 10% NaCl. Quintana et al. (1999) isolated four strains of *L. plantarum* from the brine of table olives in order to ensure appropriate conditions for fermentation of green olives.

In our study, *L. plantarum* Z80B showed the best viability in 7% NaCl concentration at 13 °C. The OD₅₉₀ was 0.77 and 1.18 at 13 and 28 °C, respectively. The pH values were about 5.39 and 4.84 at 13 and 28 °C, respectively. The other strain of *L. plantarum* Z64A also showed the best viability in 10% NaCl with the pH 5.32. Morales et al. (2011) demonstrated that *L. plantarum* and *L. pentosus* were the most acidifying microbial species which were able to reduce the pH of skim milk from 6.2 to 3.8 and 4.4 in 24 h at 32 °C, respectively. *L. acidipiscis* Z6A and *L. plantarum* 31A showed the highest growth in 8 and 9% NaCl concentration at 28 °C. Their pH values were 4.51 for *L. acidipiscis* Z6A and 3.68 for *L. plantarum* 31A. The highest OD₅₉₀ was observed as 1.96 in *L. plantarum* 31A. In this study, the results revealed that *L. plantarum* strains were able to successfully tolerate 8% NaCl at 28 °C. The lowest pH observed in 7% NaCl was 3.67 at 28 °C and highest pH was 5.55 in 13 °C for *L. plantarum* strains.

Chammem et al. (2005) determined bacterial growth in 6, 9 and 12% NaCl concentration in green olives fermentation. Authors stated that the dominant flora was *Lactobacillus* strains and optimum NaCl concentration for growth *Lactobacillus* strains was 9% NaCl. In our study, all twenty *L. acidipiscis* strains showed best salt tolerance in 7% NaCl at 28 °C and the lowest pH observed in these conditions was 3.89. *L. alimentarius* Z42A was able to tolerate to 8% NaCl and the lowest pH was 3.99 at 28 °C. *L. alimentarius* Z112B could survive in 10% NaCl and the minimum pH observed was 4.05 at 28 °C. *L. plantarum* Z100A, Z107A, *L. acidipiscis* Z46A and Z72A couldn't survive under 7, 8, 9, 10% NaCl at 13 °C. In 10% NaCl

concentration, seventeen bacteria couldn't survive at 13 °C. *E. faecium* Z8A and Z137A couldn't resist 9–10% NaCl at both 13 and 28 °C.

Microbial lipases are available in a wide potential for their use in different industrial areas. Andersen and Østdal (1995) purified lipase from *L. plantarum* MF32 strain using tributyrin as substrate. The minimum enzyme activity was determined between 1.9 and 9 U mg⁻¹. In another study, the lipolytic activity of the starter culture for meat fermentation was evaluated by Kenneally et al. (1998). The result of the study showed that tested lactobacilli and pediococcus strains didn't have lipolytic activity. Similarly, Montel et al. (1998) found that *Lactobacillus* species have been implicated weak lipolytic activity. Landeta et al. (2013) studied technological properties of LAB isolated from the cured-dried Spanish sausage. Finally, none of the isolates could break down Tween-80 or Tween-20 as substrate and showed lipolytic activity. The same experiment was performed in another medium, in results of this, only *E. faecium*, *L. plantarum*, *L. paracasei* and *L. sakei* strains showed lipolytic activity. In our study, 8 strains showed a positive result on agar medium and lipolytic activity was measured between 0.2 and 1.09 U ml⁻¹ in 27 strains. The LAB is generally advantageous for use as a starter culture in low lipolytic activity.

The cause of the softening in the olives was previously demonstrated as the pectinolytic activity of yeasts such as *Saccharomyces oleaginosus*, *S. kluyveri*, *Rhodotorula* sp., molds such as *Penicillium*, *Aspergillus*, *Geotrichum* and bacteria such as *Bacillus*, *Aerobacter*, *Escherichia* spp. (Golomb et al. 2013). One of the selection criteria for the LAB to be used for vegetable fermentation is not having pectinolytic activities. In our study, since there wasn't any inhibition zone observed in Hankins's agar medium (Hi-Media), seven *L. plantarum*, *L. acidipiscis* and *L. alimentarius* isolates showed 3.24–5.29 U ml⁻¹ pectinase activity. In another study, the highest activity of exopectinase produced by *Bacillus pumilus* was 3.20 U ml⁻¹ (Tepe and Dursun 2014). In our study, the highest enzyme activity was produced in *L. acidipiscis* Z1B as 5.29 U ml⁻¹.

Biogenic amines are also organic compounds that cause toxic effects in foods with health problems. Thapa et al. (2006) examined decarboxylase activity of LAB isolated from traditional fish products of the Eastern Himalayas using histidine, lysine, ornithin and tyrosine aminoacides. Researchers reported that none of the strains could able to produce biogen amines. Ertürkmen (2014) also found similar results that *Lactobacillus*, *Lactococcus* and *Enterococcus* strains couldn't decarboxylate lysine and histidine whereas tyrosine was decarboxylated by some strains. In our study, histidine and lysine was also not decarboxylated, but tyrosine was decarboxylated by the isolates.

Conclusion

HLAB are dominant bacteria in the fermented foods containing high levels of salt. There are no many studies about the presence of HLAB in table olives and their technological properties. The present work showed that HLAB isolates of *L. plantarum*, *L. acidipiscis* and *E. faecium* were the predominant species found in table olives in the western part of Turkey. These microorganisms may be important starter culture source for food industry due to their salt resistance capabilities, low lipolytic and pectinolytic activity features and not producing biogenic amines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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