

# Characterization of lactic acid bacteria from Turkish Pastırma

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**Abstract** A total of 92 lactic acid bacteria (LAB) isolated from Turkish Pastırma (a salted and dry-cured meat product) were identified by means of phenotypic characteristics. Of these, 45 exhibited a different phenotypic characteristic selected as representative and identified by automated ribotyping. The phenotypic characterization of predominant LAB isolated from the traditional manufactured Pastırma was based on general morphology, physiological tests and API system. The genotypic characterization of LAB was determined with automated *EcoRI* ribotyping. Identification made according to phenotypic and biochemical characteristics shows the isolates were LAB and that nine different bacterial groups could be distinguished. *Lactobacillus plantarum* was the main species detected. In addition, *Lactobacillus sakei*, *Enterococcus faecium* and *Pediococcus acidilactici* species were obtained by automated ribotyping. A few strains were phenotypically misidentified as *Lactobacillus fermentum* or *L. pentosus*, while the phenotypic characteristics were resolved in *L. sakei* and *Weissella confusa* by ribotyping. Ribotyping shows a good correlation with phenotypic methods except 4 clusters.

**Keywords** Pastırma · Automated *EcoRI* ribotyping · Lactic acid bacteria · Identification · Phenotypic identification

## Introduction

A number of traditional local dried meat processing techniques have been developed over hundreds of years,

but there is no consistent technique which would ensure that the product will always be acceptable in quality and safety (Leistner 1987, 1989; Yetim et al. 2006). Pastırma is highly regarded and very popular in most of the Middle Eastern countries. Its name is derived from the Turkish verb ‘bastırma’ which means pressing. Pastırma is one of the salted and dry-cured meat products which are pasted with cemen (the outside covered with a paste which makes it different from its counterparts) and is categorized as an intermediate moisture food (Aksu and Kaya 2001a, b; Aksu et al. 2005; El-Khateib et al. 1987; Kilic 2009; Tekinsen and Dogruer 2000).

Pastırma is an uncooked meat product produced from whole beef or water buffalo muscles and coated with cemen. Pastırma production takes place as follows. Muscles are separated from carcasses during the onset of rigor mortis and allowed to rest at room temperature for 4–8 h, following which they are divided into cuts suitable for Pastırma making. These meat cuts are salted and cured on one side, stacked and left for approximately 24 h. Then, they are salted and cured on the other side, stacked and left for an additional 24 h. Next, the cuts are rinsed with water to remove excess salt, and dried in controlled air conditions for between 3 and 10 days. After pressing and drying periods, they are covered with a paste called cemen and dried again for 1–2 days to complete the process (Kilic 2009).

The production of Pastırma takes about 1 month according to the size of muscles used. The different muscle cuts produce different types of Pastırma. The cemen [50% fenugreek (ground), 35% garlic (fresh and ground) and 25% red pepper] used to cover the Pastırma is both an important factor in the flavor and in the protection of the meat from drying and spoiling by contact with air during processing, which might cause the fat to oxidize and produce an undesirable flavor. Under normal conditions, the end product contains an average of 45% moisture, 30%

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protein, 15% fat, 5% carbohydrate (from paste) and 5% ash (Kilic 2009; Yetim et al. 2006). According to Turkish standards, such foods have zero tolerance to pathogens. Pastırma may be consumed either raw or cooked. This product is usually consumed in households over a long period. It is usually consumed for breakfast in an uncooked thinly sliced form (Anon 2005).

Lactic acid bacteria (LAB) play an important role in meat preservation and fermentation processes and are considered technologically fundamental. They are able to decrease pH by lactic acid production and produce bacteriocins to prevent the growth of pathogenic and spoilage microorganisms, thus improving the hygienic safety, food stability and the shelf life of meat products (Fontana et al. 2005). It has also been observed that LAB contributes to the flavor, color and texture development (Aymerich et al. 2003).

Nationally, some studies have been conducted to determine the microbiological biota of Pastırma (Dogruer et al. 1998; Aksu and Kaya 2001b; Elmali et al. 2007) and the effect of starter culture on its properties (Aksu and Kaya 2001a). However, there are no documented studies concerning the isolation, identification and determination of the LAB of Pastırma samples.

The aim of this work was to identify and characterize the LAB strains isolated from Pastırma produced in Turkey. The isolates were initially classified according to phenotypic and biochemical characteristics and further identified by automated *EcoRI* ribotyping. Results from both identification methods were analyzed and compared.

## Materials and methods

### Detection and enumeration of lactic acid bacteria

LAB strains were isolated from Turkish Pastırma. Ten Pastırma samples (each sample 100 g) were randomly purchased from markets in the province of Eskişehir, Turkey. Firstly, the cemen was removed from all samples, and then 25 g of the samples were added to 225 ml of sterile physiological saline and homogenized for 5 min by homogenizer. Appropriate dilutions were plated on MRS agar and M17 agar. The plates were placed in an anaerobic flask (Oxoid) in the presence of a gas-generating kit (Anaerobic System BR0038B; Oxoid) and incubated at 30°C for 2 days. Well-developed individual colonies on these plates were randomly selected and purified by streaking on MRS agar and M17 agar medium. All isolates were examined for Gram reaction, production of catalase and oxidase activity. Then, Gram-positive, catalase and oxidase-negative isolates (Harrigan and McCance 1976) were stored for further analyses. These isolates were

stocked in 20% glycerol and stored at –80°C. For analyses, isolates were grown on MRS and M17 broth or agar medium, twice consecutively.

### Phenotypic characterization

Isolates were identified using the following tests: ammonia production from arginine, CO<sub>2</sub> production from glucose, growth at different temperatures (4, 8, 10, 15 and 45°C), growth at different pH values, and growth at different NaCl concentrations (Schillinger and Lucke 1987; Stiles and Holzappel 1997). Carbohydrate fermentation tests were carried out using the API 50CHL kit according to the manufacturer's instruction (BioMerieux, France).

### Automated *EcoRI* ribotyping

The bacterial isolates were streaked onto MRS agar plates, and incubated in anaerobic conditions at 30°C for 24–48 h. Subsets of 45 lactic acid isolates, representative of the different samples, were identified by automated *EcoRI* ribotyping. *EcoRI* ribotyping was performed using the standard method of the automated RiboPrinter<sup>®</sup> Microbial Characterization System according to the manufacturer's instructions. The ribotype patterns were compared to patterns stored in the RiboPrinter<sup>®</sup> database. The identification of an isolate was predicted when the corresponding pattern matched one of those of the DuPont Identification Library Codes (labelled as DUP-IDs), with a similarity  $\geq 0.86$ . The characterization consisted of combining profiles within a similarity range, as calculated using the RiboPrinter's proprietary algorithm ( $\geq 0.93$ ), to form a dynamic ribotype or ribogroup that reflects the genetic relatedness of the isolates (Bruce 1996; De Cesare et al. 2007). The DUP-IDs and the ribogroups are calculated using different algorithms in the Riboprinter<sup>®</sup> (De Cesare et al. 2007). The generated Riboprinter<sup>®</sup> patterns were analyzed with the Finger Printing II software and a dendrogram was generated based on Unweighted Pair Group Method using arithmetic Averages (UPGMA) and Pearson correlation coefficients (optimization 1.56%).

## Results and discussion

In the present study, a total of 92 isolates were recovered from Pastırma and identified using both phenotypic and genotypic tests. Up to now, Pastırma produced in Turkey has not been deeply investigated for the characterization of the microbiota. The general properties of 92 isolates were determined by phenotypic characterization. According to the phenotypic test results (properties like growth at 4, 15 and 45°C, growth at 6.5, 7 and 10% NaCl, and NH<sub>3</sub> and

H<sub>2</sub>S production and growth at pH 3.9 and 9.6), the isolates having exactly the same phenotypic properties were determined and 45 isolates representing different phenotypes were used for genotypic study. Information of total isolate number and distribution of groups in the Pastirma samples are shown in Table 1. Evaluation of group distribution showed that Group I was present in all Pastirma samples except sample no. 4, Group VIII shows in samples 2, 4, 5, 6 and 10 and Group IV shows in samples 1, 3, 4 and 8. However, Group III shows only in sample 3, Group VI shows only in sample 5, and Groups VII and IX show only in sample 2. It is thought that the differentiations of the isolate distribution in the Pastirma samples are at a high level because the Pastirma samples were made in different regions of Turkey.

The results of the morphologic, biochemical and physiologic study of selected isolates such as the gas production from the glucose, the development of different heat and salt rate, and cell morphology are shown in Table 2. The isolates were divided into nine groups on the basis of morphological, biochemical, and physiological characteristics (Sharp 1979; Schillinger and Lucke 1987; Kunz 1989; Samelis et al. 1994). The strains of groups I and II produced gas from glucose and they could hydrolyse arginine to NH<sub>3</sub>. The strains of group III produced gas from glucose (hetero-fermentative), but they could not hydrolyse arginine to NH<sub>3</sub> (Table 2). Some groups (I, II) were considered as psychrophilic and mesophilic, since they grew at both 4 and 15°C, and they did not produce gas whereas they fermented ribose (Samelis et al. 1994). Some groups (III, VI and IX) were considered as mesophilic because they were not able to grow at 45°C (Table 2). These isolates were grouped according to their morphological, cultural, biochemical and physiological characteristics (Kandler and Weiss 1986; Montel et al. 1991; Samelis et al. 1994; Schillinger and Lucke 1987) (Table 2). Sixty percent

of the isolates which were divided into groups I, II and III seemed to belong to the genus *Lactobacillus* because they were rods. Groups VI and VII (11.1%) were tentatively identified as leuconostoc-like organisms because they were gas-producing cocci or coccobacilli and were unable to hydrolyse arginine (Table 2) (Kunz 1994). Only one isolate (%) of group IX was assigned to the genus *Leuconostoc* since this isolate presented oval cocci growing in pairs, produced gas from glucose and did not hydrolyse arginine. None of them fermented mannitol and melezitose. Group IV comprised four strains with variable cell morphology. All the bacteria presented coccobacilli shape and therefore these strains were considered to belong to the genus *Pediococcus*. Groups of *Pediococcus* were identified based on their maltose fermentation and growth at 45°C, and growth in the presence of 6.5% NaCl. *Pediococcus* groups are ADH (+). Strains in group V were identified as *Leuconostoc* spp. This group comprised 2 rod-shaped isolates, arginine positive and highly EPS producers. Six isolates (Group VIII) *Enterococcus* are able to grow at 45°C, at pH 9.6 and in the presence of 6.5% NaCl (Table 2).

The results from carbohydrate fermentation tests were revealed that 20 isolates were *Lactobacillus plantarum* (44.4 %), 6 isolates *Lactobacillus fermentum* (13.3 %), 1 isolate *Lactobacillus reuteri* (2.2 %), 6 isolates *Leuconostoc mesenteroides* subsp. *mesenteroides/dextranicum* (13.3 %), 2 isolates *Pediococcus pentosaceus* (4.4 %), 2 isolates *Pediococcus acidilactici* (4.4 %), 2 isolates *Lactobacillus pentosus* (4.4 %), and 6 isolates *Lactococcus lactis* (13.3 %).

The results obtained by *EcoRI* ribotyping confirmed the presumptive classification of the isolates within the species lactic acid bacteria. Based on the preset identification similarity threshold of 0.86, all the strains were automatically identified. *EcoRI* ribotyping differentiated the isolates into 13 distinct ribotypes (Table 3). Similarity between these 13 ribogroups ranged from 0.70 to 1.00. The 13 distinct ribotypes belonged to 11 different DUP-IDs. Considering the high similarity inherent in the *L. plantarum* strains isolated from Pastirma in particular: DUP-IDs 13075 was classified in Lineage I, whereas DUP-IDs 18766 was classified in Lineage II, DUP-IDs 15180 was classified in Lineage III, DUP-IDs 5600 and 13216 were classified in Lineage IV, DUP-IDs 18201 was classified in Lineage V, and DUP-IDs 14068 was classified in Lineage VI (Table 3). No atypical profiles or profiles belonging to Lineages I, II, III and IV were found. However, there were atypical profiles in the other lineages.

The threshold regarding the measure of similarity was fixed at 0.85% in Fig. 1 which shows the dendrogram and banding patterns of the isolates and reference strains based on *EcoRI* ribogroups. The ribogroups of one isolate and ribotypes of reference strains from Pastirma formed cluster

**Table 1** Source of isolation, number of isolates and distribution of groups in the pastirma samples

City which produced the samples	Pastirma sample no.	Isolate number	Distribution of groups
Kayseri	1	12	I, II, IV
Kayseri	2	15	I, II, VII, VIII, IX
Afyon	3	14	I, III, IV
Afyon	4	7	IV, VIII
Kayseri	5	8	I, V, VI, VIII
Ankara	6	10	I, VIII
Kastamonu	7	12	I, V
Adapazarı	8	4	I, IV
Sivas	9	5	I
İstanbul	10	5	I, VIII

**Table 2** Morphological, cultural, biochemical and physiological characteristics of the isolated strains

Properties	Group I n = 20	Group II n = 6	Group III n = 1	Group IV n = 4	Group V n = 2	Group VI n = 3	Group VII n = 2	Group VIII n = 6	Group IX n = 1
	<i>L. plantarum</i>	<i>L. fermentum</i>	<i>L. reuteri</i>	<i>Pediococcus</i> spp.	<i>L. pentosus</i>	<i>Leuconostoc</i> -like	<i>Leuconostoc</i> -like	<i>Enterococcus</i> spp	<i>Leuconostoc</i> spp
Gram reaction	+ 20	+ 6	+ 1	+ 4	+ 2	+ 3	+ 2	+ 6	+ 1
Morphology	r 20	r 6	r 1	c 4	r 2	r 3	cr 2	c 6	cr 1
Catalase	- 20	- 6	- 1	- 4	- 2	- 3	- 2	- 6	- 1
4°C	± 15+/5-	+ 5+/1-	- 1	- 4	± 1+/1-	3	+ 2	- 6	- 1
15°C	+ 20	+ 6	+ 1	+ 4	+ 2	3	+ 2	+ 6	+ 1
45°C	- 2+/18-	- 6	- 1	+ 4	± 1+/1-	3	+ 2	± 4+/2-	- 1
6.5% NaCl	+ 18+/2-	± 4+/2-	- 1	+ 4	+ 2	3	+ 2	+ 5+/1-	- 1
7% NaCl	± 7+/13-	± 2+/4-	- 1	+ 4	+ 2	3	± 1+/1-	- 6	- 1
10% NaCl	- 1+/19-	- 6	- 1	- 4	- 2	3	- 2	- 6	- 1
NH <sub>3</sub> - Arginine	± 14+/6-	+ 6	- 1	+ 4	± 1+/1-	3	± 1+/1-	+ 6	- 1
pH 3.9	± 9+/11-	± 3+/3-	+ 1	+ 4	+ 2	3	- 2	- 6	- 1
H <sub>2</sub> S	- 20	- 6	- 1	- 4	- 2	3	- 2	- 6	- 1
L-Arabinose	+ 18	+ 6	+ 1	+ 4	+ 2	1	+ 2	+ 5	+ 1
D-Ribose	+ 18	+ 6	+ 1	+ 4	+ 2	3	± 1	+ 6	+ 1
D-Xylose	- 3	- 0	- 1	± 2	+ 2	1	± 1	- 0	- 1
D-Galactose	+ 19	+ 6	- 1	+ 4	+ 2	2	± 1	- 4	- 1
D-Glucose	+ 20	+ 6	+ 1	+ 4	+ 2	3	+ 2	+ 6	+ 1
D-Fructose	+ 20	+ 6	? 1	+ 4	+ 2	3	± 1	+ 6	+ 1
D-Mannose	+ 20	+ 6	+ 1	+ 4	+ 2	3	± 1	+ 6	- 1
D-Mannitol	± 11	- 0	- 1	0	+ 2	1	- 0	+ 5	- 1
D-Sorbitol	± 9	- 0	- 1	0	+ 2	1	- 0	+ 5	- 1
Methyl- alpha D-Mannopyranoside	± 9	- 0	- 1	± 2	- 0	1	- 0	- 0	+ 1
Methyl- alpha D-Glucopyranoside	± 4	- 0	? 1	- 0	- 0	0	+ 2	- 0	- 1
N-AcetylGlucose	+ 18	+ 6	? 1	+ 4	+ 2	2	+ 2	+ 6	- 1
Amygladine	± 13	- 0	- 1	± 2	+ 2	1	- 0	- 0	- 1
Arbutine	± 13	- 0	- 1	± 3	+ 2	1	- 0	± 3	- 1
Esculine	+ 20	+ 6	+ 1	+ 4	+ 2	3	+ 2	+ 6	+ 1
Salicine	± 14	- 0	- 1	± 3	+ 2	1	- 0	+ 5	- 1
D-Cellobiose	+ 16	± 1	- 1	+ 4	+ 2	2	- 0	+ 5	- 1
D-Maltose	+ 18	± 1	? 1	+ 4	+ 2	3	+ 2	+ 6	+ 1
D-Lactose	+ 16	+ 5	- 1	± 2	+ 2	0	± 1	± 3	- 1
D-Melibiose	+ 16	+ 6	+ 1	+ 4	+ 2	1	+ 2	+ 5	+ 1
D-Saccharose	+ 16	+ 6	+ 1	+ 4	+ 2	2	+ 2	+ 6	+ 1

Table 2 (continued)

Properties	Group I n = 20	Group II n = 6	Group III n = 1	Group IV n = 4	Group V n = 2	Group VI n = 3	Group VII n = 2	Group VIII n = 6	Group IX n = 1
	<i>L. plantarum</i>	<i>L. fermentum</i>	<i>L. reuteri</i>	<i>Pediococcus</i> spp.	<i>L. pentosus</i>	<i>Leuconostoc</i> -like	<i>Leuconostoc</i> -like	<i>Enterococcus</i> spp	<i>Leuconostoc</i> spp
D-Trehalose	+ 17	+ 6	+ 1	+ 4	+ 2	+ 3	+ 2	+ 6	+ 1
D-Melezitose	± 7	- 0	- 1	0	± 1	± 1	- 0	- 0	- 1
D-Raffinose	± 10	- 0	± 1	2	± 1	± 1	± 1	- 0	- 1
Gentiobiose	± 13	± 1	- 1	3	+ 2	± 1	- 0	+ 5	- 1
D-Turanose	± 11	- 0	- 1	0	± 1	± 2	± 1	± 1	- 1
D-Tagatose	- 0	- 0	- 1	3	- 0	- 0	- 0	- 0	- 1
Potassium gluconate	± 10	- 0	- 1	0	± 1	- 0	- 0	- 0	- 1

n number of isolates, r rood, c coccus, cr coccobacillus

+ positive result, - negative result, ± variable

I at a similarity level of 92–97%. Cluster I consisted of 20 isolates. Cluster II was formed by 6 isolates (13.3%), which presented the same ribotypes. Six strains had the same pattern as the type strain and similarity level 96–92%. Cluster III consisted of 6 isolates which presented two ribotypes. Five strains had the same pattern as the type strain and the other isolates merged at the similarity level of 85% with the type strain. Cluster IV consisted of 1 isolate, cluster V consisted of 3 isolates and similarity level 86–82%, cluster VI, cluster VII and cluster VIII consisted of 1 isolate, cluster IX consisted of 2 isolates, cluster X consisted of 1 isolate, and finally, cluster XI consisted of 3 isolates. Ribotyping of 20 strains and other reference strains showed that *L. plantarum* species can be easily distinguished by this genotypic characterization method. Calculation of the similarity values between the isolates and reference strains allowed the identification of all strains which gave an identical fingerprint with the reference strain *L. plantarum* 13075. Similarity of the fingerprint patterns, however, allowed grouping of the isolates with reference strains and their identification on this basis. The results of the cluster analysis allowed us to confirm the existence of a widespread population of *L. plantarum*. 49.P3.4 showed a similar pattern (70%) with the pattern from the database of *Lactobacillus reuteri* (DUP-13154). Similarly, 163.P5.8 and 171.P7.8 showed similar patterns (70 and 75%) with the pattern from the database of *Weissella confusa*. In addition, the isolate 24.P2.1 was identified only to the genus level (*Leuconostoc*). These isolates are probably atypical strains adapted to grow in a specific Pastirma environment.

EcoRI ribotyping was confirmed as a rapid and reliable method for *L. plantarum*, *L. sakei*, *P. acidilactici*, *Weissella viridescens*, *W. hellenica*, and *Enterococcus faecium* typing. However, Table 3 and Fig. 1 when evaluated together, showed that four isolates (49.P3.4, 163.P5.8, 171.P7.8 and 24.P2.1) were not identified clearly by the riboprinter system.

In all *L. plantarum* strains, the results obtained with the phenotypical identification methods and ribotyping were in good agreement with each other and their identification was considered very reliable. Six strains of *L. sakei*, that were identified as *L. fermentum* by API 50 CHL, were found to belong to *L. sakei* by ribotyping. According to the cluster analysis results, it was determined that the 5.P1.5, 8.P1.8, 10.P1.10, 11.P1.11 and 12.P1.12 isolates were similar at a ratio of 85–91%, and that the 161.P5.5 and 167.P6.9 isolates identified as *L. fermentum* according to API data were actually *L. sakei*. Two *Pediococcus* strains that were identified as *P. pentosaceus* by API 50 CHL were found to belong to *P. acidilactici* by ribotyping. It was observed that 163.P5.8 and 171.P7.8 isolates were similar at 70 and 75%, and though both had been identified as *Lactobacillus pentosus* according to API data they were found to belong

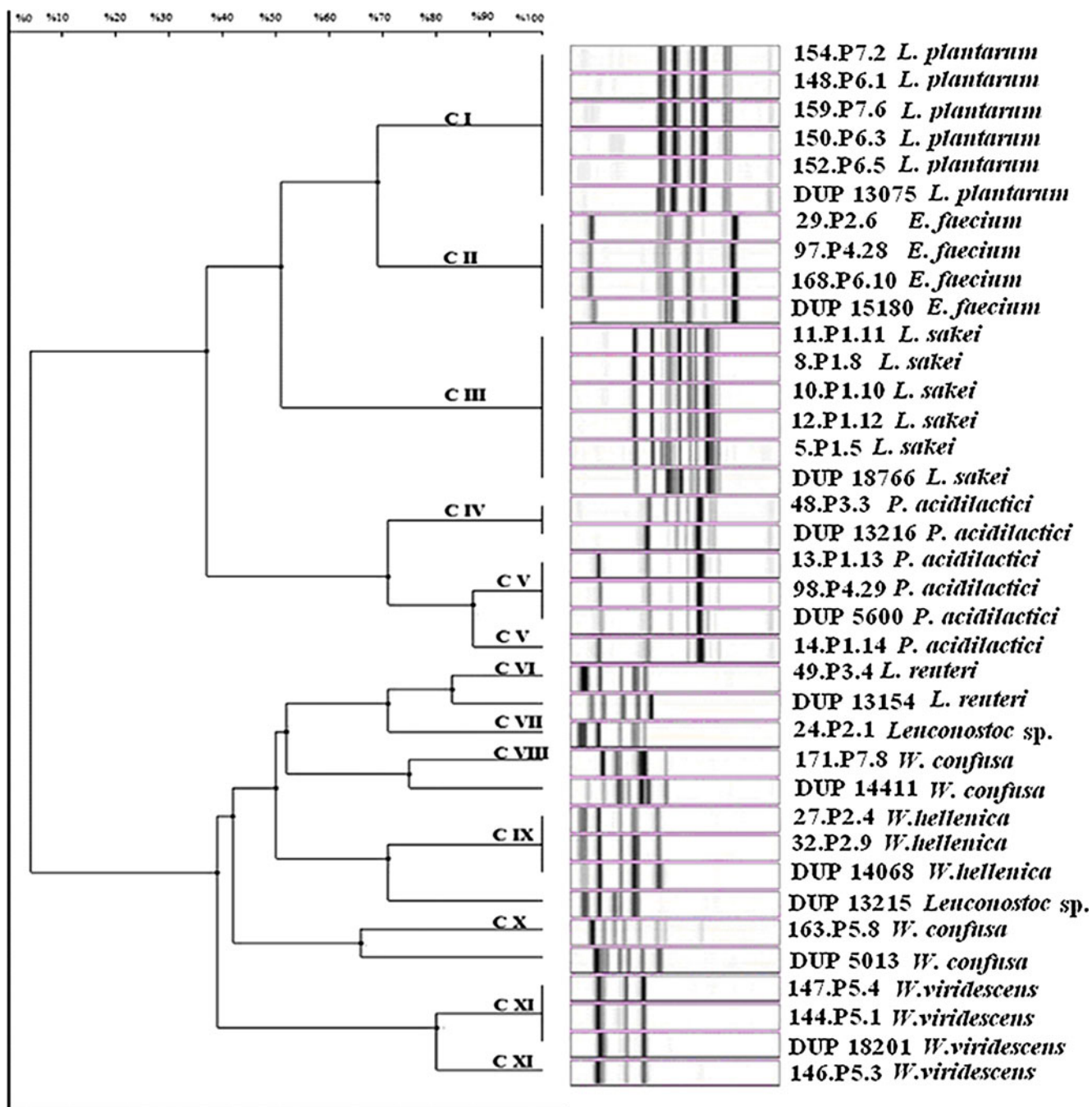
**Table 3** EcoRI ribotyping profiles of the 45 lactic acid bacteria isolated from Pastırma

Cluster	Group	Strain	DuPont ID Label	Ribogrup	DUP Number	Similarity
CI	GI	9.P1.9	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		16.P1.16	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
		25.P2.2	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
		67.P3.22	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.94
		51.P3.6	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
		148.P6.1	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
		149.P6.2	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
		150.P6.3	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.92
		151.P6.4	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		152.P6.5	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.91
		154.P7.2	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		157.P7.4	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		159.P7.6	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.95
		160.P5.5	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		161.P5.6	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		162.P5.7	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		164.P6.6	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		166.P6.8	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		167.P6.9	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.97
		172.P7.9	<i>Lactobacillus plantarum</i>	ECORI 425-12-S-5	DUP-13075	0.96
CII	G VIII	29.P2.6	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.96
		97.P4.28	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.94
		99.P4.30	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.92
		145.P5.2	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.94
		168.P6.10	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.90
		169.P6.11	<i>Enterococcus faecium</i>	ECORI 425-54-S-3	DUP-15180	0.94
CIII	G II	5.P1.5	<i>Lactobacillus sakei</i>	ECORI 425-57-S-5	DUP-18766	0.85
		8.P1.8	<i>Lactobacillus sakei</i>	ECORI 425-30-S-6	DUP-18766	0.91
		10.P1.10	<i>Lactobacillus sakei</i>	ECORI 425-30-S-6	DUP-18766	0.89
		11.P1.11	<i>Lactobacillus sakei</i>	ECORI 425-30-S-6	DUP-18766	0.92
		12.P1.12	<i>Lactobacillus sakei</i>	ECORI 425-30-S-6	DUP-18766	0.86
		28.P2.5	<i>Lactobacillus sakei</i>	ECORI 425-30-S-6	DUP-18766	0.89
CIV	G IV	48.P3.3	<i>Pediococcus acidilactici</i>	ECORI 425-57-S-7	DUP-13216	0.86
CV	G IV	13.P1.13	<i>Pediococcus acidilactici</i>	ECORI 425-53-S-8	DUP-5600	0.83
		14.P1.14	<i>Pediococcus acidilactici</i>	ECORI 425-53-S-8	DUP-5600	0.77
		98.P4.29	<i>Pediococcus acidilactici</i>	ECORI 425-53-S-8	DUP-5600	0.82
CVI	G III	49.P3.4	<i>Lactobacillus reuteri</i>	ECORI 425-30-S-5	DUP-13154	0.70
CVII	G IX	24.P2.1	<i>Leuconostoc</i> sp.	ECORI 425-30-S-5	DUP-13215	0.72
CVIII	G V	171.P7.8	<i>Weissella confusa</i>	ECORI 425- 56-S-5	DUP-14411	0.75
CIX	G VII	27.P2.4	<i>Weissella hellenica</i>	ECORI 425-30-S-7	DUP-14068	0.92
		32.P2.9	<i>Weissella hellenica</i>	ECORI 425-56-S-6	DUP-14068	0.91
CX	G V	163.P5.8	<i>Weissella confusa</i>	ECORI 425-50-S-8	DUP-5013	0.70
CXI	G VI	144.P5.1	<i>Weissella viridescens</i>	ECORI 425-55-S-2	DUP-18201	0.94
		146.P5.3	<i>Weissella viridescens</i>	ECORI 425-55-S-2	DUP-18201	0.92
		147.P5.4	<i>Weissella viridescens</i>	ECORI 425-55-S-2	DUP-18201	0.98

to *Weissella confusa* by ribotyping. *Lactobacillus fructosus* and *L. sanfrancisco* species are quite similar to *W. viridescens* according to Schillinger and Lucke (1987). Five *Weissella* spp. strains were identified as *Leuconostoc*

*mesenteroides* subsp. *mesenteroides/dextranicum* by API 50 CHL and *W. viridescens* and *W. hellenica* by ribotyping.

Among the LAB, the species most commonly determined in meat and meat products including dry sausages



**Fig. 1** Cluster analysis of the lactic acid bacteria isolates from Pastirma. Dendrogram based on UPGMA cluster analysis

processed with different technologies are *L. curvatus*, *L. plantarum* and *L. sakei* (Aymerich et al. 2003; Urso et al. 2006). In this research, the LABs isolated from Pastirma were characterized by phenotypic and genotypic methods. It was discovered that *L. plantarum* is the dominant biota as indicated by several studies (Toksoy et al. 1999; Drosinos et al. 2007; Kaban and Kaya 2008). *L. sakei* and *Enterococcus faecium* were determined to be the second dominant biota in this study. The rest of the strains isolated from Pastirma samples and characterized as *Pediococcus*

*acidilactici*, *Weissella viridescens*, *W. hellenica*, *W. confusa*, *Leuconostoc* spp., *L. plantarum*, and *L. sakei* have been reported previously as LAB and enterococci in meat products (Aymerich et al. 2003; Hammes et al. 1990; Parente et al. 2001; Schillinger and Lucke 1987; Papamanoli et al. 2003), whereas *P. acidilactici* has not been reported previously. *Weissella hellenica* and *W. viridescens* have been commonly associated with meat or meat products (Milbourne 1983; Collins et al. 1993; Adigüzel and Atasever 2009).

LAB isolated from fermented meats are well adapted to the ecology of meat fermentation (Hugas and Monfort 1997). The identification and technological characterization of LAB involved in meat fermentation are crucial to selecting the best strains to use as starters (Ammor et al. 2005; Rantsiou and Cocolin 2006).

In this study, the LAB isolated from Pastirma samples were characterized by phenotypic and genotypic methods. Our results showed that the phenotypic methods should be supported with the automated *EcoRI* ribotyping, as also indicated by Gevers et al. (2001) and Cocolin et al. (2001).

Automated ribotyping was found to be a reliable and rapid method for generating genetic fingerprints of lactobacilli, pediococci and enterococci. Some heterogeneity in the fingerprints with species was detected. This, in turn, is very useful to the meat industry for tracking meat spoilage isolates and for rapidly determining their threat to product stability. Automation decreases handling time and can increase the number of samples processed at one time, but this must be balanced with increased costs of specialized instruments and supplies.

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