Lait 87 (2007) 521–534
DOI: 10.1051/lait:2007033

Available online at:
www.lelait-journal.org

Original article

Technological characterization of wild-type *Lactococcus lactis* strains isolated from raw milk and traditional fermented milk products in Turkey

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Received 9 February 2007 – Accepted 24 May 2007

Abstract – The aim of this work was to detect technological characteristics of fifty strains of lactococci previously isolated in Turkey in order to use them as components of starter formulations. All the lactococcal strains were low acid producers from lactose except *L. lactis* subsp. *lactis* MBLL9. 22, 17 and 11 strains of *L. lactis* presented low, moderate and strong proteolytic activity, respectively. Three out of 50 strains (*L. lactis* subsp. *lactis* MBLL1, MBLL9 and MBLL57) were determined to be bacteriocin producers. The strains *L. lactis* subsp. *lactis* MBLL1 and MBLL9 were found to be lacticin 481-like producers, whereas MBLL57 was a nisin-like producer, according to their partial characterization data. *L. lactis* strains were tested against 61 lactic phages for their phage-resistance properties. Eleven strains exhibited a sensitive phenotype against different phages, whereas the remaining 39 strains were completely resistant to all phages. Among the *L. lactis* subsp. *lactis* biovar. *diacetylactis* strains, 5 strains were defined as high, 3 strains as medium and 7 strains as low-level diacetyl producers. *L. lactis* strains were found to have 1–10 plasmids with molecular weights from 1.9 to 29.2 kb.

***Lactococcus lactis* / technological characteristics / plasmid / starter culture / wild strain**

摘要 – 从土耳其原料奶和传统发酵乳制品中分离出的野生型乳酸乳球菌的特性。本文对从土耳其原料乳及传统发酵乳制品中分离出的 50 株乳酸乳球菌 (*Lactococcus lactis*) 的特性进行了研究, 目的是开发出工业生产用的发酵剂。在以乳糖为底物时, 除 *L. lactis* subsp. *lactis* MBLL9 外所有的乳酸乳球菌产酸能力较低。有 22 株乳酸乳球菌的蛋白水解活性较低, 17 株菌的蛋白水解活性适中, 11 株菌的蛋白水解活性较高。经检测在 50 株乳酸乳球菌中有3株菌 (*L. lactis* subsp. *lactis* MBLL1、MBLL9 、MBLL57) 能产生细菌素; 根据菌株的部分特性确定了菌株 *L. lactis* subsp. *lactis* MBLL1 和 MBLL9 能分泌乳链菌素-481 的类似物, 而 *L. lactis* subsp. *Lactis* MBLL57 能分泌乳链菌肽 (nisin) 的类似物。根据乳酸乳球菌对 61 种噬菌体的抵抗能力, 进而确定其抗噬菌体特性; 其中11个菌株对不同的噬菌体表现出敏感的表型, 而其余的 39 个菌株能够完全抑制所有的噬菌体, 即对所有的噬菌体均表现出抗性。在 *L. lactis* subsp. *lactis* biovar. *diacetylactis* 菌株中, 双乙酰产量高的有 5 株、产量适中的有 3 株, 有 7 株菌的产量较低。 *L. lactis* 菌株含有 1–10 个分子量在 1.9–29.2 kb 的质粒。

乳酸乳球菌 / 生产特性 / 质粒 / 发酵剂 / 野生菌株

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Résumé – Caractérisation technologique de souches sauvages de *Lactococcus lactis* isolées de lait cru et de produits laitiers fermentés traditionnels en Turquie. Le but de cette étude était de détecter les caractéristiques technologiques de 50 souches de lactocoques précédemment isolées en Turquie pour les utiliser dans des formulations de levains. Toutes les souches de lactocoques étaient peu productrices d'acide à partir du lactose, excepté la souche *L. lactis* subsp. *lactis* MBLL9. Des activités protéolytiques faibles, modérées et fortes étaient présentes chez respectivement 22, 17 et 11 souches de *L. lactis*. Trois des 50 souches (*L. lactis* subsp. *lactis* MBLL1, MBLL9 et MBLL57) produisaient des bactériocines, de type lacticine 481 pour MBLL1 et MBLL9, et de type nisine pour MBLL57, d'après leurs données de caractérisation partielle. Les souches de *L. lactis* ont été testées contre 61 phages lactiques pour leurs propriétés de résistance aux phages. Onze souches montraient un phénotype sensible à différents phages, alors que les 39 souches restantes étaient complètement résistantes à tous les phages. Parmi les souches de *L. lactis* subsp. *lactis* biovar. *diacetylactis,* 5 souches ont été définies comme hautes, 3 comme moyennes et 7 comme faibles productrices de diacétyle. Les souches de *L. lactis* se sont révélées porteuses de 1 à 10 plasmides de masses moléculaires de 1,9 à 29,2 kb.

***Lactococcus lactis* / caractéristique technologique / plasmide / levain / souche sauvage**

1. INTRODUCTION

Lactococcus lactis strains (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*) are widely used for the manufacture of dairy products including cheese, butter and fermented milks [27, 40]. Lactococcal strains used in commercial starter cultures possess numerous characteristics such as lactose and citrate fermentation abilities, proteolytic activity, bacteriocin production and immunity, bacteriophage resistance and exopolysaccharide production, which are strain-dependent [8]. These properties can contribute to the desired flavor and texture of the fermented dairy products and optimal growth on the milk components lactose and casein, as well as stability and survival [15, 34]. In the last decade, there has been an increasing interest in the screening of lactic acid bacteria from natural dairy and other fermented products in order to isolate bacteria with improved or novel properties for their potential application in traditional products, or with a view to new fermented products [9, 26, 33].

In many countries like Turkey, besides the industrial production with commercial starter cultures, cheese and other fermented dairy products are made by natural fermentations in which lactic acid bacteria are adventitiously present in milk. Therefore, Turkish dairy products could contribute to a valuable source of lactococcal strains with their improved or novel technological characteristics. In the presented work, 50 *L. lactis* strains previously isolated from raw milk and Turkish traditional fermented milk products were screened for their technological characteristics in order to provide them to researchers in the dairy industry.

2. MATERIALS AND METHODS

2.1. Bacterial strains, bacteriophages and media

L. lactis strains were isolated previously from raw milk and traditional fermented milk products in Turkey [4]. Briefly, samples were diluted and inoculated onto NRCLA [20]. After incubation at 30 °C for 48 h, red colonies with opaque zones were isolated as lactococci. Typical biochemical reactions were tested for identification by following the method described previously [21, 22]. Bacteriophages and the indicator strains were obtained from the Culture Service of Ankara University. Lactococcal strains were routinely grown

at 30 °C in M17 medium (Difco Laboratories, Detroit, Michigan, USA) supplemented with 0.5% glucose (GM17) or lactose (LM17) when necessary [37]. MRS (Difco Laboratories, Detroit, Michigan, USA) and LB (Difco Laboratories, Detroit, Michigan, USA) media were also used during the course of this study. Stock cultures were maintained at –80 °C in M17, GM17, MRS and LB broths supplemented with 40% glycerol. Bacteriophages were stocked in M17 broth containing 40% glycerol and stored at –80 °C.

2.2. Technological characterization of *L. lactis* strains

The acid-producing ability of each strain was tested by inoculating (1%, v/v) 18-h culture into 11% reconstituted skim milk (RSM) and incubating at 30 °C. The pH was measured at 6 h and 24 h.

The acidification rate was calculated as ΔpH (ΔpH = $pH_{at\ time} - pH_{zero\ time}$) [10].

The proteolytic activity of the strains was measured by the method of Citti et al. [11]. Active cultures of *L. lactis* strains were inoculated (1%, v/v) into reconstituted skim milk and incubated at 30 °C for 24 h. After incubation, the proteolytic activity of the cultures results in liberation of the amino acids tryptophan and tyrosine from the milk substrate, which then react with phenol reagent to form a blue color measured at 650 nm (results expressed as μg tyrosine·mL^{-1}).

Diacetyl production was determined by mixing 1-mL cultures of *L. lactis* strains (1% inoculated in reconstituted skim milk and incubated for 24 h at 30 °C) with 0.5 α-naphtol (1%) and KOH (16%). After incubation at 30 °C for 10 min, diacetyl producer strains showed a red ring at the top of the tubes [23].

Bacteriophage sensitivities of *L. lactis* strains were determined by the phage titration and efficiencies of plaquing (EOPs) calculated by dividing the phage titer on the test strain by the titer produced when the phage was grown on the original host strain [40].

Bacteriocin activity was examined as described by van Belkum et al. [38]. By using sterile toothpicks, *L. lactis* strains were spotted onto GM17 plates and grown overnight at 30 °C. Then, 3 mL of appropriate soft agar media (0.7% agar) containing 100 μL of overnight culture of indicator strains were poured on the surface. After incubation for 24 h at a favorable temperature for the indicator strains, inhibition zones were measured. The effects of pH, temperature and enzymes on the bacteriocin activity were identified by the procedure of Franz et al. [18]. One arbitrary activity unit (AU) was defined as the reciprocal of the highest dilution yielding a clear zone of inhibition on the indicator lawn, and was multiplied by a factor of 100 to obtain the AU·mL^{-1} of the sample.

2.3. Plasmid DNA analyses

Plasmid DNA was isolated from the lactococcal strains by the method of Anderson and McKay [6]. For the bacterial lysis 10 mg·mL^{-1} lysosyme and 20% SDS solutions were applied separately. Phenol solution (saturated with 3% NaCl) and a mixture of chloroform:isoamylalcohol (24:1) were used for the precipitation step. The extraction of plasmid DNA was done by ethanol (100%, ice-cold). To avoid RNA contamination, 5 mg·mL^{-1} of RNaseA was used. The plasmid DNA samples were subjected to electrophoresis in 0.7% agarose gels. A dendrogram analysis was performed by using the software Minitab v.14 (Minitab, Inc., State College, PA, USA) to test similarities of the *L. lactis* strains, based on correlation coefficient distance with complete linkage. Strains were clustered by the arbitrary homology of 0.95.

Table Ia. Acidifying activities of wild *L. lactis* strains expressed as the decrease in pH (ΔpH) after 6 h of incubation at 30 °C in reconstituted skim milk.

Strains	ΔpH ranges (6 h)	Acid production
MBLL1, MBLL3, MBLL6, MBLL8, MBLL11, MBLL12, MBLL14, MBLL16, MBLL18, MBLL25, MBLL26, MBLL27, MBLL30, MBLL32, MBLL37, MBLL40, MBLL43, MBLL44, MBLL45, MBLL46, MBLL52, MBLL56, MBLL57, MBLL58, MBLL60, MBLL61, MBLL62, MBLL64, MBLL65, MBLD4, MBLD5, MBLD7, MBLD10, MBLD17, MBLD19, MBLD21, MBLD29, MBLD35, MBLD51, MBLD36, MBLD54, MBLD55, MBLD59, MBLD63, MBLC15, *MBL34, MBLC38, MBLC47, MBLC50	< 1.00	Slow
–	1.00–1.5	Moderate
MBLL9	> 1.5	Fast
L. lactis IL1403**	0.16	–
L. lactis SIK-83**	0.13	–

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.
*MBL34: Atypic *L. lactis* subsp. *lactis* strain.
**Control strains: *L. lactis* IL 1403 [17]; *L. lactis* SIK-83 [5].

3. RESULTS AND DISCUSSION

The acidifying activity of the *L. lactis* strains was determined by measuring pH changes (ΔpH) after 6 h and 24 h of incubation in reconstituted skim milk. The cultures were considered as fast, medium or slow acid producers from lactose when ΔpH was achieved >1.5, 1.00–1.50 and < 1.00, respectively [10]. Most of the strains (49 out of 50) are slow acid producers since their growth in reconstituted skim milk reduced pH by < 1.00 units in 6 h of incubation at 30 °C (Tab. Ia). The control strains IL1403 and SIK83 are also slow acid producers. By contrast, 46% of strains are able to reduce the pH by over 1.5 units (fast acid producers) after 24 h of incubation (Tab. Ib). These results supported the data presented in the literature that acidifying activities of wild-type lactococcal strains are rather low [7, 27]. Acidification activity is an important criterion for the selection of lactic starter culture strains. Fast acid producers are frequently used as starter culture strains, whereas poor or medium acid producers can be used as adjunct cultures depending on their other technological properties [8, 25].

The proteolytic activity of *L. lactis* strains revealed appreciable differences. Eleven of them (*L. lactis* subsp. *lactis* MBLL8, MBLL9, MBLL44, MBLL60, MBLL61 and MBLL64; *L. lactis* subsp. *lactis* biovar. *diacetylactis* MBLD5, MBLD17, MBLD21 and MBLD63, and *L. lactis* subsp. *cremoris* MBLC38) rendered between 25 and 50 $\mu g \cdot mL^{-1}$ tyrosine

Table Ib. Acidifying activities of wild *L. lactis* strains expressed as the decrease in pH (ΔpH) after 24 h of incubation at 30 °C in reconstituted skim milk.

Strains	ΔpH ranges (24 h)	Acid production
MBLL1, MBLL3, MBLL11, MBLL12, MBLL14, MBLL16, MBLL30, MBLL32, MBLL37, MBLL45, MBLL56, MBLD19, MBLD35, MBLD36, MBLD59, MBLC15	< 1.00	Slow
MBLL27, MBLL64, MBLD4, MBLD5, MBLC50, MBLD51, MBLD54, MBLD63, *MBL34, MBLC38, MBLC47	1.00–1.5	Moderate
MBLL6, MBLL8, MBLL9, MBLL18, MBLL25, MBLL26, MBLL40, MBLL43, MBLL44, MBLL46, MBLL52, MBLL57, MBLL58, MBLL60, MBLL61, MBLL62, MBLL65, MBLD7, MBLD10, MBLD17, MBLD21, MBLD29, MBLD55	> 1.5	Fast
L. lactis IL1403**	0.47	–
L. lactis SIK-83**	1.44	–

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.
*MBL34: Atypic *L. lactis* subsp. *lactis* strain.
**Control strains: *L. lactis* IL 1403 [17]; *L. lactis* SIK-83 [5].

equivalents after 24 h of incubation in reconstituted skim milk at 30 °C, while the other 39 produced concentrations of tyrosine equivalents below 25 $\mu g \cdot mL^{-1}$ (Tab. II). The proteolytic activity of dairy lactococci is essential for bacterial growth in milk and it is involved in the development of organoleptic properties of fermented milk products [8, 16]. In this respect, the 11 *L. lactis* strains mentioned above generated in excess of 25 $\mu g \cdot mL^{-1}$ tyrosine equivalents after 24 h of incubation in reconstituted skim milk, thus fulfilling the requirements to be considered useful as proteolytic starters. After 24 h of incubation, 19 out of 23 fast acid producers and 5 out of 11 moderate acid producers showed moderate or high proteolytic activity. Thus, proteolytic activity correlated quite well with the acidification for 48% of strains (Tab. II).

For assessment of the antagonistic activity of the *L. lactis* strains, isolated colonies of 50 strains were overlaid with a lawn each of 23 Gram-positive and Gram-negative indicator bacteria. The results showed that 24 out of 50 *L. lactis* strains appeared to have antimicrobial activity (Tab. III). Based on proteolytic enzyme treatments, 3 out of these 24 strains (*L. lactis* subsp *lactis* MBLL1, MBLL9 and MBLL57) were identified as bacteriocin producers (Tab. IV). Bacteriocins produced by lactic acid bacteria can

Table II. Proteolytic activities of wild *L. lactis* strains expressed as μg tyrosine mL^{-1} after 24 h of incubation at 30 °C in reconstituted skim milk.

L. lactis strains	Proteolytic activity (μg tyrosine (Tyr)·mL^{-1})
MBLL1, MBLL3, MBLL27, MBLL30, MBLL32, MBLL37, MBLL40, MBLL12, MBLL16, MBLL45, MBLL52, MBLL56, MBLD7, MBLD29, MBLD35, MBLD36, MBLD51, MBLD54, MBLC15, *MBL34, MBLC47, MBLC50	Low (< 10)
MBLL14, MBLL18, MBLL25, MBLL26, MBLL6, MBLD19, MBLD10, MBLL11, MBLL43, MBLL46, MBLD55, MBLD59, MBLL57, MBLL58, MBLL62, MBLL65, MBLD4	Moderate (10–25)
MBLL60, MBLL61, MBLL64, MBLD5, MBLD17, MBLD21, MBLL8, MBLL9, MBLL44, MBLD63, MBLC38	High (25–50)
LMG 2132**	16.65

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.
*MBL34: Atypic *L. lactis* subsp. *lactis* strain.
**Control strain: *L. lactis* subsp. *cremoris* [5].

be defined as biologically active proteins or protein complexes displaying a bactericidal mode of action exclusively towards Gram-positive bacteria and particularly closely related species. Bacteriocin-producing *L. lactis* strains have been used in starter cultures for cheese-making to improve the quality of the cheese. On the other hand, these strains are added with sensitive adjunct cultures to increase their autolysis in order to accerelate cheese ripening [19, 29]. Partial characterization of the bacteriocins was performed according to the response of these bacteriocins against different enzyme, pH and temperature treatments and their host range (Tabs. III and IV). Depending on the obtained data, bacteriocins of *L. lactis* subsp. *lactis* MBLL1 and MBLL9 were found to be similar to lacticin 481, while the bacteriocin of *L. lactis* subsp. *lactis* MBLL57 was similar to nisin [30, 31, 36]. Nisin is the only lantibiotic to date that has been approved for commercial use. Like nisin, lacticin 481 is also a broad-spectrum lantibiotic with potential uses in the food industry and medicine [19].

The flavoring compound diacetyl is an end product of citrate metabolism by certain lactic acid bacteria, such as *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*. Such strains are used in butter-making processes [28]. Among the *L. lactis* subsp. *lactis* biovar. *diacetylactis* strains, 5 strains were defined as high-, 3 strains as medium- and 7 strains as low-level diacetyl producers (data not shown).

Fifty *L. lactis* stains were tested for their susceptibility to 61 lactic bacteriophages by comparing bacteriophage EOPs at 30 °C. Thirty-nine out of 50 strains (20 *L. lactis* subsp. *lactis*, 15 *L. lactis* subsp. *lactis* biovar. *diacetylactis* and 4 *L. lactis* subsp. *cremoris*) showed complete resistance to all bacteriophages. Two *L. lactis* subsp. *lactis* (MBLL56 and

Table III. Antibacterial activities of *L. lactis* strains.

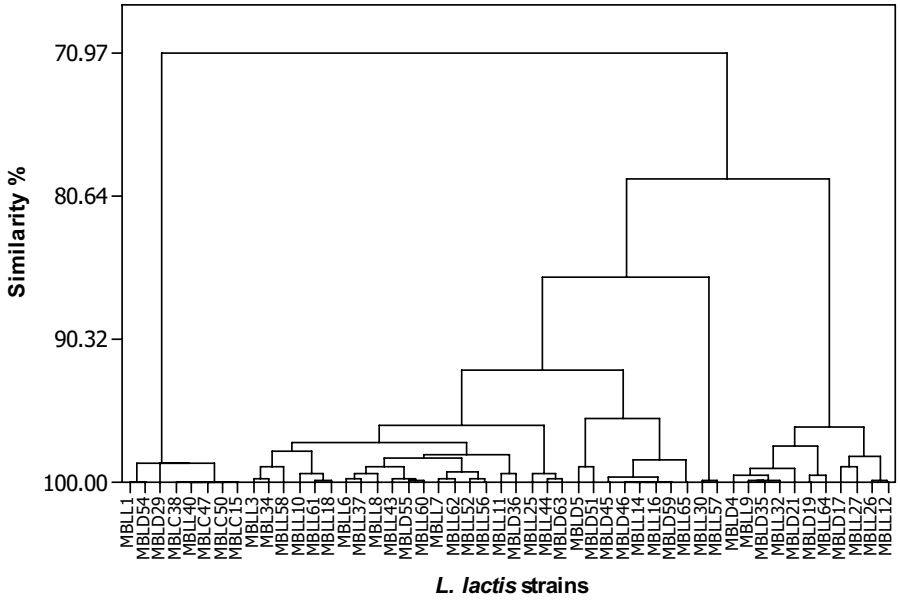
Indicator bacteria	*L. lactis* strains											
	MBLL1	MBLL3	MBLD4	MBLD5	MBLL6	MBLD7	MBLL8	MBLL9	MBLD10	MBLL11	MBLL14	MBLL16
Pediococcus pentosaceus LMG2001	-	-	-	-	-	-	-	11 mm	-	-	-	-
Lactobacillus plantarum LMG2003	14 mm	-	-	-	-	-	-	13 mm	-	-	-	-
L. lactis LMG2088 (laktokokisin G producer)	8 mm	-	-	-	-	-	-	-	-	-	-	-
L. lactis subsp. *cremoris* LMG2132 (lacticin A+B producer)	14 mm	-	-	-	-	-	-	17 mm	-	-	-	-
L. lactis IL1403	16 mm	-	-	-	-	-	-	18 mm	-	-	-	-
L. lactis SIK-83 (nisin producer)	-	-	-	-	-	-	-	7 mm	-	-	-	-
Lactobacillus sake NCDO 2714	10 mm	-	-	-	-	-	-	10 mm	-		-	-
Enterococcus faecalis LMG2602	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis LMG2708	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus carnosus LMG2709	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus LMG2732	-	-	-	-	-	-	-	-	-	-	-	-
Listeria innocua LMG2813	18 mm	8 mm	9 mm	8 mm	9 mm	9 mm	7 mm	18 mm	11 mm	9 mm	7 mm	6 mm
L. lactis LMG2907 (lacticin 3147 producer)	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis LMG2908	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis LMG2909	8 mm	-	-	-	-	-	-	-	-	-	-	-
L. lactis LMG2910 (lacticin 3147 producer)	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis LMG2911 (lacticin 3147 producer)	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis LMG2912 (lacticin 3147 producer)	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens LMG3020	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus LMG3022	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli LMG3083	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella enterica Typhimurium LMG3085	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis JC17 (lacticin 481 producer)	-	-	-	-	-	-	-	-	-	-	-	-

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.

Table III. Antibacterial activities of *L. lactis* strains.

Indicator bacteria	*L. lactis* strains											
	MBLD17	MBLL18	MBLD21	MBLD29	MBLD36	MBLL37	MBLC38	MBLL40	MBLL46	MBLD51	MBLL57	MBLL58
Pediococcus pentosaceus LMG2001	-	-	-	-	8 mm	4 mm	-	-	-	-	18 mm	-
Lactobacillus plantarum LMG2003	-	-	-	-	16 mm	-	-	-	-	-	19 mm	-
L. lactis LMG2088 (lactococcin G producer)	-	-	-	-	-	-	6 mm	-	-	-	-	-
L. lactis subsp. *cremoris* LMG2132 (lacticin A+B producer)	-	-	-	-	2 mm	2 mm	9 mm	-	-	-	16 mm	-
L. lactis IL1403	-	-	-	-	4 mm	9 mm	9 mm	-	5 mm	-	12 mm	-
L. lactis SIK-83 (nisin producer)	-	-	-	-	-	9 mm	11 mm	-	6 mm	-	-	-
Lactobacillus sake NCDO 2714	-	-	-	-	15 mm	-	-	-	-	-	18 mm	-
Enterococcus faecalis LMG2602	-	-	-	-	3 mm	-	12 mm	-	7 mm	-	7 mm	-
Enterococcus faecalis LMG2708	-	-	-	-	2 mm	-	13 mm	-	6 mm	-	8 mm	-
Staphylococcus carnosus LMG2709	-	-	-	-	3 mm	2 mm	-	-	-	-	10 mm	-
Bacillus cereus LMG2732	-	-	-	-	2 mm	-	-	-	-	-	6 mm	-
Listeria innocua LMG2813	7 mm	8 mm	7 mm	7 mm	4 mm	-	8 mm	12 mm	-	8 mm	14 mm	4 mm
L. lactis LMG2907 (lacticin 3147 producer)	-	-	-	-	2 mm	-	-	-	-	-	10 mm	-
L. lactis LMG2908	-	-	-	-	3 mm	-	-	-	-	-	10 mm	-
L. lactis LMG2909	-	-	-	-	3 mm	8 mm	8 mm	-	-	-	6 mm	-
L. lactis LMG2910 (lacticin 3147 producer)	-	-	-	-	5 mm	-	9 mm	-	-	-	10 mm	-
L. lactis LMG2911 (lacticin 3147 producer)	-	-	-	-	2 mm	-	9 mm	-	-	-	8 mm	-
L. lactis LMG2912 (lacticin 3147 producer)	-	-	-	-	2 mm	-	9 mm	-	-	-	8 mm	-
Pseudomonas fluorescens LMG3020	-	-	-	-	3 mm	-	-	-	-	-	-	-
Staphylococcus aureus LMG3022	-	-	-	-	-		-	-	-	-	6 mm	-
Escherichia coli LMG3083	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella enterica Typhimurium LMG3085	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis JC17 (lacticin 481 producer)	-	-	-	-	4 mm	13 mm	13 mm	-	-	-	14 mm	-

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.



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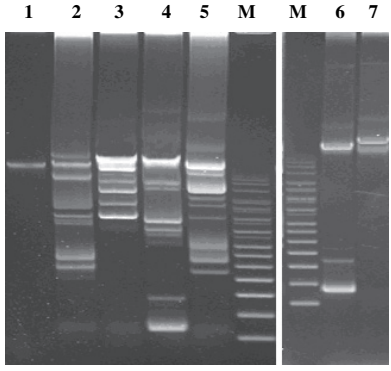

1 MBLL1 (Cluster I)	(kb):	21.7
2 MBLL3 (Cluster II)	(kb):	24.2, 21.8, 18.1, 10.8, 8.8, 7.8, 5.5, 5.0, 4.6
3 MBLD4 (Cluster III)	(kb):	23.9, 20.7, 17.1, 14.1, 11.4, 8.6, 7.4
4 MBLD5 (Cluster IV)	(kb):	24.8, 16.5, 14.8, 8.0, 7.2, 3.8, 2.5
5 MBLL6 (Cluster II)	(kb):	26.7, 21.2, 19.1, 14.8, 12.6, 11.0, 8.4, 6.0, 5.3, 4.8
6 MBLD29 (Cluster I)	(kb):	22.0, 4.4, 2.7
7 MBLL30 (Minor Cluster)	(kb):	23.1, 21.6
M ccc plasmid DNA Marker	(kb):	16.2, 14.2, 12.1, 10.1, 8.0, 7.0, 6.0, 5.0, 4.0, 3.0, 2.1

(b)

Figure 1. (a) Dendogram based on complete linkage cluster analysis with 0.95 association coefficient of the 50 plasmid DNA patterns. (b) Representative DNA plasmid patterns of *L. lactis* strains for different clusters.

Table IV. The effects of pH, temperature and enzyme treatments on bacteriocin activity.

Treatments	Bacteriocin activity (AU·mL^{-1})			
	MBLL1	MBLL9	MBLL57	SIK-83
Control	3200	200	6400	6400
Enzymes				
Trypsin	3200	200	6400	6400
α-Chymotrypsin	0	0	3200	3200
Pepsin	3200	200	6400	6400
α-Amylase	3200	200	800	800
Lipase	3200	200	3200	3200
Catalase	3200	200	6400	6400
Lysozyme	3200	200	6400	6400
Proteinase K	0	0	0	0
Heat				
100 °C, 5 min	3200	200	6400	6400
100 °C, 10 min	3200	200	6400	6400
100 °C, 15 min	3200	200	6400	6400
100 °C, 20 min	3200	100	6400	6400
pH				
pH 2	3200	800	12 800	12 800
pH 3	3200	800	12 800	12 800
pH 4	3200	400	12 800	12 800
pH 5	3200	400	6400	6400
pH 6	3200	200	6400	6400
pH 7	3200	200	6400	6400
pH 8	3200	200	6400	6400
pH 9	1600	0	3200	3200
pH 10	1600	0	1600	1600
pH 11	1600	0	800	800

AU: Arbitrary unit.
MBLL: *L. lactis* subsp. *lactis*.
SIK-83: Nisin producer control strain (*L. lactis* subsp. *lactis*) [5].

MBLL66) and 2 *L. lactis* subsp. *lactis* biovar. *diacetylactis* strains (MBLD54 and MBLD59), conferring a complete bacteriophage-resistant phenotype, were found to be lysogenic, which were induced spontaneously (data not shown). The other 11 strains of *L. lactis* showed different susceptibility profiles against 12 out of 61 lactic phages (Tab. V). The susceptibility of lactococcal starter cultures to infection by bacteriophages remains a major problem facing the dairy fermentation industry worldwide [12]. This problem is compounded by phage biodiversity, which is driven by rapid growth rates, large burst size and genomic plasticity. These traits work synergistically to enable phages to rapidly evolve resistance to existing phage-defence systems by mutation and recombination. Over the years, the use of natural phage-defence systems has proved to be invaluable for the protection of strains that are expected to perform consistently and over extended time frames within industrial applications [35]. Our results and previous research with the Turkish isolates [1–4] of lactococci showed similarities with respect to bacteriophage resistance characteristics. High bacteriophage resistance levels of

Table V. Bacteriophage sensitivity of wild *L. lactis* strains.

L. lactis strains	Bacteriophages											
	Φpll36-3	Φpll67-14	Φpll98-22	Φpll36-58	Φplc61-50	Φplc61-52	Φplc61-55	Φpld67-38	Φpld67-42	Φpld67-45	Φpll98-25	Φpll98-32
MBLL3	+	-	-	-	-	+	-	-	+	-	-	+
MBLL14	-	-	+	-	+	-	-	-	-	-	+	-
MBLL16	-	-	+	-	+	+	-	-	-	-	+	-
MBLL18	+	-	-	-	-	-	+	-	-	-	-	+
MBLL25	-	+	-	-	-	-	-	-	-	-	-	-
MBLL26	-	+	-	-	-	-	-	-	-	-	-	-
MBLL27	-	-	-	-	-	+	-	-	-	-	-	-
MBLL32	+	-	-	+	-	-	-	+	-	-	-	-
MBLL37	-	-	-	-	-	+	-	-	-	-	-	-
MBLD54	-	-	-	-	+	-	-	-	-	+	-	-
MBLD59	-	-	-	-	-	-	+	-	-	-	-	-

MBLL: *L. lactis* subsp. *lactis*.
MBLD: *L. lactis* subsp. *lactis* biovar. *diacetylactis*.
MBLC: *L. lactis* subsp. *cremoris*.
-: Resistant.
+: Sensitive.

lactococcal strains originating in Turkey may result from their non-industrial strain characters. These strains have high industrial starter culture potential with regards to bacteriophage resistance properties. On the other hand, lysogenic strains must be investigated in detail because they represent a renewable source of bacteriophages in the dairy industry and can cause a lytic effect for lactococcal starter culture strains.

L. lactis strains generally carry a number of different plasmids of 2 to more than 100 kb in size, some of which encode properties essential for the manufacture of fermented dairy products and for metabolic functions such as lactose and citrate fermentation, proteolytic activity, bacteriocin production and bacteriophage resistance. Therefore, plasmid biology has become an intensive research area for starter culture strain improvement studies [24, 32, 35]. The strains used in this study harbored 1–10 plasmids with molecular weights from 1.9 to 29 kb (Figs. 1a and 1b). Cluster analysis of the plasmid DNA patterns showed the genetic variability within the 50 strains. The similarity level ranged from 0.71 to 1.00, depending on the strains. Four major clusters and one minor cluster were obtained considering an arbitrary homology of 0.95 (Fig. 1a). Furthermore, a key plasmid profile at subspecies level could not be obtained. The reason for variability in plasmid profiles could be their origin, as the samples were collected from different regions of Turkey. It is also known that plasmids are the most unstable genetic elements which are affected by environmental changes because of their possible gain or loss by horizontal or vertical transfer [13, 14, 39].

The data reported indicate that the strains used in this study demonstrate considerable differences according to their industrially important traits such as lactose and citrate fermentation, proteolytic activity, bacteriocin production and bacteriophage resistance. Some of these strains, especially *L. lactis* subsp. *lactis* MBLL9, showing potentially important properties, are valuable for practical application as starter, adjunct and protective cultures. The traditional fermented dairy environment in Turkey seems to be a rich source of valuable strains with good combinations of properties which, upon optimization, might help to improve the starter cultures for the fermented dairy industry.

Acknowledgements: This study was supported by The Scientific and Technological Research Council of Turkey (Project No. TBAG-HD/90 105T506). We would like to thank Hande Ersöz for critical reading of the manuscript.

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