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Identification of selected *Lactobacillus* strains isolated from Siahmazgi cheese and study on their behavior after inoculation in fermented-sausage model medium

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ABSTRACT

Seventy-one lactic-acid bacteria (LAB) were isolated from a sample of "Siahmazgi", an Iranian traditional cheese. Lactobacilli were then screened for some technological properties such as rapid and adequate production of lactic acid, growth in different salt concentrations, gas production from carbohydrates, catalase activity and antimicrobial activity. Finally, the selected lactobacilli were tested for their growth profile in a fluid model-medium modified according to the special conditions of fermented sausages. LSCD5, LSCD7, LSCD10, LSCD11 and LSCD14 showed high acidifying activity, and reduced the pH of MRS broth medium to ~4.0 in 24 h at 30 °C and in 48 h at 20 °C. These strains were identified by biochemical and molecular methods (16s rDNA sequencing) as *Lactobacillus plantarum*. The screened lactobacilli, except LSCD11, were able to grow at a high salt concentration of 10%. They also showed antimicrobial activities against *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157:H7. Relatively acceptable souring properties and good growth properties in simulated fermented sausage (SFS) medium were found for LSCD7 and LSCD14, with maximum numbers of 7.21, and 7.99 log CFU/ml, respectively. As a result, LSCD7 and LSCD14 exhibited the best technological properties to act as starter cultures for manufacturing fermented meat products.

1. Introduction

Lactic-acid bacteria (LAB), economically a very important group of bacteria for food and feed production, has long been used as a starter culture in the production of fermented sausages and other meat-derived commodities. In meat fermentation, its main function is to initiate rapid acidification of the batter, since the lower pH values that characterize the end products enhance microbial stability by inactivating pathogens, and it creates the biochemical and physicochemical transformations to attain the unique and specific sensory properties of the ripe products during fermentation and maturation (Leroy, Geyzen, Janssens, Vuyst, & Scholliers, 2013).

Isolation and screening of microorganisms from naturally occurring processes have been the most common means for obtaining beneficial cultures for scientific and commercial purposes (van den Berg et al., 1993). The most promising microorganisms for fermented-meat starter cultures are those that are well adapted to the meat environment and to the specific manufacturing process, and are capable of dominating the microbiota of the product due to their specific composition and metabolic activity (Babić et al., 2011). The modern meat industry has to ensure high quality, reduce variability and enhance organoleptic characteristics in large-scale, low-cost sausage production, which is not achievable using spontaneous fermentation methods: it is almost impossible to make sure that the population and variety of microorganisms present in the raw material will always be similar and behave in the same way (Baka, Papavergou, Pragalaki, Bloukas, & Kotzekidou,

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2011). Therefore, it is still worthwhile to search for optimal lactic-acid starter cultures.

The advancement of molecular biology has enabled DNA-based molecular techniques to replace the tedious, ambiguous and time-consuming phenotypic methods. Molecular identification and characterization tools can discriminate even between closely related groups of species, which are indistinguishable on the basis of phenotype (Singh, Goswami, Singh, & Heller, 2009). Among these methods, 16S rDNA analysis is a powerful tool that has enabled a rapid and more precise identification and classification of genera and species, especially in samples with a wide variety of microbiota (de Garnica, Sáez-Nieto, González, Santos, & Gonzalo, 2014). In the present study, 16S rDNA analysis was applied to the genetic identification of selected lactobacilli isolated from traditional Siahmazgi cheese. Dairy products are often reported as good carriers for LAB cultures. While several investigations have been performed based on the screening and characterization of sausage isolates (Babić et al., 2011; Benito et al., 2007; Kaban & Kaya, 2008; Landeta, Curiel, Carrascosa, Muñoz, & de Las Rivas, 2013), there is not yet enough research on their isolation from dairy products to be applied in the manufacture of fermented sausages. Siahmazgi cheese is an artisanal cheese originating in the mountainous area of Talesh-Guilan in Iran. It is traditionally manufactured from ewe's milk or a mixture of ewe's and goat's milks in a one-month period when the milk becomes plentiful. Brine-ripening takes approximately six months in special bags made of sheepskin (Kargozari et al., 2014). Partovi et al. (2014) Identified and characterized the dominant isolated LAB from Siahmazgi cheese. The cheese's firm texture, high salt content and low pH value (Farahani, Ezzatpanah, & Abbasi, 2014; Partovi et al., 2014) suggest a potential for the existing LAB flora to be well adapted to the environment of, and produce optimum acid levels in, fermented sausages. Although researchers have published a few detailed studies about the growth properties and efficiency of LAB isolated from non-meat origin products, including kimchi (a Korean plant-material fermented product) (Lee, Kim, & Kunz, 2006), dairy origin and human intestinal origin (Cenci-Goga, Ranucci, Miraglia, & Cioffi, 2008; Rubio, Jofré, Martín, Aymerich, & Garriga, 2014) as starter culture in fermented sausages, to our knowledge this is the first study of the suitability of nontraditional starter cultures from Siahmazgi cheese for use as starter cultures in fermented-sausage production.

The aim of this study was to evaluate the LAB strains isolated from Siahmazgi cheese to determine their suitability for use as starter cultures in fermented-sausage production. The isolated lactobacilli were screened for technological properties such as rapid and adequate production of lactic acid, growth in different salt concentrations, gas production from carbohydrates, catalase activity and antimicrobial activity. The selected lactobacilli were eventually tested for their growth profile in simulated fermented sausage (SFS) medium modified according to the special conditions of fermented sausages.

2. Materials and methods

2.1. Cheese samples

Six samples of Siahmazgi cheese with the same ripening period (six months) were collected aseptically, in both their original fermentation vessels and sterile sample bottles, from six different small-scale facilities producing traditional cheese located in separate regions. The physicochemical and microbial characteristics of the studied cheese samples have been previously reported (Partovi et al., 2014). The samples were coded SCA-SCF (Siahmazgi cheese A-F), and their pH was checked at the sampling site using a Corning pH meter (model no. 220, Corning Science Products, Corning, NY, USA). Samples were kept at 4 °C after collection and analyzed in a laboratory immediately upon arrival. For microbial analysis three cheese samples with lower pH values ($P < 0.05$) were selected.

2.2. Isolation and enumeration of LAB from Siahmazgi cheese

LAB enumeration and isolation was performed from three cheese samples: SCB, SCD and SCE. Fifty grams of each cheese sample was removed aseptically from the center of each sample; these samples were ground together to produce a composite sample, of which 25 g was aseptically transferred to a sterile stomacher bag containing 225 ml sterile buffered peptone water, and homogenized in a Lab blender stomacher (BagMixer®400, Interscience, Saint Nom, France) for 2 min. Serial dilutions of the homogenates were prepared in the same diluent, and appropriate dilutions were spread-plated on de Man, Rogosa and Sharpe (MRS, Merck, Darmstadt, Germany). LAB were enumerated by plating on MRS agar incubated for 48 h at 30 °C in anaerobiosis. Anaerobic conditions were achieved using anaerobic jars (GasPak, BBL, Cockeysville, MD) equipped with a GasPak envelope (GasPak, BBL, Cockeysville, MD). The microbial experiments were done in duplicate. The isolates were stored in Microbank™ vials (Pro-labo Diagnostics, Neston, Wirrall, UK) at −80 °C in MRS broth plus 20% (v/v) sterile glycerol until further analysis. Isolates from stocks were sub-cultured in MRS broth for daily use.

2.3. Technological properties

Twenty to 30 single colonies per sample were randomly selected from MRS plates corresponding to the highest dilution at which growth occurred. The selection was based on color, shape and colony size, and the resultant colonies were purified by streak plating at least three times on the same medium. All the strains were tested for cell morphology, gram-stain and catalase production. Cell morphology was observed using an Olympus BX41 light microscope (Olympus, Tokyo, Japan). Gram-positive and rod-shaped LAB considered as lactobacilli were further investigated for catalase test using 3% H_2O_2 (Saginur, Clecner, Portnoy, & Mendelson, 1982) and gas (CO_2) production from glucose and sodium gluconate in phenol red broth (Merck, Germany) containing inverted Durham tubes. Lactobacilli were then classified into obligate homo-fermentative, facultatively hetero-fermentative and obligate hetero-fermentative lactobacilli. The homo-fermentative and facultatively hetero-fermentative lactobacilli were subsequently checked for their acidifying capacity. Acid productions of the lactobacillus strains were determined in 25 ml of sterilized 10% reconstituted skim milk and 25 ml of MRS broth. One percent inoculums from an overnight culture were used and incubation proceeded for 72 h at 30 °C (optimal growth temperature) and 20 °C (a more restrictive and technologically relevant temperature) (Rubio et al., 2014). Titratable acidity was determined after 6, 12, 24, 48 and 72 h of incubation. In parallel, the acidifying ability of the strains was determined by measurement of pH. Three trials for acidifying activity were conducted for each strain. Those strains that were able to rapidly produce acid at higher levels (as the most important selection criteria) were then tested for the capacity to grow in MRS broth in the presence of 3, 6.5 and 10% (w/v) NaCl.

2.4. Antimicrobial activity assay

To detect antimicrobial activity, an agar-well diffusion assay was used. *Listeria monocytogenes* (PTCC No. 1298), *Staphylococcus aureus* (PTCC No. 1112) and *Escherichia coli* O157:H7 (PTCC No. 1110) obtained from the Persian Type Culture Collection (Tehran, Iran) were used as the indicator strains. Briefly, sterile MRS agar (20 ml, with

8 g/l agar) was mixed with 100 μl of freshly prepared nutritive broth of the indicator strains at 46–52 °C and poured into Petri dishes. After solidification, the wells were punched with a sterile 7 mm cork borer, and fifty microliters of an overnight culture of each strain of LAB was injected (Zeng et al., 2014). The plates were then stored at 4 °C for 2 h to allow uniform diffusion into the agar (Kalyoncu, Minareci, & Minareci, 2010). After that, the plates were incubated aerobically at 30 °C, and examined after 24 h for clear zones of the food-borne pathogen strains' inhibition. The diameters of the inhibition zones were measured and the diameter of the well, 7 mm, was subtracted from the total zone diameter.

2.5. Identification of the isolates

Lactobacilli isolates were first compared on the basis of esculin hydrolysis using esculin agar at 35 °C for 24 h, and on the basis of their sugar-fermentation profiles. Carbohydrate utilization was assessed at 24, 48, and 72 h after incubation of the tested isolates at 30 °C based on Kandler and Weiss (1986). The abilities of the isolated strains to ferment amygdalin, arabinose, cellobiose, fructose, galactose, glucose, gluconate, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose and xylose were observed. Triplicate reactions were prepared for each sugar-fermentation experiment. For genotype identification, the chromosomal DNA of the selected lactobacillus strains was extracted using the Microlysis kit (Labogen, Rho, Italy) according to the manufacturer's instructions. The 16S ribosomal DNA (16S rDNA) was amplified using standard PCR protocol, and the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGATTCCAAGCC-3′) used to obtain 1500 bp PCR amplicons (Stackebrandt & Goodfellow, 1991). The PCR was carried out in a thermal cycler Mastercycler (Eppendorf, Hamburg, Germany) as follows: one cycle of initial heating at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 90 s, annealing at 62 °C for 90 s and extension at 72 °C for 120 s. PCR products were separated by electrophoresis (1 h at 85 V) on 1% (w/v) agarose gel electrophoresis (Invitrogen, Cergy-Pontoise, France), and the DNA was visualized under UV light after staining with ethidium bromide (0.5 μg/ml) followed by digital image capture using a camera (UVP CA91786, USA). The size of the amplified fragments was determined by comparison with a 1 Kb DNA Ladder (CinnaGen, Iran). The obtained PCR products were excised and eluted from the gel. Amplicons were purified with a gel DNA GF-1 recovery kit (Vivantis, Malaysia) and sequenced by both primers on ABI 3700 automated sequencers (Applied Biosystems, Foster City, CA). Homology comparisons were made using the Basic Local Alignment Search Tool (BLAST), online at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

2.6. The growth profile and acidification activity of selected lactobacilli in model-media

The complex microbial communities of fermented raw sausages alter considerably according to different factors such as meat quality, manufacturing process and the use of accompanying ingredients. Since dealing with these factors is very sophisticated, a model system was substituted to provide LAB isolated from Siahmazgi with environmental conditions similar to those of fermenting sausages. The acidification activity was determined in an SFS medium including meat extract (12.0 g/l); glucose (10.0 g/l); NaCl (20.0 g/l); K_2HPO_4 (2.0 g/l); $MgSO_4.7H_2O$ (0.15 g/l); and sodium glutamate (0.5 g/l). The pH of the medium was adjusted to 5.8. Each isolate was separately sub-cultured twice in MRS broth at 30 °C for 48 h prior to inoculation. The tubes containing 250 ml of SFS broth were then inoculated (1% (v/v)) with the revitalized strains. The growth of the test inoculates was investigated by checking the evolution of viable cell counts in the single cultures. To evaluate the growth rates of the corresponding LAB, the generation time and division rate were calculated. The sampling for determination of CFU and pH was carried out in triplicate after 0, 4, 8, 24, 48, 75, 100 and 150 h of incubation at 20 °C.

2.7. Data analysis

The data was analyzed by one way ANOVA using Statgraphics Plus version 3 for Windows (Statistical Graphics Co., Rockville, Maryland, USA). Differences between the mean values of the measured properties were compared using Duncan's Multiple Range test; a probability value of $P < 0.05$ was considered significant. The data presented in the current study are the means of three replications and are expressed as the mean ± standard deviation.

3. Results and discussion

3.1. Selection and identification of the isolated LAB from Siahmazgi

The LAB present in the Siahmazgi cheese were enumerated by cultivating on MRS agar. Mean counts of the cheese samples' LAB (per gram of cheese) were 1.92×10^7 for SCB, 5.30×10^3 for SCD and 3.81×10^6 CFU/g for SCE. LAB isolation was performed from these three cheese samples with lower pH values (pH < 5.0), which led to the isolation of 71 colonies on selection plates. Rod-shaped, Gram-positive cells were observed in 52 isolates (73.20%) and classified as lactobacilli. *Lactobacillus* has been reported to be the predominant genus in raw-milk, or artisanal, cheeses, since it can grow under the highly selective conditions of the cheese environment (e.g. low a_w, high salt concentration, anaerobic conditions, reduced availability of nutrients, etc.) (Badis, Guetarni, Moussa Boudjema, Henni, & Kihal, 2004). All of the isolates in this study were catalase-negative. However, the presence of catalase activity in LAB might be an important property of starter-culture strains because it eliminates hydrogen peroxide, which can interfere with the organoleptic properties of fermented meat products by increasing rancidity and the discoloration of the final product. Some LAB strains involved in meat fermentation have heme-dependent catalase activity (Abriouel et al., 2004). The isolates were then tested to produce gas from sodium gluconate and from glucose, which would allow us to allocate the lactobacilli according to their type of fermentation (homofermentative, facultative heterofermentative and obligatory heterofermentative lactobacilli) (Badis et al., 2004). The hetero-fermentative strains that formed about 61.5% of the total lactobacilli in Siahmazgi cheese were considered somewhat undesirable in fermented sausages because of the formation of carbon dioxide, which leads to several small holes in the ripe product (Danilović et al., 2011), and high production of acetic acid, which gives rise to pungent odors (Lee et al., 2006). Moreover, the production of sufficient amounts of lactic acid to reduce the pH of the meat to values between 4.8 and 5.0 is the major role of potential starter strains (Casquete et al., 2011). The dominant acidic conditions, which result in the development of curing color and texture and the inhibition of pathogenic and spoilage bacteria, are mainly a consequence of the accumulation of lactic acid. Therefore, for further examinations, hetero-fermentative strains and strains with weak acidifying capacity were excluded. The level of pH induced by lactobacillus strains after 72 h incubation at 30 °C in MRS and skim milk media, ranged from 3.68 to 4.91 and from 3.89 to 6.52 respectively (Fig. 1). The average pH induced by the SCD isolates was lowered to 6.59 to 4.01 within 24 h and remained unchanged afterwards (Fig. 1-a). Although the isolates from SCB indicated a higher decrease rate in average pH within 24 h

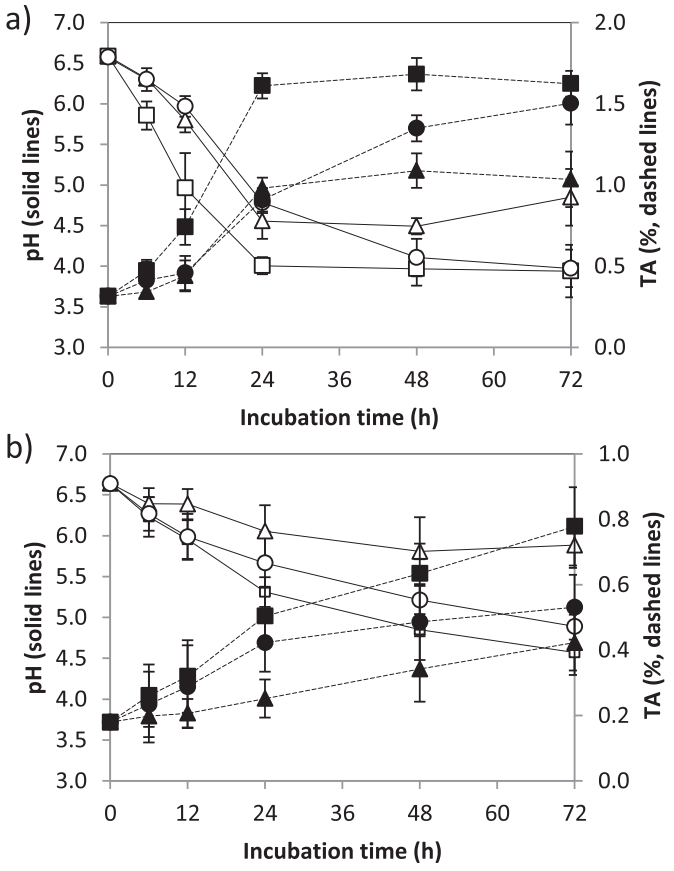

Fig. 1. pH and total acidity (TA) in MRS culture broth (a) and skim milk broth (b) of selected *Lactobacillus* strains during 72 h incubation at 30 °C. ▲, △: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCB; ■, □: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCD; ●, ○: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCE. The white and black symbols represent pH and acidity, respectively. The symbols represent means ± standard deviation (error bars) obtained from three independent experiments.

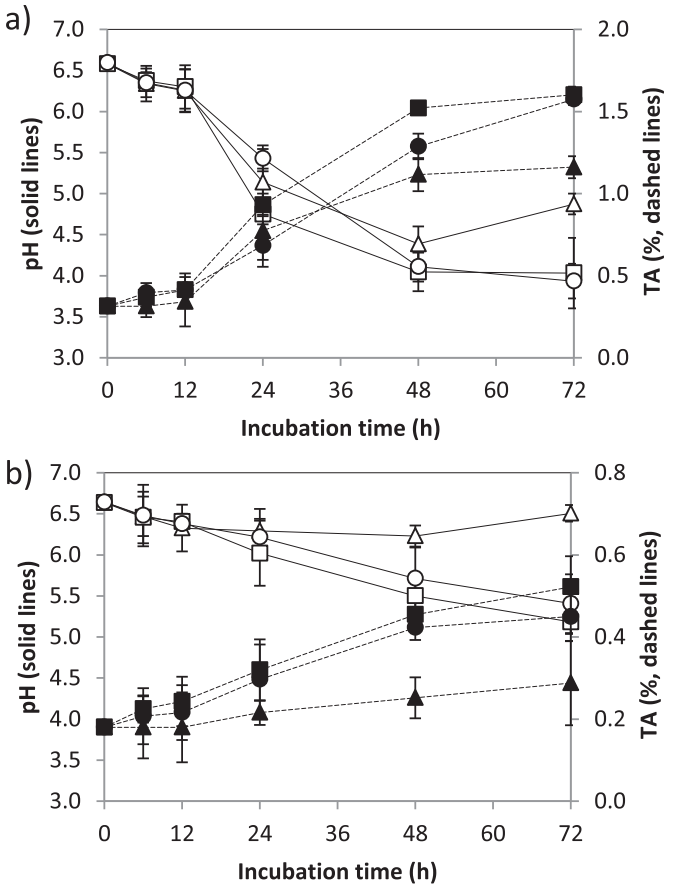

Fig. 2. pH and total acidity (TA) in MRS culture broth (a) and skim milk broth (b) of selected *Lactobacillus* strains during 72 h incubation at 20 °C. ▲, △: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCB; ■, □: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCD; ●, ○: *Lactobacillus* isolates originated from Siahmazgi cheeses coded SCE. The white and black symbols represent pH and acidity, respectively. The symbols represent means ± standard deviation (error bars) obtained from three independent experiments.

compared to the SCE isolates, the final pH was not lowered below 4.49 (Fig. 1a). The general trend in milk pH was the same as that in MRS medium but with higher final pH values compared to those of the MRS medium (Fig. 1b). The pH induced by lactobacillus strains after 72 h incubation at 20 °C ranged from 3.68 to 4.97 in MRS and from 4.61 to 6.60 in milk media (Fig. 2). The SCD isolates revealed high acidifying activity in the MRS medium within 24 h, followed by SCB isolates (Fig 2a). In the skim milk medium, the pH-lowering activity was much slower compared to the MRS medium (Fig. 2b). The strong acid-producing isolates in the SCD samples may be responsible for the low pH (4.99) of SCD cheese samples in spite of their low LAB count (5.3×10^3 CFU/g). Titratable acidity (%) in the same conditions was between 0.71 and 1.80 in MRS and between 0.50 and 0.72 in milk, after incubation at 30 °C for 24 h. Similarly, Neviani, Divizia, Abbiati, and Gatti (1995) have reported that the acidification activity of the lactobacillus strains of grana cheese (Parmesan) incubated at constant temperature varied according to the strain used. Conforming with the screening results obtained, we selected five isolates that produced acid rapidly – LSCD5, LSCD7, LSCD10, LSCD11 and LSCD14 – to further investigate the salt tolerance of the selected isolates. The selected isolates produced no gas from glucose with a broad carbohydrate-fermentation pattern (Table 1). They all hydrolyzed esculin and fermented cellobiose, fructose, glucose, maltose, mannose, melezitose and sucrose. The carbohydrate fermentation pattern of the strains in this study were very similar to those described by Xanthopoulos et al. (2000). It is widely accepted that the identification of lactobacilli to species or strain level based on physiological and biochemical measures is very complicated, though some patterns seem to be species-related. Sequencing of the 16S rDNA gene showed that the five selected isolates were closely related to *Lactobacillus plantarum* (99% similarity to GeneBank sequences). All *L. plantarum* strains used in this study showed different accession numbers, indicating they were different strains (Fig. 3, Table 1). *L. plantarum* is one of the non-starter LAB species most frequently isolated from ripened cheeses, and is likewise among the most frequently isolated LAB from dry sausages processed with different technologies (Ruiz-Moyano, Martín, Benito, Nevado, & de Guía Córdoba, 2008). This probably reflects the greater tolerance of *L. plantarum* to salt and low pH. In this study, the *L. plantarum* strains had similar acidification ability to those found by Xanthopoulos et al. (2000). Furthermore, the tolerance of meat-starter LAB to salt concentrations of 2–10% (max 15%) is a limiting factor that influences the stability and competitive properties of the starter culture over the entire fermentation and ripening process (Ammor & Mayo, 2007). All remaining strains grew in media containing 3, 6.5 and 10% (w/v) NaCl except LSCD11, which did not show any growth at 10% (w/v) NaCl. It has been reported that *Lactobacillus casei* and *L. plantarum* can grow in high salt concentration and long-ripened cheeses (Navidghasemizad, Hesari, Saris, & Nahei, 2009).

Table 1
Biochemical and physiological characteristics of LAB isolated from Siahmazgi cheeses; antimicrobial activity of strains against undesirable bacteria.

Isolate code	Fermentation of																									Growth in			Identification based on 16s rDNA sequencing (% identity)	Indicator strain		
	Gas from glucose	Gas from Na-gluconate	Esculin hydrolysis	Amygdalin	Arabinose	Cellobiose	Fructose	Galactose	Glucose	Gluconate	Lactose	Maltose	Mannitol	Mannose	Melizitose	Melibiose	Raffinose	Rhamnose	Ribose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose	3% NaCl	6.5% NaCl	10% NaCl		*Listeria monocytogenes*	*Staphylococcus aureus*	*Escherichia coli*
LSCD5[a]	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	L. plantarum JDM1 (99)	5.53b ± 0.61[c]	5.65a ± 0.44	6.04b ± 0.57
LSCD7	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	L. plantarum WCFS1 (99)	10.17c ± 0.41	8.82b ± 0.21	7.14b ± 0.63
LSCD10	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	L.plantarum B23 (99)	4.13a ± 0.23	6.29a ± 0.64	4.47a ± 0.37
LSCD11	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	−	L. plantarum C11 (99)	N.D.[b]	N.D.	N.D.
LSCD14	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	L. plantarum WCFS1 (99)	11.56d ± 0.70	12.61c ± 0.45	7.19b ± 0.94

a–d Different letters in the same column indicate significant differences ($P < 0.05$).
[a] LSCD5-14: Selected *Lactobasillus* strains isolated from Siahmazgi cheese (SC) coded D.
[b] N.D. not detected. +: Positive, −: Negative.
[c] The values represent the diameter of inhibition halo in mm as mean ± standard deviation from triplicate determinations.

3.2. Antimicrobial activity

LAB have major potential for use in bio-preservation because of their production of lactic acid or lactic and acetic acid. They may likewise produce other inhibitory substances such as diacetyl, hydrogen peroxide, carbon dioxide, reuterin (b-hydroxypropionaldehyde) and bacteriocins, which are ribosomally produced proteinaceous compounds exhibiting antimicrobial activity (Castellano, Belfiore, Fadda, & Vignolo, 2008; Stiles, 1996). According to this study's tests of the ability to produce antimicrobial substances, four selected isolates from Siahmazgi cheese showed growth-inhibition zones on indicator *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7. All strains showed inhibition-zone diameters larger than 4 mm (Table 1). For different strains, statistically significant different inhibitory activities were observed ($P < 0.05$). Strain LSCD14 showed strong antibacterial activities against indicator bacteria compared with other strains. Larger zones of inhibition against *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 (11.56 ± 0.70, 12.61 ± 0.45, 7.19 ± 0.94 mm, respectively) were found. Maldonado-Barragán, Caballero-Guerrero, Lucena-Padrós, and Ruiz-Barba (2013) reported different spectrum of antimicrobial activity for different *L. plantarum* strains, considering the panel of indicator bacterial strains proposed. In this study, all the *L. plantarum* strains had a listeriostatic effect, which is an essential characteristic of the strains selected as starter cultures because *L. monocytogenes* is a frequent pathogenic microorganism in the environment that can grow at refrigeration temperature, as well as tolerate low pH (<5.0) and high levels of NaCl (10%) (Zeng et al., 2014). As reported by Ahmadova et al. (2013), *Lactobacillus curvatus* A61 isolated from homemade Azerbaijani cheese inhibited the growth of *Listeria innocua*, *Listeria ivanovii* and *L. monocytogenes S. aureus* is low a_w- salt- and nitrite-tolerant, and can grow under anaerobic conditions. Thus there is an increased risk that it will grow and produce toxins (Güzin Kaban & Kaya, 2006). The mechanism involved in the inhibitory action of *L. plantarum* against *S. aureus* in fermented products might be associated with its bacteriocins (Tulini, Winkelströter, & De Martinis, 2013 and Zhu, Zhao, Sun, & Gu, 2014). Purification and characterization of the antimicrobial metabolites should be carried out to confirm the bacteriocin production by the culture.

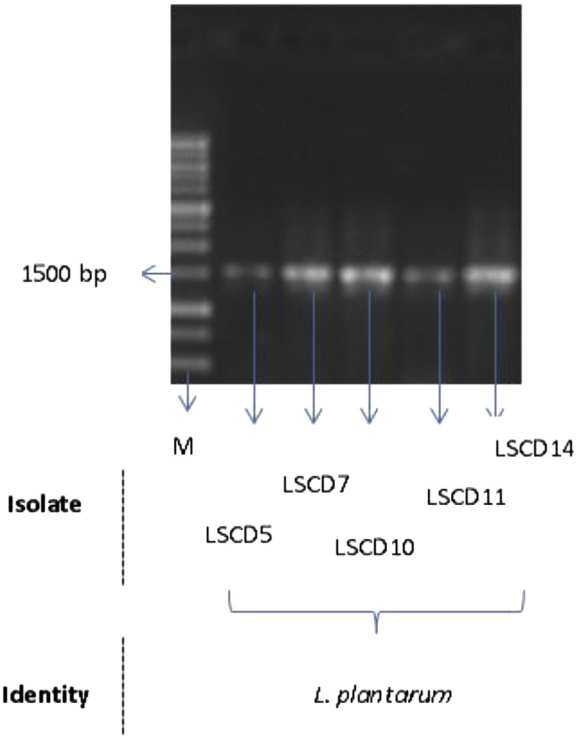

Fig. 3. PCR amplification products obtained from five selected lactobacilli with the primer pair 27F and 1525R. Molecular marker size (lane M) in base pairs is given below the picture.

3.3. Growth profile of selected strains in submerged model medium

Fig. 4 shows the changes in the counts of each strain of the selected four *L. plantarum* isolates after inoculation into SFS medium. Among the studied strains, LSCD7 and LSCD14 initiated growth without considerable lag phases after inoculation, while LSCD10 exhibited a lag phase of approximately 12 h, and the counts of LSCD5 decreased to about 2.5 log CFU/ml within 4 h. Therefore, the results suggest that the two latter strains needed time to adapt to the new habitat. Furthermore, the bacterial count of LSCD7 was

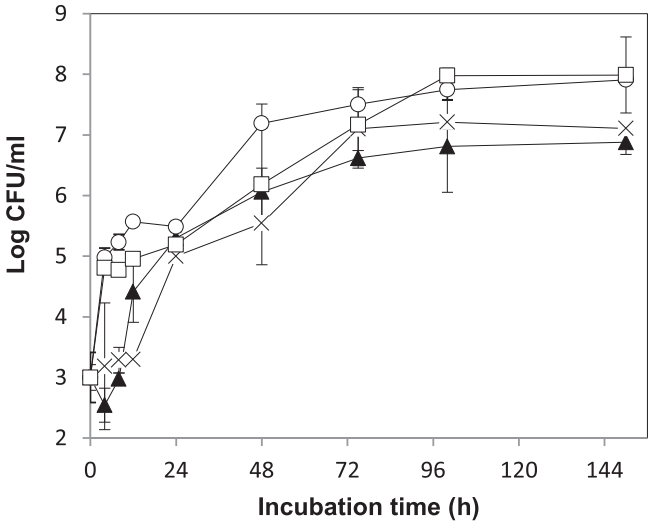

Fig. 4. The growth profile of the selected *Lactobacillus plantarum* strains isolated from Siahmazgi cheese samples. ▲: LSCD5; ○: LSCD7; ×: LSCD10; □: LSCD14. Values are means ± standard deviation of three replicate evaluations for each bacterial strain.

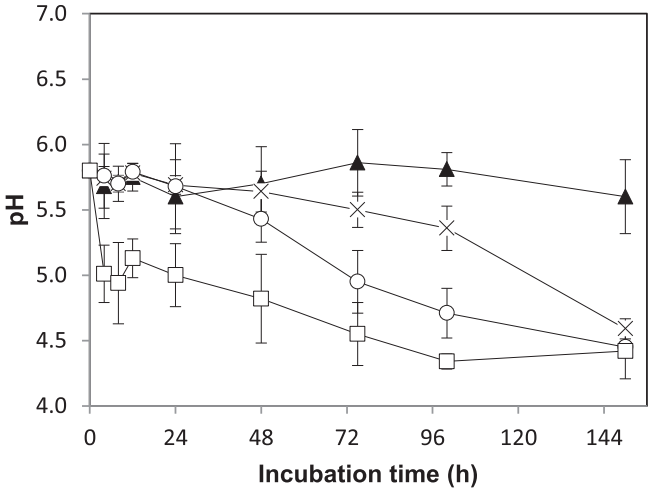

Fig. 5. pH changes of the meat model medium inoculated with the selected *Lactobacillus plantarum* strains isolated from Siahmazgi cheese samples. ▲: LSCD5; ○: LSCD7; ×: LSCD10; □: LSCD14. Values are means ± standard deviation of three replicate evaluations for each bacterial strain.

~1 log higher than that of other isolates at 24 and 48 h after inoculation. The maximum numbers for LSCD7 and LSCD14 were 7.91 and 7.99 log CFU/ml, whereas those of the two other strains ranged from 6.88 to 7.21 log CFU/ml. LSCD7 showed the best growth profile, with 7.19 log CFU/ml in 48 h. This is in agreement with the fact that *L. plantarum* naturally occurring in meat has been previously reported to be among the competitive and predominant species during sausage fermentation (Danilović et al., 2011; Zeng et al., 2014). Meanwhile, the generation times of all strains tested were quite high (4.23–13.27 h), indicating their rather slow growth (Table 2). Lee et al. (2006) reported a lower generation time value (2.58 h) for *L. plantarum* strains isolated from kimchi which has the same SFS medium as this work. This might suggest variability among *L. plantarum* strains from different origins. The lowest generation time, and thus the highest division rate, was for LSCD7, followed by LSCD14 ($P < 0.05$). In a study by Zeng et al. (2014), a similar variability among strains of the same species was observed. With regard to souring properties, the tested strains generally produced slow pH drops, with the exception of LSCD14, since the pH decreased to 5.0 after 4 h of incubation (Fig. 5). The SFS media treated with LSCD7 and LSCD10 reached pH values of 5.43 and 5.64 after 48 h of incubation, respectively. In SFS medium inoculated with LSCD5, only a slight decrease in pH (0.20 units from the start pH value of 5.80) was observed even after 150 h of fermentation. Marroki, Zúñiga, Kihal, and Pérez-Martínez (2011) and Kafili, Emam Djomeh, and Mayo (2013) reported the possibility of finding groups of strains with different acidifying ability in different strains of LAB within the same species.

Table 2
Parameters of growth rate of the LAB strains isolated from Siahmazgi cheese in SFS model medium.

Parameter	*L. plantarum* LSCD5	*L. plantarum* LSCD7	*L. plantarum* LSCD10	*L. plantarum* LSCD14
Generation time (h)	$9.51^{c} \pm 0.96$	$4.26^{a} \pm 0.51$	$11.90^{d} \pm 0.85$	$7.03^{b} \pm 1.29$
Division rate (h^{-1})	$0.17^{ab} \pm 0.01$	$0.24^{c} \pm 0.03$	$0.08^{a} \pm 0.01$	$0.15^{b} \pm 0.02$

Different letters in the same raw indicate significant differences ($P < 0.05$).

4. Conclusions

The results from this study showed that selected *L. plantarum* strains isolated from Siahmazgi cheese – specifically, namely LSCD7 and LSCD14 – appeared to have good potential to be used as a lactic-acid starter culture for sausage production. It was determined that these strains have particularly marked technological properties, being rapid acid producers and salt tolerant, and having antimicrobial activities against some Gram-positive and Gram-negative bacteria. Likewise they had the capacity to grow, and to decrease pH values, under simulated conditions of fermented sausages. The *lactobacillus* isolates as starter cultures might improve safety by inactivating pathogens and spoilage microorganisms via acid production and bacteriocins. Therefore further studies are required to put to the test the mechanisms involved in the protective effect of *L. plantarum*, especially in a real sausage environment, but this study confirms that the selection of an effective strain can be enhanced by using intra-species diversity.

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