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Full Length Research Paper

Phenotypic and genotypic characterization of lactic acid bacteria isolated from Azerbaijani traditional dairy products

Amarela Terzic-Vidojevic[1*], Maja Tolinacki[1], Milica Nikolic[1], Jelena Lozo[1], Jelena Begovic[1], Sahib Gurban oglu Gulahmadov[2], Akif Alekperovich Kuliev[2], Michèle Dalgalarrondo[3], Jean-Marc Chobert[3], Thomas Haertlé[3] and Ljubisa Topisirovic[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, P. O. Box 23, 11010 Belgrade, Serbia.
[2]Department of Biochemistry and Biotechnology, Baku State University, 23, Khalilov Street, 370602 Baku, Azerbaijan.
[3]UR 1268 Biopolymers Interactions Assemblies, National Institute of Agronomic Research, Functions and Interactions of Dairy Proteins, B.P. 71627, 44316 Nantes Cedex 3, France.

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Studied lactic acid bacteria (LAB) were isolated from two types of final ready-to-eat artisanal dairy products (cheeses – Agdas, Sheki and yogurts - Karabakh, Ganja and Baku) manufactured in Azerbaijan. The Agdas cheese belongs to the group of semi hard cheeses whilst the Sheki cheese belongs to hard cheeses. Both of cheeses were produced from cow's milk without the addition of the starter cultures. The Karabakh and Baku yogurts were produced from bovine's milk and the Ganja yogurt from buffalo's milk. Overall 378 isolates were collected from these dairy products and 296 of them were Gram-positive and catalase-negative. It was determined using biochemical tests and molecular methods that four species of LAB were mostly present in these cheeses: *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus paraplantarum* and *Enterococcus faecium* while in yogurts, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* and *Enterococcus faecium* dominated. Five enterococci were producers of antimicrobial compounds.

Key words: Antimicrobial activity, Azerbaijan, dairy products, DGGE, non-starter lactic acid bacteria (NSLAB), proteolytic activity, rep-PCR, 16S rDNA.

INTRODUCTION

The protein breakdown, fat hydrolysis and lactose metabolism are very important processes for production of high value dairy products. These processes are catalyzed by agents such as residual coagulant, indigenous milk enzymes, starter or non-starter microflora and secondary organisms (El Soda et al., 1995; McSweeney and Sousa, 2000). Lactobacilli generally dominate the non-starter lactic acid bacteria (NSLAB) population (Williams and Banks, 1997; Fitzsimons et al., 1999; Swearingen et al., 2001). Other bacterial groups for instance, pediococci, micrococci and leuconostocs have been also found in the microflora of artisanal dairy products (Manolopoulou et al., 2003; Callon et al., 2004).

NSLAB influence flavor and texture development especially of homemade fermented dairy products manufactured at specific ecological localities. These bacteria represent the local, specific microflora and it is believed that differences between qualities of such products arise from the presence of NSLAB (Cogan et al., 1997; Beresford et al., 2001). However, they certainly exert unpredictable effect on the quality of artisanal fermented products. That is why the study of strain heterogeneity in natural cheese starters is of great importance due to their possible wider application in the dairy industry (Fortina et

*Corresponding author. E-mail: lab6@eunet.rs. Tel.: +381 11 3975960. Fax: +381 11 3975808.

al., 1998).

Azerbaijan represents a geographical and natural interface between Europe and Asia. It is situated in the south of Caucasus. More than half of its territory is covered with mountains while the rest of the country is situated either above 1000 m of altitude or at Caspian Sea level. There are 9 climate zones in Azerbaijan which are contributing to a development of important biodiversity. Immemorial pastoral traditions of multiple ethnic groups which were transiting or dwelling in this region are still maintained. Azeri pastoral populations developed traditional ways of different dairy products production, particularly cheeses and yogurts. Recently, lactobacilli isolated from Azerbaijani cheeses Motal and Brunza which produce antimicrobial compound were characterized (Gurban et al., 2006).

The Agdas and Sheki cheeses, and Karabakh, Baku and Ganja yogurts are dairy products from Azerbaijan, manufactured solely in country households without the addition of any starter culture. Thus, LAB in these dairy products correspond to the microflora in localities where dairy products are manufactured. Samples of Agdas and Sheki cheeses, and Karabakh, Baku and Ganja yogurts were gathered from 5 specific geographic locations belonging to different climate zones.

Taking into account that no data on the microflora composition of Agdas and Sheki cheeses as well as Karabakh, Baku and Ganja yogurts exist. The aim was to study the indigenous LAB population involved in the production of artisanal dairy products, in order to set preliminary description of LAB species carrying out the traditional fermentation of those cheeses and yogurts. This study represents a first step towards the understanding of LAB populations responsible for specific organoleptic qualities of these dairy products. Characterization of isolated LAB at phenotypic and genotypic levels including the strain determination would help in the selection of autochthonous strains which could be potentially used as starter cultures for the dairy production at the industrial level.

MATERIALS AND METHODS

Manufacturing and sampling of cheeses and yogurts

LAB isolation was done from final ready-to-eat dairy products (2 kinds of cheese and 3 kinds of yogurt). They were collected from the distinct geographical regions in Azerbaijan. The first cheese sample named Agdas was obtained from Agdas region, located in the northwest of Azerbaijan which has mountainous relief at the altitude of approximately 1500 m. Cheese type named Sheki was obtained from the area of Sheki city. Localization, relief and sea level status of Sheki are similar to Agdas.

The Karabakh yogurt originates from city of Fuzuly and Baku yogurt is from Baku city region located near the Caspian Sea. Ganja yogurt is from region of Ganja city which is settled in foothills of Caucasus at 600 m of altitude.

Agdas and Sheki cheeses were manufactured from cow's milk. The pasteurization of milk was done immediately after milking by warming the milk to 70°C which took 15 min. These cheeses were made by addition of the 1% (v/v) homemade rennet to pasteurized milk which was subsequently cooled to 37°C. The curd formation took 2 h under conditions of cheese making. Afterwards the curd was cut into small pieces and transferred in cotton bag and pressed for whey extraction for 6 - 8 h. Then, the curd without whey was put into a barrel with brine containing 2% NaCl (for Agdas cheese) and cheese ripening was taking 1.5 month. After ripening period, this cheese was white with a semi-hard consistence. The ripening of Sheki cheese was performed (after whey extraction) in sheepskin with 3% NaCl for 5 months. It had hard consistence and yellow color and could be classified between hard cheeses. The temperature at which the ripening occurred was 8 - 17°C in both cases.

The manufacturing of all three yogurts is very similar and it is done for years in the same traditional way. Karabakh and Baku yogurts were made from bovine milk, whereas, Ganja yogurt was prepared from buffalo milk. The production of yogurt was done as follows: 10 ml of old, previously produced yogurt was added to 1 L of pasteurized milk chilled to 37 - 40°C. After the inoculation, the milk was stored at this temperature in glass containers or saucepan vessels and incubation was carried out for 4 - 8 h until the curd was formed.

The samples of cheeses and yogurts were collected from farmhouses in sterile plastic containers and transported to the laboratory in portable refrigerator.

Bacterial strains, media and growth conditions

Bacterial strains used in this study are listed (Table 1). ***Lactobacillus*** strains were cultured in MRS broth (pH 5.7) (Merck GmbH, Darmstadt, Germany), whereas, ***Lactococcus, Enterococcus*** and ***Streptococcus*** strains were grown in M17 broth (pH 7.2) (Merck GmbH) supplemented with glucose (0.5%, w/v; GM17 broth). To each medium, agar (2.0% w/v; Torlak, Belgrade, Serbia) was added when used as a solid medium. The plates were incubated overnight at corresponding temperatures depending on the strain.

Microbiological analysis

For the isolation of bacteria 10 g of each sample from the cheeses interior and 10 ml of each yogurt samples were taken and homogenized with pestle in sterile mortar and transferred to 90 ml sterile 2.0% trisodium citrate solution in a sterile conical flask. Decimal dilutions of the homogenates were prepared with sterile 0.85% (w/v) sodium chloride and were plated on media most suitable for the isolation of LAB. LAB was isolated on both MRS agar plates and GM17 agar plates after incubation at 30°C and at 45°C for 3 days in aerobic and in anaerobic conditions. Incubation in anaerobic conditions was carried out by using the anaerobic jars with Anaerocult A (Merck GmbH).

The enumeration of total mesophilic and thermophilic bacteria was performed by using MRS and GM17 agar plates and incubation at 30 and 45°C for 72 h. Results are expressed as colony forming units (cfu) per gram of cheese or per milliliter of yogurt.

LAB isolates from Agdas and Sheki cheeses are designated as BGAZES1 and BGAZES2 respectively and those isolated from Karabakh, Ganja and Baku yogurts are designated as BGAZEJ1, BGAZEJ2 and BGAZEJ3 respectively.

50 - 100 colonies per cheese or yogurt sample were randomly taken from both MRS and GM17 agar plates corresponding to the highest dilution at which growth occurred. Overall, 378 isolates were isolated and after catalase test, Gram staining and microscopic observation (Olympus U-RFL-T, BX51, GmbH, Hamburg, Germany), 296 were chosen for further analyses. It was confirmed according to the catalase test and Gram staining that the rest of 82 isolates did not belong to the lactic acid bacteria. Gram-positive and catalase-negative isolates were sub-cultured to purity on MRS or GM17 agar plates and stored at –80°C in GM17 (for cocci-like LAB)

Table 1. The list of strains used in this study.

Bacterial strains	Source of reference
L. plantarum A112[a,b,c]	Vujcic and Topisirovic (1993)
L. paracasei subsp***. paracasei*** BGBUK2-16[a,b] and BGBUK2-16/K4[a]	Lozo et al. (2004)
L. paracasei subsp***. paracasei*** BGBUK2-8[a]; BGLI15[a]; BGKP20[a]	Laboratory collection[d]
L. lactis subsp. ***lactis*** BGMN1-5[a] and BGMN1-596[a]	Gajic et al. (1999)
L. lactis subsp. ***cremoris*** NS1[a]	Laboratory collection
L. lactis subsp. ***lactis*** biovar. ***diacetylactis*** S50[a]	Kojic et al. (1991b)
L. paraplantarum BGKP15[b]; BGDA17a[b]	Laboratory collection[d]
L. brevis BGHI3a[b,c]	Laboratory collection[d]
E. durans BGZLS20-35b[b]	Laboratory collection[d]
E. faecium BGGJ8-3[b]	Laboratory collection[d]
S. thermophilus S80[b]	Laboratory collection

[a]Used for BLIS-activity detection.
[b]Used for rep-PCR.
[c]Used for DGGE-PCR.
[d]These strains were identified by molecular methods, AFLP, SDS-PAGE and rep-PCR with $(GTG)_5$ primer in the Laboratorium voor Microbiologie, Universitet Gent, Gent, Belgium.

or in MRS (for rods-like LAB) broth containing 15.0% of glycerol (v/v).

For 46 chosen isolates of LAB, further characterization and tests were performed as follows: (a) growth at 30 and 45°C in MRS and GM17 broth, (b) salt tolerance: 4 and 6.5% (w/w) NaCl in MRS and GM17 broth, (c) production of carbon dioxide from glucose by sub-culturing the isolates in tubes with MRS broth Durham's bells, (d) L-arginine hydrolysis, (e) black zone formation on bile esculin agar (Himedia, Mumbai, India) performed only for cocci-like LAB, (f) activity in milk (for all 296 isolates). Activity in milk was measured by pH Meter HI 9311 (Hanna Instruments, Lisbon, Portugal) in skimmed-milk medium prepared from reconstituted skimmed milk powder 10% (w/v) and sterilized by autoclaving at 110°C for 20 min. Sterilized milk was inoculated with 0.2% liquid culture of LAB incubated overnight at optimal growth temperature. The changes of pH values were observed after 4, 4.5, 5, 5.5, 6, 16 and 24 h of incubation. Identification of the isolates was performed according to the methods and criteria of Sharpe (1979); Hardie (1986); Kandler and Weiss (1986); Mundt (1986a, b); Sneath et al. (1986). The tests (a) and (b) were repeated 3 times.

The fermentation of carbohydrates was determined on modified MRS media but containing bromocresol purple (0.04 g/l) as a pH indicator. The carbon sources were added to the medium to give a final concentration of 1% (w/v) as described previously (Badis et al., 2004). The carbohydrates used in the test were L-arabinose, cellobiose, esculin, fructose, galactose, glucose, glycerol, inuline, lactose, maltose, mannitol, mannose, melibiose, refinish, rhamnose, ribose, salicin, sorbose, sorbitol, starch, sucrose, trehalose and D-xylose.

Detection of antimicrobial activity

Antimicrobial activity of isolated LAB was screened for all 296 LAB isolates by agar-well diffusion method (Tagg and McGiven, 1971) using ***L. lactis*** subsp. ***lactis*** BGMN1-5/BGMN1-596, ***L. lactis*** subsp. ***cremoris*** NS1, ***L. lactis*** subsp. ***lactis*** biovar. ***diacetylactis*** S50, ***L. plantarum*** A112, ***L. paracasei*** subsp ***paracasei*** BGBUK2-16/K4, BGKP20, BGLI15 and BGBUK2-8 as indicator strains. Soft GM17 and MRS agar (0.7%, w/v) containing ***Lactococcus*** or ***Lactobacillus*** indicator strains were overlaid onto GM17 and MRS plates respectively. Wells were made in the lawn of hardened soft agars. Aliquots (50 µl) of supernatant of overnight cultures (16 h) were placed into the wells. To confirm the production of proteinaceous substance, a crystal of proteolytic enzyme pro-tease TYPE XIV (Sigma Chemie GmbH, Deisenhofen, Germany) was placed close to the edge of supernatant containing well. The plates were incubated overnight at 30°C. A clear zone of inhibition around the well but not in vicinity of protease TYPE XIV crystal was taken as an indication of proteinaceous nature of produced antimicrobial substance, that is, a potential bacteriocin-like compound.

Assays of proteolytic activity

Proteolytic activities of the isolates were assayed as described previously (Kojic et al., 1991a). Briefly, collected fresh cells (10 mg approximate density 10^{10} cells/ml) were re-suspended in 0.1 mol/l sodium-phosphate buffer, pH 6.5. The cell suspension was mixed with β-casein (5 mg/ml in 0.1 mol/l sodium-phosphate buffer, pH 6.5) (Sigma, St. Louis, MO, USA) and incubated for 3 h at 30°C. After incubation, the cells were pelleted by centrifugation (5 min at 13000 rpm), the clear supernatant was taken and prepared for the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was carried out on 12.5% polyacrylamide gel on standard vertical electrophoresis unit (Hoefer SE 600, Amersham Biosciences, San Francisco, Minnesota, USA). Gels were stained with Coomassie brilliant blue R250 (Serva, Heidelberg, Germany) and distained in a mix of methanol (20%) and acetic acid (7%).

Total DNA isolation

Total DNA from cheese was extracted as described by Randazzo et al. (2002). Total DNA from LAB isolates was purified by the method of Hopwood et al. (1985). Plasmid profiles were screened according to the method of O'Sullivan and Klaenhammer (1993). Total DNA and plasmid profiles were analyzed by electrophoresis on 1% agarose gels containing ethidium bromide and visualized by CCD

camera Biometra BDR2/5/6 (Bio Doc Analyze GmbH, Göttingen, Germany).

DGGE analysis of PCR amplicons

To investigate the dominant bacterial communities by denaturing gradient gel electrophoresis (DGGE) analysis, polymerase chain reaction (PCR) products were generated with PCR primers U968-GC (5'-CGCCGGGGGCGCGCCCCGGGCGGGGCGGGGGCAC-GGGGGGAACGCGAAGAACCTTAC-3') and L1401 (5'-GCGTGTGTACAAGACCC-3') to amplify the V6 - V8 regions of eubacterial 16S rDNA (Randazzo et al., 2002). All PCR reaction mixtures (50 µl) consisted of 20 mmol/l Tris-HCl (pH 8.4), 50 mmol/l KCl, 3 mmol/l $MgCl_2$, 50 mmol/l each of the four deoxy-nucleotide triphosphates (dNTP), 1 U of ***Taq*** polymerase, 5 pmol/l of each primer (Fermentas UAB, Vilnius, Lithuania). Template DNA (1 µg) was added to a reaction. The samples were amplified in GeneAmp PCR System 2700 (Applied Biosystems, Foster City, California, USA) programmed as follows: initial denaturation of DNA for 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and extension of incomplete products for 7 min at 72°C. PCR products were quantified by electrophoresis on a 1% agarose gel containing ethidium bromide and visualized by CCD camera Biometra BDR2/5/6 (Bio Doc Analyze).

The presence of ***prtB*** gene was determined by PCR with prtB10 and prtB20 primers as previously described (Pastar et al., 2003).

DGGE analysis was performed on the DGGE System (DGGE System, C.B.S. Scientific Co., Del Mar, CA, USA) essentially as described previously by Randazzo et al. (2002). Samples were added to a 6.5% polyacrylamide gel (acrylamide-bis acrylamide, 19:1) in 1 x TAE buffer. Optimal separation of the PCR products for the species in the cheese samples was achieved with a 40 - 60% urea-formamide denaturant gradient increasing in the direction of electrophoresis. A 100% denaturant corresponds to 7 mol/l urea and 40% (v/v) formamide. Electrophoresis was performed at a constant voltage of 90 V at 60°C for 16 h. The DNA bands were visualized by silver staining according to manufacturer's instructions (PlusOne™ DNA Silver Staining Kit, Amersham Biosciences, Uppsala, Sweden).

rep-PCR analysis of LAB isolates

For the repetitive extragenic palindromic-polymerase chain reaction (rep-PCR) analysis, total DNA from different isolates of LAB was used as template for PCR amplifications with BOXA1R (5´-CTACGGCAAGGCGACGCTGAG-3´) and $(GTG)_5$ (5´-GTGGTGGTGGTGGTG-3´) oligonucleotide primers, each with its optimal PCR program (Versalovic et al., 1994), using the same PCR reaction mixture (50 µl) as described previously for the DGGE analysis. Reactions were carried out according to previously described procedure (Terzic-Vidojevic et al., 2007).

A statistical matrix was formed with the isolated and reference strains as variables (columns of input matrix) and the position of bands as statistical cases (rows of the matrix). In the statistical matrix, only 2 characteristics of the bands were used, 0 (no band present) and 1 (band present). Clustering was carried out in Statistica 7.0 for Windows (StatSoft Inc. USA) using the algorithm "unweighed pair-group average linkage analysis". Distances between the clusters were performed using "Percent of disagreement".

16S rDNA sequencing

For the 16S rRNA region sequencing, total DNA of LAB isolates was used as a template for PCR amplifications by using U968 (5´-AACGCGAAGAACCTTAC-3´) and L1401 (5´-GCGTGTGTACAA-GACCC-3´) primers (Zoetendal et al., 1998; Randazzo et al., 2002), using the same PCR reaction mixture (50 µl) as described previously in chapter "DGGE analysis of PCR amplicons". Reactions were carried out in thermal cycler as formerly described (Terzic-Vidojevic et al., 2007). Obtained PCR product was purified by QIAquick PCR Purification Kit/250 (Qiagen GmbH, Hilden, Germany), and sequenced by CRIBI-BMR servizio sequenziamento DNA, Universita di Padova, Italy. The sequence was aligned in the NCBI database using the standard nucleotide-nucleotide homology search BLAST (The Basic Local Alignment Search Tool) (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS

Total counts of bacteria

Total counts of viable bacteria grown on MRS and GM17 agar plates were the most uniform in Agdas cheese regardless of temperature at which agar plates were incubated. The average count of total bacteria in both of cheese samples ranged between 2.01×10^7 and 1.03×10^8 cfu/g on MRS, 1.74×10^7 and 4.16×10^7 cfu/g on GM17 agar plates. The highest variation in total counts of viable bacteria was found in the Karabakh yogurt. In this yogurt, the total counts of viable bacteria varied in the range 10^4 - 10^8 cfu/ml depending on both the agar plates used for bacterial growth (MRS or GM17) and temperature of incubation. Results also showed that abundance of thermophilic microflora in all yogurt samples were similar. The exception is Karabakh yogurt in which total counts of viable thermophilic bacteria was considerably higher on GM17 agar plates than on MRS agar plates when incubation was performed at 45°C (Table 2). In addition, the analysis of yogurt samples showed the presence of a significant number of yeasts (1.9×10^6 cfu/ml in Karabakh yogurt, 1.8×10^5 cfu/ml in Ganja yogurt and 1.4×10^6 cfu/ml in Baku yogurt) and molds (4×10^4 cfu/ml in Ganja yogurt and 3×10^4 cfu/ml in Baku yogurt) grown on GM17 agar plates with other bacterial colonies.

Isolation of LAB from different kinds of Azerbaijani dairy products

From 2 cheese and 3 yogurt samples used in this study, 378 randomly chosen isolates of LAB were taken for their analysis. Results showed that 296 isolates were Gram-positive and catalase-negative; they were taken for further examination of proteolytic activity, activity in milk and production of antimicrobial compounds.

One hundred randomly selected colonies were isolated from each cheese samples. Out of 100 tested colonies isolated from Agdas cheese, 98 isolates showed to be Gram-positive and catalase-negative while 83 Gram-positive and catalase-negative isolates were found among 100 tested colonies from Sheki cheese. In the case of analysis of yogurt samples, among 70 isolated LAB from Karabakh yogurt, 52 were Gram-positive and catalase-

Table 2. Number of total viable bacteria in samples of different Azerbaijani cheeses and yogurts on MRS and GM17 agar plates.

Sample	cfu/g (ml) of sample[a]			
	MRS at 30°C	MRS at 45°C	GM17 at 30°C	GM17 at 45°C
Agdas cheese	2.01×10^7	2.28×10^7	1.74×10^7	2.93×10^7
Sheki cheese	6.56×10^7	1.03×10^8	2.38×10^7	4.16×10^7
Karabakh yogurt	1.55×10^4	1.49×10^4	7.24×10^7	3.86×10^8
Ganja yogurt	4.02×10^6	3.04×10^7	3.96×10^6	3.58×10^7
Baku yogurt	9.20×10^5	2.16×10^7	2.58×10^6	1.78×10^7

[a]Average values of three independent experiments.

Table 3. Distribution of lactic acid bacteria isolated from samples of two kinds of Azerbaijani dairy products.

Groups of LAB	Agdas cheese	Sheki cheese	Karabakh yogurt	Ganja yogurt	Baku yogurt
Number of cocci	50 (51.0%)	13 (15.7%)	41 (78.8%)	24 (72.7%)	20 (66.7%)
Number of lactobacilli	48 (49.0%)	70 (84.3%)	11 (21.2%)	9 (27.3%)	10 (33.3%)
Total number of tested isolates	98 (100%)	83 (100%)	52 (100%)	33 (100%)	30 (100%)

negative. From a total of 55 isolates from Ganja yogurt, 33 were Gram-positive and catalase-negative, whereas 30 out of 53 isolates from Baku yogurt showed the same features (Table 3).

Phenotypic characterization of LAB

Activity in milk: Activity in milk was tested for all 296 LAB isolated from Azerbaijani Agdas and Sheki cheeses as well as from Karabakh, Ganja and Baku yogurts. Results showed that 161 of 296 LAB isolates formed curd in skimmed milk after 16 h of incubation. Among them, 8 enterococci isolates (BGAZES1-30, BGAZES1-34, BGAZES1-39, BGAZES1-41, BGAZEJ3-28, BGAZEJ3-29, BGAZEJ3-31 and BGAZEJ3-38) and 3 lactobacilli isolates (BGAZES1-80, BGAZES1-82 and BGAZES1-85) as well as 3 streptococci isolates (BGAZEJ3-27, BGAZEJ3-30 and BGAZEJ3-43) exhibited very good acidification activity in milk and curd after 4.5 - 6 h of incubation (data not shown).

Phenotypic characteristics and carbohydrate fermentations

According to the analysis of proteolytic activity on β-casein, plasmid profiles and rep-PCR method, 46 LAB isolates were chosen for the analysis of some phenotypic characteristics (growth at 30 and 45°C growth in corresponding broth with 4 and 6.5% of salt concentration ability of L-arginine hydrolysis and production of CO_2 from glucose) and fermentation of already mentioned carbohydrates. The results of phenotypic characterization of LAB are shown in Table 4. The LAB identification based on this analysis showed that in samples of different kinds of Azerbaijani dairy products, 6 species of LAB were present. ***L. brevis***, ***L. plantarum***, ***L. paraplantarum*** and ***E. faecium*** species were present in Agdas and Sheki cheese samples while the species ***L. delbrueckii*** subsp. ***lactis***, ***S. thermophilus*** and ***E. faecium*** were identified in yogurt samples. 4 LAB isolates isolated from Ganja and Baku yogurt samples could not be identified according to phenotypic characteristics since they fermented tested carbohydrates poorly. Nevertheless, those 4 isolates (BGAZEJ3-7, BGAZEJ3-30, BGAZEJ3-31 and BGAZEJ3-38) showed a very good acidification activity in milk and after 4.5 h of incubation the pH reached a value of 4.8.

Proteolytic activity and plasmid profiles

Examination of proteolytic activity of all 296 LAB isolates revealed that 2 lactobacilli from Agdas cheese, 8 lactobacilli from Sheki cheese, 8 lactobacilli and 10 cocci from Karabakh yogurt, 9 lactobacilli from Ganja yogurt and 2 isolated lactobacilli from Baku yogurt exhibited a very good proteolytic activity as observed by β-casein hydrolysis by whole cells for 3 h of incubation. All strains within species ***L. delbrueckii*** (identified by 16S rDNA sequencing data given in Table 6) showed very good proteolytic activity and PCR with ***prtB*** specific primers confirmed the presence of ***prtB*** gene in all isolates (data not shown). A small number of lactobacilli (5.7%) and of cocci (7.7%) isolated from all cheese and yogurt samples showed a good proteolytic activity while the rest of lactobacilli and the large number of cocci degraded β-casein poorly or did not degrade it at all (data not shown).

Table 4. Some phenotype characteristics and carbohydrate patterns among LAB isolates from samples of different Azerbaijani dairy products.

Test	Group I 9 isolates	Group II 1 isolate	Group III 11 isolates	Group IV 5 isolates	Group V 1 isolate	Group VI 5 isolates	Group VII 1 isolate	Group VIII 1 isolate	Group IX 8 isolates	Group X 4 isolates
Growth at 45 °C	- (8)	+	- (10)	- (5)	-	+ (4)	+	-	+ (8)	+ (2); +/- (2)
Growth in 4% NaCl	+ (9)	+	+ (11)	+ (5)	+	- (5)	+	-	+ (8)	- (4)
Growth in 6.5% NaCl	+ (6)	-	+ (11)	+ (3)	+	- (5)	+	-	+ (8)	- (4)
Hydrolysis of L-arginine	+ (9)	+	- (11)	- (4)	-	+ (3); +/- (1)	-	±	+ (8)	- (2); + (2)
Production of CO_2 from glucose	+ (9)	+	- (10)	- (4); +/- (1)	-	- (5)	+	-	- (8)	- (4)
L-Arabinose	+ (9)	+	- (2); +/- (1)	- (5)	-	- (3); +/- (1)	-	-	+ (8)	- (4)
Ribose	+ (8)	+	+ (6); +/- (1)	- (3); +/- (1)	±	+ (2); +/- (1)	-	-	+ (8)	- (4)
D-Xylose	+ (9)	+	- (11)	- (5)	-	+ (2); +/- (1)	-	-	- (8)	- (4)
Galactose	+ (7)	+	+ (11)	+ (5)	+	+ (4)	±	-	+ (8)	- (4)
Glucose	+ (8); +/- (1)	+	+ (11)	+ (5)	+	+ (4); +/- (1)	+	+	+ (8)	+/- (4)
Fructose	+ (8); +/- (1)	+	+ (11)	+ (4)	+	+ (5)	+	-	+ (8)	- (4)
Mannose	+ (3)	+	+ (11)	+ (5)	+	+ (5)	+	-	+ (8)	- (4)
Sorbose	- (9)	-	- (11)	- (5)	-	- (5)	-	-	- (8)	- (4)
Mannitol	- (5); +/- (1)	-	+ (11)	+ (4); +/- (1)	+	+ (3)	-	-	- (6); +/- (2)	- (4)
Sorbitol	- (6)	-	+ (8)	- (3)	+	- (5)	-	-	- (8)	- (4)
Esculin	- (6)	+	+ (10)	+ (4)	+	- (3)	-	-	+ (8)	- (4)
Salicin	- (8)	-	- (8); +/- (1)	- (5)	-	- (3); +/- (1)	-	-	+/- (8)	- (4)
Cellobiose	- (6)	+	+ (11)	+ (4); +/- (1)	+	- (4); +/- (1)	-	-	+ (8)	- (4)
Maltose	+ (8); +/- (1)	+	+ (11)	+ (5)	+	+ (4)	-	-	+ (8)	- (4)
Lactose	+ (6); +/- (2)	+	+ (10)	+ (5)	+	+ (5)	+	+	+ (8)	+ (4)
Melibiose	+ (6); +/- (1)	+	+ (11)	- (5)	+	- (4)	-	-	+ (8)	- (4)
Sucrose	- (4); +/- (1)	-	+ (11)	+ (3); +/- (2)	+	+ (5)	+	+	+ (3)	- (4)
Trehalose	- (6)	+	+ (11)	+ (5)	+	+ (4)	-	-	+(2); +/- (6)	- (4)
Raffinose	- (7)	-	+ (11)	- (5)	+	- (2); +/- (3)	±	-	- (8)	- (4)

+: Positive reaction. -: Negative reaction. ±: Weak reaction. ( ): Number of isolates. All isolates grew at 30 °C. Most of the isolates were glycerol negative except two isolates (BGAZES1-93 and BGAZES2-36), which showed weak reaction. Most of the isolates were rhamnose negative except one isolate (BGAZEJ2-43), which showed weak reaction. Most of the isolates were inuline negative except one isolate (BGAZEJ2-89), which showed weak reaction. All isolates were starch negative. Group I: ***L. brevis*** (BGAZES1-51/1, BGAZES1-76, BGAZES1-57, BGAZES1-96, BGAZES1-58, BGAZES1-52, BGAZES1-75/2, BGAZEJ2-77, BGAZES1-92). Group II: ***L. fermentum*** (BGAZES1-93). Group III: ***L. plantarum*** (BGAZES2-3, BGAZEJ2-89, BGAZEJ3-32/1, BGAZES2-4, BGAZES2-7, BGAZES2-8, BGAZES2-87, BGAZES1-75, BGAZES2-83, BGAZES2-86, BGAZES2-67). Group IV: ***L. plantarum***/ ***L. paracasei*** (BGAZES1-89, BGAZES2-31, BGAZES2-63, BGAZES2-36, BGAZES1-83). Group V: ***L. paraplantarum*** (BGAZES2-95). Group VI: ***L. delbrueckii*** subsp. (BGAZEJ3-92, BGAZEJ1-49, BGAZEJ1-56, BGAZEJ1-62, BGAZEJ3-76). Group VII: ***L. delbrueckii*** (BGAZEJ2-96). Group VIII: ***S. thermophilus*** (BGAZEJ1-35). Group IX: ***E. faecium*** (BGAZEJ3-34, BGAZEJ2-2, BGAZEJ3-27, BGAZEJ3-29, BGAZEJ2-43, BGAZEJ3-43, BGAZEJ2-41, BGAZEJ2-3). Group X: Unidentified isolates of LAB (BGAZEJ3-7, BGAZEJ3-30, BGAZEJ3-31, BGAZEJ3-38).

According to their proteolytic activity, 127 LAB isolates were chosen for the analysis of their plasmid profiles. Plasmid bands in cocci isolates varied from 0 - 2 while the number of plasmid bands in lactobacilli isolates varied between 1 and 9 (data not shown).

Antimicrobial activity of LAB isolates

It was confirmed that only 5 out of 296 analyzed

Table 5. Antimicrobial activity of lactic acid bacteria from samples of Agdas cheese and Karabakh yogurt.

Indicator strain	Isolates				
	BGAZES1-5	**BGAZES1-12**	**BGAZES1-38**	**BGAZES1-44**	**BGAZEJ1-48**
BGMN1-5	-	Clear zone (2 mm)	Clear zone (2 mm)	Clear zone (2 mm)	Clear zone (1 mm)
BGMN1-596	-	Clear zone (2 mm)	Clear zone (2 mm)	Clear zone (2 mm)	Clear zone (3 mm)
S50	Clear zone (2 mm)	-	-	-	-
NS1	Clear zone (1.5 mm)	-	-	-	-
BGBUK2-8	Clear zone (1.5 mm)	-	-	-	-
BGBUK2-16	-	-	-	-	Turbid zone (3 mm)
A112	-	-	-	-	Clear zone (3 mm)

Three more indicator strains, ***L. paracasei*** subsp. ***paracasei*** BGBUK2-16/K4, BGLI15 and BGKP20 (Table 1), were used in the test, but none of BLIS producers showed antimicrobial activity towards these indicator strains.

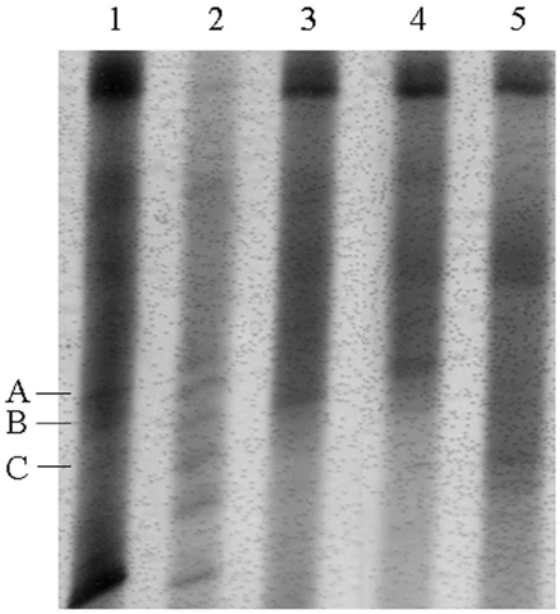

Figure 1. DGGE patterns of PCR-amplified 16S rDNA segments derived from total DNA of Agdas (lane 1) and Sheki (lane 2) cheeses, and from reference strains: lane 3, ***L. plantarum*** A112; lane 4, ***L. paraplantarum*** BGZLS60-58; lane 5, ***L. brevis*** BGHI3a. The positions of bands discussed in the text are indicated by letters that correspond to species of bacteria: A, ***L. paraplantarum***, B, ***L. plantarum*** and C, ***L. brevis***.

LAB isolates showed antimicrobial activity. Experiments with protease revealed a proteinaceous nature of antimicrobial compounds indicating the possibility they could be bacteriocin-like substances (BLIS).

Four of BLIS producers were isolated from Agdas cheese and one was isolated from Karabakh yogurt. All BLIS producers belonged to species ***E. faecium*** as identified by rep-PCR and 16S rDNA sequencing. Tested isolates exhibited clear or turbid zones of inhibition on indicator strains including those producing bacteriocins such as BGMN1-5, S50 or BGBUK2-16. Among these BLIS producers the widest spectrum of antimicrobial activity appeared to be displayed by isolate BGAZEJ1-48 that gave zone of inhibition on 4 out of 10 indicator strains used in the test (Table 5).

Molecular identification of LAB isolates

PCR-DGGE identification of LAB isolates: To investigate the diversity of the dominating microbial community in Sheki and Agdas cheeses, cheese samples were analyzed by PCR-DGGE. DGGE pattern indicated the presence of three lactobacilli species in the Sheki and in the Agdas cheeses (Figure 1). The intense bands from DGGE profiles of the strains belonging to ***L. paraplantarum*** (band A), ***L. plantarum*** (band B), and ***L. brevis*** (band C) corresponded precisely to the bands in the DGGE pattern of the Agdas and the Sheki cheeses.

rep-PCR analysis

According to results of proteolytic activity and different plasmid profiles, 51 different rods-like LAB and cocci-like LAB were chosen for molecular identification by rep-PCR (Figures 2 and 3). The results revealed that 13 of 33 chosen lactobacilli were identified as ***L. plantarum***, 4 as ***L. paraplantarum***, 8 as ***L. brevis*** and 1 isolate of lactobacilli were identified as ***L. paracasei*** subsp. ***paracasei.*** 10 isolates of lactobacilli were not identified by this method (BGAZES2-87, BGAZEJ1-49, BGAZEJ1-56, BGAZEJ1-62, BGAZEJ2-89, BGAZEJ2-96, BGAZEJ3-32, BGAZEJ3-32/2, 2BGAZEJ3-76 and BGAZEJ3-92). Based on the rep-PCR, 18 cocci-like LAB were identified as follows: 12 belonged to the species ***E. faecium,*** 1 isolates as ***E. durans***, 3 were identified as ***S. thermophilus*** (Table 6); 2 isolates of cocci-like LAB were not identified by the rep-PCR method (BGAZEJ3-36 and BGAZEJ3-48).

16S rDNA sequence analysis

Since discrepancies in band patterns between analyzed

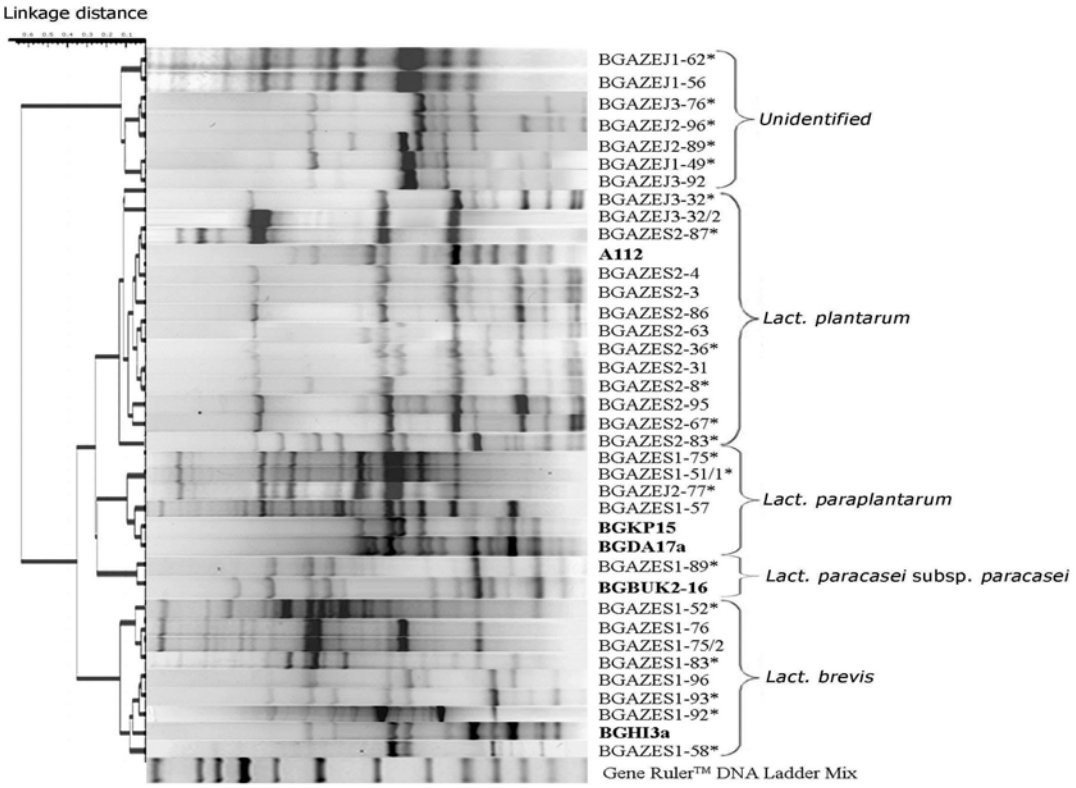

Figure 2. Dendrogram based on statistical analysis of the BOXA1R - PCR fingerprints of lactobacilli isolated from different kinds of Azerbaijani dairy products. Reference strains used in the test are given in bold letters. ***** Isolates which are also identified by 16S rDNA sequencing.

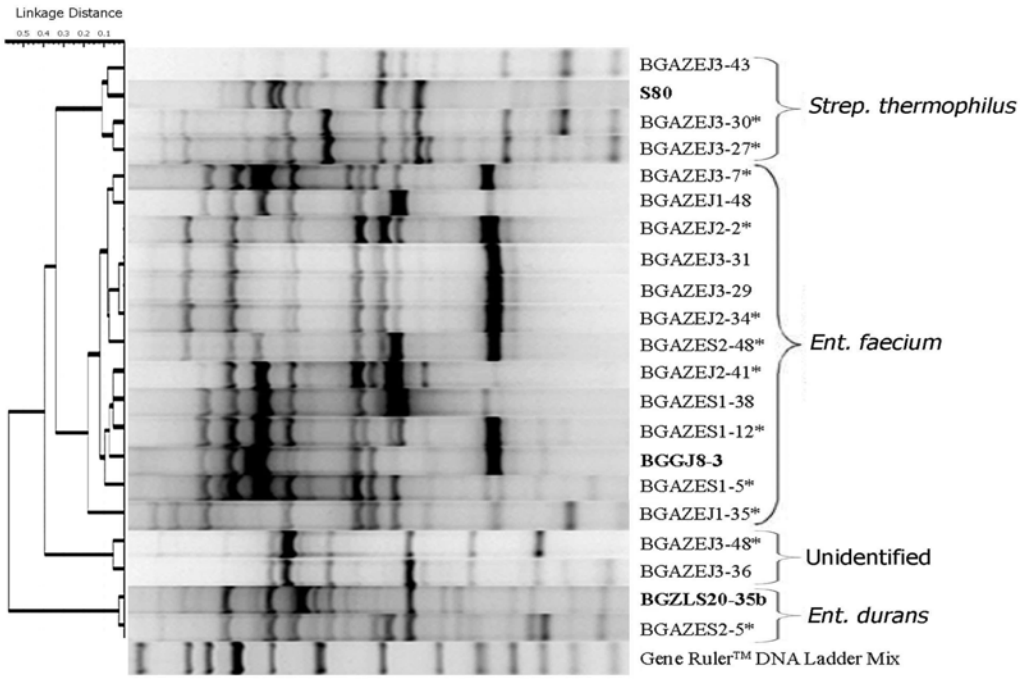

Figure 3. Dendrogram based on statistical analysis of the $(GTG)_5$ - PCR fingerprints of cocci-like isolated from different kinds of Azerbaijani dairy products. Reference strains used in the test are given in bold letters. ***** Isolates which are also identified by 16S rDNA sequencing.

isolates by rep-PCR and references strains were obtained, 36 isolates were subjected to 16S rDNA gene sequencing. The results of sequencing showed that 8 of 21 chosen lactobacilli were identified as ***L. plantarum***, 3 as ***L. plantarum/ L. paraplantarum/ L. pentosus***, 2 as ***L. rhamnosus/ L. paracasei/ L. casei***, 3 as ***L. brevis*** and 5 isolates of lactobacilli belonged to the species ***L. delbrueckii.*** Based on the same method, 15 cocci-like LAB were identified as follows; 9 belonged to the species ***E. faecium,*** 1 isolate was identified as ***S. thermophilus/S. salivarius,*** 3 isolates were identified as ***S. thermophilus*** and 2 isolates of cocci-like LAB (BGAZES2-5 and BGAZEJ3-48) were identified as ***E. durans/ E. faecium/ E. carnosus*** and ***E. durans/ E. avium/ E. carnosus***, respectively. The data demonstrated high identity (93 – 100%) with 16S rDNA sequences of relevant species in GenBank database (NCBI) confirming the rep-PCR results (Table 6).

DISCUSSION

It is known that in the process of cheese production, the ripening is probably the most significant period during which starter and non-starter bacteria, chymosin and the indigenous milk enzymes develop the organoleptic and textural properties of the cheese (Brennan et al., 2002). In order to isolate and to analyze NSLAB from Azerbaijani traditionally manufactured dairy products, Agdas and Sheki cheeses as well as Karabakh, Ganja and Baku yogurts were used since these products were prepared without addition of any known starter culture.

Before the isolation of LAB, its populations in Agdas and Sheki cheeses were determined, being between 10^7 and 10^8 cfu/g. These values correspond to those usually found in cheeses during ripening (Williams and Banks, 1997; Fox et al., 1998; Fitzsimons et al., 2001). However, the total number of bacteria in three yogurt samples has broader span ranging between 10^4 and 10^8 cfu/ml, regarding the composition of bacterial population in Azerbaijan dairy products. It was ascertained that lactobacilli and enterococci were present in cheeses while in yogurts species ***S. thermophilus*** was also present.

The analysis of the proteolytic activity revealed that relatively small number (about 20%) of lactobacilli and cocci isolated from all 5 samples of Azerbaijan dairy products, showed very good activity in β-casein hydrolysis. Nevertheless, numerous enterococci identified as ***E. fae-cium*** present in these products showed very low or no proteolytic activity. This result is in accordance with recent investigations showing that ***E. faecalis*** strains showed generally better performance in comparison to ***E. faecium*** and ***E. durans*** in respect to some biochemical properties such as the acidifying ability and proteolytic activity (Suzzi et al., 2000; Sarantinopoulos et al., 2001).

The PCR-DGGE analysis of mixed bacterial populations is widely used in environmental microbiology (Muyzer, 1999). This analysis revealed that in the Agdas and in the Sheki cheese ***L. plantarum*** is more present than the other species from this genus. Comparing the previous results and the data from literature, we determined that this species is dominant in cheeses originated from the region of Egypt (Ayad et al., 2004), Marocco (Ouadghiri et al., 2005), Caucasus (Terzic-Vidojevic, A., unpublished data) and Tibet (Duan et al., 2008) while in the cheeses originate from Northern Europe (Fitzsimons et al., 2001; Østlie et al., 2004), Spain (Arizcun et al., 1997; Pérez-Elortondo et al., 1998; Ortigosa et al., 2006), Italy (Marino et al., 2003) and the Balkans (Terzic-Vidojevic et al., 2007; Nikolic et al., 2008) the most present species is ***L. paracasei.***

The presence of ***L. brevis*** and ***L. paraplantarum*** species in the Agdas and in the Sheki cheese was also confirmed by PCR-DGGE analysis. Based on phenotypic characteristics and fermentation of carbohy-drates, the same LAB isolated from these cheeses was identified as ***L. brevis, L. plantarum*** and ***E. faecium.***

Phenotypic characterization and carbohydrates fermentation of LAB isolated from all three yogurts (Karabakh, Ganja and Baku) revealed that lactobacilli belonged to the species ***L. delbrueckii*** subsp. ***lactis*** and cocci-like LAB were represented by two species ***S. thermophilus*** and ***E. faecium.***

Molecular identification methods had great significance where high heterogeneity between isolated stains was present. Using the rep-PCR method it was established that in both cheeses species ***L. brevis L. plantarum*** and ***E. faecium*** were present and in yogurts ***S. thermophilus*** and ***E. faecium*** were also present.

A certain number of LAB isolates (mostly lactobacilli isolated from yogurt samples) was not identified by this method. Besides that, some cheese and yogurt cocci-like LAB isolates, identified as ***S. thermophilus*** based on phenotypic characterization and the fermentation of carbohydrates was identified as ***E. faecium*** by the rep-PCR method and vice versa. It has been reported that natural lactococci can show atypical phenotypic characteristics (for example, growth in presence of 6.5% NaCl) and commonly survive in hostile condition (Corroler et al., 1998). Additionally, the phenotypic characterization highlighted a high degree of variability in ***S. thermophilus*** despite the analyzed strains being principally isolated from yogurt samples. As recently reported, some strains were able to ferment galactose, suggesting that this phenotypic trait should be considered as variable in the taxonomic description of the species ***S. thermophilus*** (Giraffa et al., 2001; Mora et al., 2002). The difficulties in the identification of some isolates by rep-PCR method occurred because of the interspecies differences of band patterns which are most probably the result of genetic variability within the species. Because of the mismatch of the LAB determination results, large number of isolates (36 of them) was identified by 16S rDNA sequencing. In that occasion, it was confirmed that in the Agdas cheese, the most prevalent species were ***L. plantarum. brevis***, ***L. paraplan-***

Table 6. Isolates of LAB identified by rep-PCR and 16S rDNA sequencing.

Isolates	Identification by rep-PCR	Identification by 16S rDNA (% identity)
BGAZES1-5	***E. faecium***	***E. faecium*** (100%)
BGAZES1-12	***E. faecium***	***E. faecium*** (99%)
BGAZES1-38	***E. faecium***	NA
BGAZES1-44	NA	***E. faecium*** (100%)
BGAZES1-51/1	***L. paraplantarum***	***L. plantarum/ L. paraplantarum/ L. pentosus*** (99%)
BGAZES1-52	***L. brevis***	***L. rhamnosus/ L. paracasei*** (100%)
BGAZES1-57	***L. paraplantarum***	NA
BGAZES1-58	***L. brevis***	***L. brevis*** (100%)
BGAZES1-75	***L. paraplantarum***	***L. plantarum/ L. paraplantarum/ L. pentosus*** (99%)
BGAZES1-75/2	***L. brevis***	NA
BGAZES1-76	***L. brevis***	NA
BGAZES1-83	***L. brevis***	***L. rhamnosus/ L. paracasei/ L. casei*** (95%)
BGAZES1-89	***L. paracasei*** subsp. ***paracasei***	***L. plantarum/ L. paraplantarum/ L. pentosus*** (99%)
BGAZES1-92	***L. brevis***	***L. brevis*** (98%)
BGAZES1-93	***L. brevis***	***L. brevis*** (96%)
BGAZES1-96	***L. brevis***	NA
BGAZES2-3	***L. plantarum***	NA
BGAZES2-4	***L. plantarum***	NA
BGAZES2-5	***E. durans***	***E. durans/ E. faecium/ E. carnosus*** (99%)
BGAZES2-7	NA	***L. plantarum*** (100%)
BGAZES2-8	***L. plantarum***	***L. plantarum*** (100%)
BGAZES2-31	***L. plantarum***	NA
BGAZES2-36	***L. plantarum***	***L. plantarum*** (100%)
BGAZES2-48	***E. faecium***	***E. faecium*** (99%)
BGAZES2-63	***L. plantarum***	NA
BGAZES2-67	***L. plantarum***	***L. plantarum*** (100%)
BGAZES2-83	***L. plantarum***	***L. plantarum*** (100%)
BGAZES2-86	***L. plantarum***	NA
BGAZES2-87	Unidentified	***L. plantarum*** (100%)
BGAZES2-95	***L. plantarum***	NA
BGAZEJ1-35	***E. Faecium***	***E. faecium*** (100%)
BGAZEJ1-48	***E. Faecium***	NA
BGAZEJ1-49	Unidentified	***L. delbrueckii*** subsp. ***lactis*** (100%)
BGAZEJ1-56	Unidentified	NA
BGAZEJ1-62	Unidentified	***L. delbrueckii*** subsp. ***lactis***/subsp. ***bulgaricus***/ subsp. ***delbrueckii*** (98%)
BGAZEJ2-2	***E. Faecium***	***S. thermophillus*** (100%)
BGAZEJ2-34	***E. Faecium***	***E. faecium*** (99%)
BGAZEJ2-41	***E. Faecium***	***E. faecium*** (100%)
BGAZEJ2-43	NA	***E. faecium*** (100%)
BGAZEJ2-48	NA	***E. faecium*** (99%)
BGAZEJ2-77	***L. paraplantarum***	***L. plantarum*** (99%)
BGAZEJ2-89	Unidentified	***L. delbrueckii*** subsp. ***bulgaricus*** (100%)
BGAZEJ2-96	Unidentified	***L. delbrueckii*** subsp. ***bulgaricus*** (99%)
BGAZEJ3-7	***E. Faecium***	***S. thermophilus*** (93%)
BGAZEJ3-27	***S. thermophilus***	***S. thermophilus/ S. salivarius*** (99%)
BGAZEJ3-29	***E. Faecium***	NA
BGAZEJ3-30	***S. thermophilus***	***S. thermophilus*** (99%)
BGAZEJ3-31	***E. Faecium***	NA
BGAZEJ3-32	Unidentified	***L. plantarum*** (100%)
BGAZEJ3-32/2	Unidentified	NA
BGAZEJ3-36	Unidentified	NA

Table 6. Contd.

BGAZEJ3-43	***S. thermophilus***	NA
BGAZEJ3-48	Unidentified	***E. durans/ E. avium/ E. carnosus*** (99%)
BGAZEJ3-76	Unidentified	***L. delbrueckii*** subsp***. bulgaricus/*** subsp***. lactis/*** subsp***. delbrueckii*** (99%)
BGAZEJ3-92	Unidentified	NA

NA: Not applicable.
The rep-PCR pattern of isolate BGAZEJ1-56 was the same as pattern of isolate BGAZEJ1-62, like as rep-PCR patterns of isolates BGAZEJ3-92 and BGAZEJ1-49.

tarum and ***E. faecium***, while the Sheki cheese primarily contained species ***L. plantarum*** and ***E. faecium.*** In cheeses, the species ***L. plantarum*** gains entrance through post-pasteurization contamination but may also constitute part of the raw milk flora (Chapman and Sharpe, 1981; Turner et al., 1986). In the samples of all three yogurt types, (Karabakh, Ganja and Baku) the species ***L. delbrueckii*** subsp. ***lactis***, ***L. delbrueckii*** subsp. ***bulgaricus***, ***S. thermophilus*** and ***E. faecium*** were identified by 16S rDNA sequencing.

Enterococci are recognized as an essential part of the natural microflora of many dairy products and in some cheeses, they dominate over lactobacilli and lactococci (Poullet et al., 1993; Centeno et al., 1995; Terzic-Vidojevic et al., 2007; Nikolic et al., 2008). It has been reported that enterococci are one of the most resistant microbiological groups to adverse conditions such as salt and acidity which explains their predominance in some cheeses (Cogan et al., 1997). The largest number of enterococci (100 isolates in total) was isolated from Agdas and Sheki cheeses.

Although the genus ***Enterococcus*** comprises the most controversial group of LAB, the contribution of enterococci to the organoleptic properties of fermented food-stuffs (cheeses, sausages, vegetables and olives) and also their ability to produce enterocins are important cha-racteristics for their application in food technology (Giraffa, 2002, 2003; Foulquié Moreno et al., 2006). Strains of enterococci including ***E. faecium*** and ***E. faecalis*** are known to produce bactericidal peptides which are called enterocins that generally belong to class II bacteriocins (Franz et al., 1999).

Overall, 5 enterococci isolated from Agdas cheese and Karabakh yogurt, identified as ***E. faecium,*** showed anti-microbial activity and created inhibition zones on 2, 3 or even 4 different indicator strains. In the present study, both phenotypic and genotypic approaches were used to identify LAB in two kinds of Azerbaijani dairy products. The results of the present research indicate that lactobacilli and enterococci have a critical role in the manufacturing and ripening of Agdas and Sheki cheeses. In particular, ***L. plantarum*** and ***L. brevis*** were found as well as the species ***E. faecium***. In addition, ***L. paraplantarum*** was found in Agdas but not in Sheki cheeses. Yogurts Karabakh, Ganja and Baku were rich with species ***L. delbrueckii*** subsp. ***lactis***, ***L. delbrueckii*** subsp. ***bulgaricus*** and ***S. thermophilus***. The species ***E. faecium*** was also present in yogurts but in a smaller number than in cheeses.

Results also demonstrated that Azerbaijani dairy products may be used as a source of strains that could constitute a starter culture for the industrial cheese and yogurt production. Namely according to phenotypic and molecular characterization results, 55 isolates were chosen for further studies (data shown in Table 6) where the attention will be focused on detailed examinations of technological characteristics as well as the production of diacetyl and acetoin, citrate utilization, growth in the conditions of different salt concentrations, exopolysac-charide forming e.t.c.

Consequently, the strains having most desirable tech-nological properties will be tested as starter cultures for the production of mentioned dairy products with typical organoleptic characteristics of artisanal products. The future productions of dairy products will be organized first on small scale and than in industrial conditions. Since daily consumption of those products in human alimen-tation is significant and consumers also request the improvements in output of food production as well as in its quality, standardization of these dairy products will enable their future industrial production. This is particu-larly important for the production of cheeses so popular in Azerbaijan and its neighborhood.

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