Selection of enhanced antimicrobial activity posing lactic acid bacteria characterised by (GTG)₅-PCR fingerprinting

Joana Šalomskienė · Asta Abraitienė ·
Dovilė Jonkuviienė · Irena Mačionienė ·
Jūratė Repečkienė

Revised: 1 August 2014 / Accepted: 5 August 2014 / Published online: 20 August 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract The aim of the study was a detail evaluation of genetic diversity among the lactic acid bacteria (LAB) strains having an advantage of a starter culture in order to select genotypically diverse strains with enhanced antimicrobial effect on some harmful and pathogenic microorganisms. Antimicrobial activity of LAB was performed by the agar well diffusion method and was examined against the reference strains and foodborne isolates of *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhimurium*. Antifungal activity was tested against the foodborne isolates of *Candida parapsilosis*, *Debaromyces hansenii*, *Kluyveromyces marxianus*, *Pichia guilliermondii*, *Yarrowia lipolytica*, *Aspergillus brasiliensis*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Scopulariopsis brevicaulis*.

A total 40 LAB strains representing *Lactobacillus* (23 strains), *Lactococcus* (13 strains) and *Streptococcus* spp. (4 strains) were characterised by repetitive sequence based polymerase chain reaction fingerprinting which generated highly discriminatory profiles, confirmed the identity and revealed high genotypic heterogeneity among the strains. Many of tested LAB demonstrated strong antimicrobial activity specialised against one or few indicator strains. Twelve LAB strains were superior in suppressing growth of the whole complex of pathogenic bacteria and fungi. These results demonstrated that separate taxonomic units offered different

possibilities of selection for novel LAB strains could be used as starter cultures enhancing food preservation.

Keywords Lactic acid bacteria · (GTG)₅-PCR fingerprinting · Indicator bacteria and fungi · Antimicrobial activity

Introduction

Lactic acid bacteria (LAB) are represented by the huge number of widely distributed bacterial species. They were detected on plants, in different soils, animal and human guts. Such variety of ecological niches is favored by their ability to outcompete the other microorganisms. Antibacterial or antifungal activity of LAB had been intensively described in many studies. Several of them examined an antagonistic effect of LAB against both bacterial and fungal indicator species.

Cakir (2010) tested antibacterial activity against different bacteria species (*Kocuria rhizophila*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella enteritidis*) and 20 yeast strains. After isolation of 21 wild LAB strain from naturally fermented herbs, 18 of them showed antibacterial activity against the tested bacteria and none of them had an antifungal activity against yeast cultures. Smaoui et al. (2010) characterized nine strains from the collection of 54 LAB isolates (isolated from meat, fermented vegetables and dairy products) by the broad-spectrum inhibition against *Lactobacillus casei*, *Listeria ivanovii*, *Salmonella enterica*, *Fusarium* spp. and *Candida tropicalis*. Strains of *Enterococcus* and *Weissella* spp. tested by Fhoula et al. (2013) demonstrated strong activity against *Stenotrophomonas maltophilia*, *Pantoea agglomerans*, *Pseudomonas savastanoi*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Penicillium expansum*, *Verticillium dahliae* and *Aspergillus brasiliensis*. Yang et al. (2012) studied 138 LAB isolates and identified 28 strains belonging to

J. Šalomskienė · A. Abraitienė · D. Jonkuviienė (✉) · I. Mačionienė
Kaunas University of Technology, Food institute, Taikos av.
92LT 50118 Kaunas, Lithuania
e-mail: dovile.jonkuvienne@yahoo.com

A. Abraitienė
Vilnius University, Institute of Biotechnology, Vilnius, Lithuania

J. Repečkienė
Nature Research Center, Vilnius, Lithuania

Enterococcus faecium, *Streptococcus thermophilus*, *Lactobacillus casei* and *Lactobacillus sakei* subsp. *sakei*, that produced bacteriocins. Out of selected 28 strains, eight were tested for the substances against *Listeria innocua*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Erwinia carotovora*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Penicillium expansum*, *Botrytis cinerea* and *Monilinia frusicola*. Nine LAB strains out of 94 studied expressed a broad spectrum of activity against gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella gallinarum*), moulds (*Aspergillus*, *Fusarium*, *Penicillium* genera) and yeasts (*Rhodotorula*, *Candida* spp.) (Stoyanova et al. 2010). That study also showed that selected lactococci could prevent contamination of raw smoked sausages by the foodborne pathogens. Thus, the above mentioned studies illustrate that only a limited number of LAB strains possessing enhanced antibacterial activity inhibited the growth of fungi as well. However, the detailed genetic background of LAB strains had not been examined in the above mentioned studies.

The genetic analysis had been done in the other studies. REP-1R-D and REP-2R-D primers were used to assess genetic diversity, combined with the examining of LAB antimicrobial activity (Valerio et al. 2009). These primers were not very informative, therefore, we applied the polytrinucleotide (GTG)₅ as a primer because (GTG)₅ had been previously reported as a suitable tool for rapid and reliable specification of LAB (Gevers et al. 2001).

Our study was aimed at finding out the impact of genetic diversity on antibacterial and antifungal activity of LAB strains that possess technological advantages in use as a starter culture. The comprehensive knowledge on the impact of LAB genetic background on their antagonistic effect could enhance the degree of exploiting novel strains in biopreservation. Because of the practical significance, we have tested activity of LAB against the harmful microorganisms, present in various food products. For LAB used as starter cultures, it is

important to confront many species of the undesirable microorganisms for obtaining highly qualified and controlled fermentation process. This would ensure the proper taste and extend the shelf-life of the products.

Materials and methods

Lactic acid bacterial strains and culture conditions

A group of 40 phenotypically characterized strains from the Food Institute's (Kaunas University of Technology) collection were selected for this study because of their advantages in using as starter cultures. All LAB strains were stored at -72°C in „Microbank“ system (*Pro-Lab Diagnostic*, UK). Selected strains were revived for 24 h in the sterilised milk under the temperature optimal for studied species (Table 1).

(GTG)₅-PCR fingerprinting

Total genomic DNA was extracted from LAB cell culture (CC) and *Bacillus cereus* cultures after incubating them for 24 h in the brain-heart infusion broth (*BHI*, *Oxoid*, UK) under the optimal for each species temperature. DNA was extracted from 1 mL of the broth centrifuged at 900 rpm for 15 min following the automated sample processing with QIAcube instrument using a QIAamp stool DNA mini kit (*Qiagen*, Germany) according to manufacturer's recommendations. DNA samples were quantified using a Thermo Scientific NanoDrop 2000 spectrophotometer and stored at -20°C until further analyses.

The repetitive sequence based polymerase chain reaction (rep-PCR) fingerprinting was accomplished using (GTG)₅ primer (5'-GTG GTG GTG GTG GTG-3'). The reaction mixture consisted of 8 μL of PCR grade water, 2.5 μL of 10 $\times$ reaction buffer without MgCl_2 , 5 μL of MgCl_2 (25 mM), 0.5 μL of dimethyl sulfoxide (*DMSO*; *Sigma-Aldrich*, USA), 1 μL of dNTP mix (10 mM each), 2 μL of (GTG)₅ primer (10

Table 1 Number of the representative strains and the optimal growth temperature of LAB species used in this study

Species	Number of strains	Optimal growth temperature, $^{\circ}\text{C}$
<i>Lactobacillus plantarum</i>	1	30
<i>Lactobacillus acidophilus</i>	7	37
<i>Lactobacillus helveticus</i>	4	37
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	5	37
<i>Lactobacillus reuteri</i>	2	30
<i>Lactobacillus casei</i>	3	37
<i>Lactobacillus brevis</i>	1	30
<i>Lactococcus lactis</i>	13	30
<i>Streptococcus thermophilus</i>	4	37
Total	40	

pmol μ L), 1 μ L of 5 U/ μ L recombinant Taq DNA polymerase (*Thermo Fisher Scientific Baltic*) and 5 μ L of bacterial DNA adjusted to 10 ng/ μ L.

The reaction conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min. Final extension step was made at 72 °C for 10 min. PCR amplifications were performed using an Eppendorf Mastercycler 5330 Plus PCR thermal cycler (*Eppendorf, Germany*). PCR reactions, prepared with *Lactobacillus helveticus* 305 and *Lactobacillus plantarum* 1 strains, were twice repeated in order to check the repeatability of rep-PCR fingerprints. The obtained PCR products were twice loaded on two different gels. The differences in band intensity only were noticed.

The amplified products were analyzed by agarose gel electrophoresis (1.5 %) in 1×TBE at 10 V/cm. For better resolution of the small rep-PCR products, they were additionally loaded on the 5 % polyacrilamide gel. DNA markers GeneRuler 100bp DNA Ladder Plus and pUC Mix 8 (both *Thermo Fisher Scientific Baltic*) were run along with the samples on the gel during electrophoresis. After electrophoresis, PCR products were stained with ethidium bromide (10mg/ml) and scanned using FLA-5000 laser-based imaging system. Bands obtained by rep-PCR were scored manually. The results exposed by both agarose and polyacrilamide gels were analyzed. Resulted banding patterns of the gels were subjected to the numerical analysis by cluster analysis performed with an on-line software <http://genomes.urv.cat/UPGMA/> (Garcia-Vallve and Palau 1999). The dendrogram was generated by unweighted pair group method with arithmetic average (UPGMA) based on Jaccard's genetic coefficient.

Sampling, isolation and identification of bacterial and fungal isolates

The foodborne isolates and reference strains of each bacterial species were used in screening. The reference strains used were: *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19111, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella Typhimurium* ATCC 13076. Antifungal activities against the foodborne yeasts (*Candida parapsilosis*, *Debaromyces hansenii*, *Kluyveromyces marxianus*, *Pichia guilliermondii*, *Yarrowia lipolytica*), reference strain *Candida albicans* ATCC 10231, the foodborne micromycetes (*Aspergillus brasiliensis*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*) and reference strain *Aspergillus brasiliensis* ATCC 16404 were tested in this experiment. Reference strains were obtained from the collection of microorganisms of KTU Food institute while sources of the all foodborne isolates are listed in Table 2.

For isolation of the foodborne bacteria, 10 g of food sample was homogenized in 90 mL of physiological solution. Suitable serial dilutions were plated onto following media: *L. monocytogenes* onto Agar Listeria Ottaviani Agosti (ALOA, Biolife, Italy), *E. coli* onto Tryptone Bile X-Glucuronide Agar (TBX Agar, Liofilchem, Italy), *B. cereus* onto Mannitol Egg Yolk Polymyxin Agar (MYP Agar, Liofilchem, Italy), and *S. aureus* onto Baird-Parker agar (Liofilchem, Italy). For isolation of *Salmonella* spp., 25 g of food sample were added into buffered peptone water (Liofilchem, Italy), reinoculated to Rappaport Vassiliadis soya peptone broth (Liofilchem, Italy) and Muller-Kauffmann tetrathionate-novobiocin broth (Merck, Germany). Then the samples were plated out on selective Xylose Lysine Deoxycholate Agar (XLD Agar, Oxoid, UK) and Nutrient Agar (Liofilchem, Italy). The plates were incubated aerobically under the optimal temperature (Table 2). Identification of the several randomly picked up bacterial strains was carried out according to the standard methods (ISO 16649–2:2001 for *E. coli*, ISO 11290–1:1996, EN ISO 11290–1:1996/AM1:2004 for *L. monocytogenes*, ISO 6579:2002, ISO 6579:2002/Cor.1:2004 for *Salmonella* spp., ISO 6888–1:1999+Amd:2003 for *S. aureus* and ISO 7932:2004 for presumptive *B. cereus*).

Foodborne isolates of *B. cereus* were characterized by pyrosequencing. PCR reaction and pyrosequencing of the obtained PCR amplicons were performed with 3B Blacklight sepsis bacterial kit (3B Blackbio Biotech Ltd., India) according to the manufacturer's recommendations. PCR conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 20 s, 54 °C for 20 s, 72 °C for 30 s. Final extension step was made at 72 °C for 5 min. PCR products were stored at –20 °C until further analyses. Pyrosequencing was performed on the PyroMark Q24 automatic pyrosequencing instrument (Qiagen, Germany) using Gold24 (Qiagen, Germany) kit. Nucleotide sequence analysis was performed using the PyroMark Q24 software and Basic Local Alignment Search Tool from the National Center for Biotechnology Information (NCBI BLAST; <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). Enterotoxins, produced by *B. cereus*, were detected using the BCET-RPLA (Oxoid, UK) commercial immunoassay kit. *B. cereus* isolates producing enterotoxins were used for further testing.

Yeasts and micromycetes were isolated from food raw material according to the previously described methods (Kurtzman et al. 2011; Samson et al. 2000). Yeasts were identified following Kurtzman et al. (2011) and Barnett et al. (2000). Micromycetes were identified conventionally according to their macroscopic and microscopic features. Manuals of Domsch et al. (1980), Watanabe (2002) and Pitt (2007) were used. All isolates of bacteria, yeast and micromycetes were maintained on Plate Count Agar (PCA, Liofilchem, Italy) slants at 4 °C as pure cultures.

Table 2 Cultivation conditions and isolation sources of the foodborne microorganisms used in the current study

Species	Number of strains	Cultivation medium	Optimal growth temperature, °C	Isolation source
Bacteria				
<i>Bacillus cereus</i>	10	PCA	30	Skim milk powder, ready-to-eat foods
<i>Listeria monocytogenes</i>	10	PCA	37	Smoked fish products
<i>Salmonella typhimurium</i>	10	PCA	37	Fresh meat
<i>Staphylococcus aureus</i>	2	PCA	37	Milk, herring
<i>Escherichia coli</i>	10	PCA	37	Fast frozen meat stuffed cabbage, mashed potato dumplings, ground meat, cultured milk
Yeasts				
<i>Candida parapsilosis</i>	1	SDA	25	Sweet cream cheese
<i>Debaromyces hansenii</i>	1	SDA	25	Yoghurt
<i>Kluyveromyces marxianus</i>	1	SDA	25	Cottage cheese
<i>Pichia guilliermondii</i>	1	SDA	25	Sweet cream cheese
<i>Yarrowia lipolytica</i>	1	SDA	25	Cheese
Micromycetes				
<i>Aspergillus brasiliensis</i>	1	MEA	25	Barley grains
<i>Aspergillus versicolor</i>	1	MEA	25	Dried thyme
<i>Cladosporium herbarum</i>	1	MEA	25	Cheese
<i>Penicillium chrysogenum</i>	1	MEA	25	Cheese
<i>Scopulariopsis brevicaulis</i>	1	MEA	25	Peas

PCA Plate count agar, SDA Sabouraud dextrose agar, MEA Malt extract agar

Agar well diffusion assay

For determining antibacterial activity, 1 mL of LAB (in sterile milk) was added to 10 mL of MRS broth (Oxoid, UK) and incubated for 48 h under the optimal temperature (Table 1). Cell-free supernatants (CFS) were obtained, removing cells by centrifugation at 6000 rpm for 15 min. Then supernatant was filtered through 0.2 µm pore filter. The part of the CFS was used for the antimicrobial/antifungal assays, the other part was neutralized with NaOH to pH 6.5. The detected antimicrobial activity of cell-free neutralisate (CFN) was tested also after adding catalase (Sigma-Aldrich, USA) at concentration 20 mg/mL to CFN and incubating at 25 °C for 30 min.

The foodborne and reference bacterial strains were cultivated on PCA slants for 18 h under the optimal temperature (Table 2). Then each bacterial culture was washed with sterile physiological solution and a density of the cell suspension was adjusted according to McFarland Standard No. 0.5 (corresponding to 1.5×10^8 cfu/mL). One millilitre of bacterial cell suspension was added to 100 mL of the melted PCA cooled to 45 °C. Then 10 mL of the mixture was poured into a Petri dish. Isolated yeast and micromycete cultures were cultivated for 48 h at 25 °C on Sabouraud Dextrose Agar (SDA, Liofilchem, Italy) or Malt Extract Agar (MEA, Liofilchem, Italy) slants. Inoculums of 1.0×10^6 cfu/mL were prepared. One millilitre of the tested culture was added to 100 mL of the

melted SDA or MEA cooled to 45 °C. Then 10 mL of the mixture was poured into a Petri dish. Fifty microlitres of LAB cell culture (CC), CFS or CFN were placed into wells of 7.5 mm diameter made in the solidified agar. An antibacterial activity was assessed following 24 h of incubation at 30 °C or 37 °C. An antifungal activity was determined following 48 h of incubation at 25 °C. It was quantified by measuring in millimetre diameter around the wells formed by a zone of inhibition (ZOI). All experiments were performed in triplicate and the results displayed as the mean values.

Results and discussion

PCR fingerprinting

Genotypic analysis by (GTG)₅-PCR fingerprinting generated a total of yielding 93 bands with size ranging from 290 to 4200 bp. Two strains (*Lb. acidophilus* L 59–30 and *Lb. delbrueckii* subsp. *bulgaricus* 14), the ones among exposing the highest levels of antibacterial and antifungal activity, failed to generate PCR products even after repeated PCR reactions for three times with standard 50 ng of chromosomal DNA, twice diluted or 3 and 5 times concentrated chromosomal DNA used per reaction. Number of bands generated by other

strains ranged from 6 to 25 with an average of 11 bands per sample. (GTG)₅-PCR fingerprinting technique generated highly discriminatory profiles and revealed high genotypic heterogeneity among the studied strains (Fig. 1).

In the other study, REP-1R-D and REP-2R-D primers scored 39 diverse polymorphic bands and generated 17 different patterns (Valerio et al. 2009). However, no clear influence of genetic relatedness on antifungal activity against *Aspergillus brasiliensis*, *Penicillium roqueforti*, *Endomyces fibuliger* and production of the organic acids or pH had been revealed. On the contrary, the polytrinucleotide (GTG)₅, which we applied in our research, was efficient in the studies of molecular typing, species identification of *Lactobacillus* (Scheirlinck et al.

2007; Svec et al. 2009), *Lactococcus* (Prodelalova et al. 2005; Rademaker et al. 2007; Svec and Sedlacek 2008) and *Streptococcus* (Svec et al. 2008). It allowed identification of many, but not all, isolates of *Lactobacillus* species isolated from the cervix of healthy women (Svec et al. 2011).

In our study, numerical analysis grouped all the studied strains into separate clusters according to their respective taxonomic designations. *Lactococcus* (*L.*) *lactis* spp. formed one big cluster which was further subdivided into three subclusters of different subspecies (*L. lactis* subsp. *lactis*: Nos. 8–11, *L. lactis* subsp. *lactis* biovar. *diacetylactis*: Nos. 12–15, and *L. lactis* subsp. *cremoris*: Nos. 16–20). *L. lactis* subsp. *lactis* biovar. *diacetylactis*

Fig. 1 Dendrogram of the LAB strains generated from rep-PCR with (GTG)₅ primer fingerprints using the unweighted pair group method with arithmetic average (UPGMA) based on Jaccard's genetic coefficient

(Nos. 12–14) contained the most closely genetically related cultures except for strain No. 768/5 (Fig. 1). All tested strains of *Streptococcus thermophilus* (Nos. 4–7) were placed into one separate cluster as well as expected based on low number of tested samples. However, bacterial strains of *Lactobacillus* (*Lb.*) genus were divided into three different clusters. LAB strains belonging to *Lb. plantarum* (No. 1) and *Lb. reuteri* (Nos. 2 and 3) formed the first cluster, *Lb. delbrueckii* (Nos. 21–24) and *Lb. casei* (Nos. 25–27) - the second, *Lb. acidophilus* (Nos. 28–33), *Lb. helveticus* (Nos. 34–37) and *Lb. brevis* (No. 38) - the third. Our results correspond well a close genotypic relationship based on relatedness of coat proteins, RNA polymerase subunits and 16S rRNA genes between *Lb. acidophilus* and *Lb. helveticus* as well as between *Lb. plantarum* and *Lb. reuteri* which had been published previously (Claesson et al. 2008). Profiles obtained by (GTG)₅-PCR fingerprinting formed separate clusters of *Lactobacillus* according to their taxonomic units. However, the results of such analysis did not reveal the evolutionary divergence among taxones as determined by the studies of conservative sequences (Claesson et al. 2008; Mori et al. 2004).

Antibacterial activity of LAB

Our study showed that the tested LAB strains exposed antibacterial activity against all reference strains (Table 3) and foodborne isolates (Table 4) of *B. cereus*, *S. Typhimurium*, *L. monocytogenes*, *E. coli* and *S. aureus*. In many cases, activity against reference strains was slightly higher than the activity against the foodborne isolate of the same species. In Tables 3 and 4 it is evident that antibacterial activity among CC and CFS of LAB against gram-positive bacteria was much more diverse (ZOI ranged from 0 to 31 mm) than against gram-negative bacteria (ZOI ranged from 9 to 21 mm). Antibacterial activity against gram-negative bacteria, in the contrary to the gram-positive bacteria, was not possessed by all examined strains (Table 3).

Comparing the highest antibacterial activity among LAB strains, *Lb. plantarum* and *Lb. helveticus* 305 demonstrated the highest activity against *E. coli* foodborne isolate (ZOI reached 19 mm for CC and 18 mm for CFS) and against *S. Typhimurium* foodborne isolate (ZOI reached 20 mm for CC and 19 mm - for CFS), respectively. *Lactobacillus* spp., thus, being able to inhibit the adhesion of *E. coli* and *S. Typhimurium* and to induce the secretion of anti-inflammatory cytokines, showed their practical advantage (Petros et al. 2006).

Following the results of immunological BCET-RPLA test we found that 5 out of 10 analyzed *B. cereus* cultures, identified using the genetic pyrosequencing method, produced diarrheal toxins. This finding indicates that inhibition of

B. cereus growth in food products was important for health care. Antibacterial effect against *B. cereus* was not induced by all tested *Streptococcus thermophilus* and 2 *Lactococcus lactis* strains. High ZOI of CFS (exceeding 15 mm) was characteristic to 15 LAB strains. The highest activity against *B. cereus* foodborne isolate was demonstrated by *Lb. acidophilus* LM1 (ZOI reached 25 mm for CC and 24 mm - for CFS). These results are in accordance with the other reports on *Bacillus* spp. inhibited by LAB isolated from the bread products (Suomalainen and Mayra-Makinen 1999), milk (Rossland et al. 2003), and fermented cheese (Neyriz-Nagadehi et al. 2012; Rukure and Bester 2001).

The highest activity against *L. monocytogenes* and *S. aureus* was demonstrated by *Lb. acidophilus* 336. Its activity against *L. monocytogenes* ATCC 19111 reached 31 mm for CC, ZOI against the foodborne isolate was 30 mm for CC and 27 mm for CFS, respectively (Tables 3, 4). *Lb. acidophilus* 336 showed the highest ZOI against *S. aureus* foodborne isolate of 26 mm for CC and maintained ZOI of 25 mm in all other tests. Our finding that LAB showed antibacterial activity against *L. monocytogenes* is in a close agreement to the previously reported results (Sip et al. 2012; Tadesse et al. 2008; Todorov 2008).

LAB supernatant with pH 3.7–4.5 showed the highest antibacterial activity (ZOI ranged from 12 mm to 30 mm), CFS with pH 4.6–5.4 showed lower ZOI (from 9 mm to 21 mm). Analyzing the results of CFN, antibacterial activity in most of cases was lost. Comparing the results of CFN and CC/CFS, it is evident that antibacterial activity in most of cases was lower (Table 4). CFN of LAB did not inhibit growth of *S. Typhimurium* and *B. cereus*. However, antibacterial activity of CFN against *E. coli* was lost after treatment with catalase. Catalase-treated CFN was able to inhibit the growth of *L. monocytogenes* (Tables 3 and 4). Antibacterial activity of CFN after treatment with catalase was possessed by *Lb. brevis* 43 (ZOI 25 mm and 13 mm for reference and foodborne isolates, respectively), *Lb. reuteri* 3, 7 (ZOI 25 mm and 20 mm for reference and foodborne isolates, respectively), *Lb. acidophilus* L 59–30 7 (ZOI 16 mm for the both reference and foodborne isolates) and *Lb. acidophilus* L 41-2B 2v (ZOI 16 mm and 13 mm for reference and foodborne isolates, respectively). So these strains were selected for further investigation in the role of biopreservatives for fresh fruits and vegetables, since fruits and vegetables can harbor pathogenic *L. monocytogenes*, capable to survive at refrigeration temperatures (Vescovo et al. 2006).

Thus, the same LAB strain was identified as possessing the highest antibacterial activity against both reference strains and foodborne isolates of the same gram-positive bacteria species (*B. cereus*, *L. monocytogenes* and *S. aureus*). Different LAB strains appeared to be the best inhibitors against gram-

Table 3 Antibacterial activity of different LAB strains shown against the reference strains of *Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 13076, *Listeria monocytogenes* ATCC 19111, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923

No.	Lactic acid bacterial strain	ZOI of cell culture, mm/ ZOI of cell-free supernatant, mm				
		Gram-negative bacteria		Gram-positive bacteria		
		<i>E. coli</i>	<i>S. Typhimurium</i>	<i>L. monocytogenes</i>	<i>B. cereus</i>	<i>S. aureus</i>
1.	<i>Lb. plantarum</i> 1	17/17	16/16	20/18	20/20	24/23
2.	<i>Lb. reuteri</i> 3	19/18	16/16	30/25//25///25	22/22	20/20
3.	<i>Lb. reuteri</i> 7	19/18	16/16	30/25//25///25	22/22	20/20
4.	<i>S. thermophilus</i> 24-36-13	11/12	12/12	0/0	0/0	15/15
5.	<i>S. thermophilus</i> 10KC-1	12/10	10/11	0/0	0/0	9/12
6.	<i>S. thermophilus</i> 182 II	12/13	12/13	0/0	0/0	12/10
7.	<i>S. thermophilus</i> 10 KC-2	12/13	11/11	0/0	0/0	9/12
8.	<i>L. lactis</i> subsp. <i>lactis</i> 140/2	17/16//9	21/20	21/20	15/16	19/16
9.	<i>L. lactis</i> subsp. <i>lactis</i> 142/21	15/14	20/19	17/17	13/12	17/20
10.	<i>L. lactis</i> subsp. <i>lactis</i> Z 264	12/13	17/18	0/0	0/0	18/17
11.	<i>L. lactis</i> subsp. <i>lactis</i> Z 252	16/18	16/17	13/12	17/16	18/17
12.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 57	15/15//11	21/20	1/18	13/13	17/17//11
13.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 130	16/15	12/11	10/10	15/12	16/15/10
14.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 149/1	14/13	11/11	0/0	0/0	13/15
15.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 768/5	17/16//9	21/19	18/17	14/14	17/17//12
16.	<i>L. lactis</i> subsp. <i>cremoris</i> 260/Z	12/9	16/16	16/13	12/11	18/16
17.	<i>L. lactis</i> subsp. <i>cremoris</i> 88/218	12/10	12/13	14/12	11/11	18/17
18.	<i>L. lactis</i> subsp. <i>cremoris</i> 206/10	12/9	13/15	18/17	11/14	17/16
19.	<i>L. lactis</i> subsp. <i>cremoris</i> 67/15	12/10	12/15	0/0	0/0	17/17
20.	<i>L. lactis</i> subsp. <i>cremoris</i> 73/64	14/11	15/15	17/15	13/14	18/16
21.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 3	18/17	19/18	25/26	26/27	24/23
22.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 148/3	19/18	19/19	23/24	26/25	24/24
23.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 21	19/18	20/19	20/24	20/23	25/25
24.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> R	18/18	20/20	24/24	25/29	26/25
25.	<i>Lb. casei</i> 24	17/14	18/17	27/25	21/19	25/23
26.	<i>Lb. casei</i> L	15/14	18/17	27/24	26/23	23/24
27.	<i>Lb. casei</i> 12	12/14	19/18	23/21	14/14	22/21
28.	<i>Lb. acidophilus</i> L	20/20	19/19	27/27	26/21	24/23
29.	<i>Lb. acidophilus</i> 308	21/19	18/17	27/25	13/11	25/25
30.	<i>Lb. acidophilus</i> BL 97	17/17	22/18	29/28	30/28	22/18
31.	<i>Lb. acidophilus</i> 336	21/20	19/18	31/29	25/27	25/25
32.	<i>Lb. acidophilus</i> L41-2B-2v	16/16	15/15	27/21//11///17	20/14	17/16
33.	<i>Lb. acidophilus</i> LM1	20/19	21/20	26/30	30/27	24/23
34.	<i>Lb. helveticus</i> 5	21/19	27/26	25/27	17/17	25/24
35.	<i>Lb. helveticus</i> 19	19/19	24/24	23/25	20/19	26/24
36.	<i>Lb. helveticus</i> 13	19/18	26/25	25/26	22/19	26/26
37.	<i>Lb. helveticus</i> 305	20/19	19/26	23/25	19/17	26/25
38.	<i>Lb. brevis</i> 43	15/19	14/13	25/25//12///25	22/21	18/17
39.■	<i>Lb. acidophilus</i> L 59–30	16/17//10	18/16	27/20//15///16	20/14	17/16//10
40.■	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 14	18/18	17/17	24/26	30/29	24/23

Strains selected for the future studies are marked by bold font and listed in the text

■ – strains of *L. acidophilus* L 59–30 and *L. delbrueckii* subsp. *bulgaricus* 14 which failed in obtaining PCR product. // – ZOI of cell-free neutralisate, mm (if showed), /// – ZOI of cell-free neutralisate after the treatment with catalase, mm (if showed)

Table 4 Antibacterial activity of different LAB strains shown against the foodborne isolates of *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhimurium*

No.	Lactic acid bacteria strain	ZOI of cell culture, mm/ ZOI of cell-free supernatant, mm				
		Gram-negative bacteria		Gram-positive bacteria		
		<i>E. coli</i>	<i>S. Typhimurium</i>	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
1.	<i>Lb. plantarum</i> 1	19/18	17/16	18/18	18/18	20/20
2.	<i>Lb. reuteri</i> 3	16/17	15/15	23/21	23/22//17///20	18/17
3.	<i>Lb. reuteri</i> 7	16/17	15/15	23/21	23/21//17///20	18/17
4.	<i>S. thermophilus</i> 24-36-13	12/12	13/11	0/0	0/0	10/10
5.	<i>S. thermophilus</i> 10KC-1	12/12	10/11	0/0	0/0	11/9
6.	<i>S. thermophilus</i> 182 II	13/13	11/12	0/0	0/0	10/10
7.	<i>S. thermophilus</i> 10 KC-2	12/12	10/11	0/0	0/0	0/0
8.	<i>L. lactis</i> subsp. <i>lactis</i> 140/2	12/14	19/18	15/14	20/19	19/17
9.	<i>L. lactis</i> subsp. <i>lactis</i> 142/21	16/15	20/19	12/12	15/16	16/15
10.	<i>L. lactis</i> subsp. <i>lactis</i> Z 264	14/14	17/17	0/0	0/0	15/13
11.	<i>L. lactis</i> subsp. <i>lactis</i> Z 252	15/15	16/17	15/11	11/11	14/15
12.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 57	16/15	19/18	13/11	15/11	14/12
13.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 130	15/13	12/11	10/10	12/10	15/13/10
14.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 149/1	11/12	11/10	0/0	0/0	13/11
15.	<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 768/5	15/15	20/18	13/13	15/12	13/12
16.	<i>L. lactis</i> subsp. <i>cremoris</i> 260/Z	14/14	12/12	12/13	16/11	14/13
17.	<i>L. lactis</i> subsp. <i>cremoris</i> 88/218	14/14	10/11	10/10	12/11	14/16
18.	<i>L. lactis</i> subsp. <i>cremoris</i> 206/10	13/13	10/11	10/12	15/12	12/12
19.	<i>L. lactis</i> subsp. <i>cremoris</i> 67/15	13/13	10/11	0/0	0/0	10/11
20.	<i>L. lactis</i> subsp. <i>cremoris</i> 73/64	14/14	11/11	12/13	15/13	13/14
21.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 3	16/16	18/18	19/21	23/23//15	21/22
22.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 148/3	18/17	18/18	19/14	23/21//14	24/21
23.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 21	18/16	19/19	14/14	21/17	24/22
24.	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> R	15/15	19/19	17/18	20/20//12	23/23
25.	<i>Lb. casei</i> 24	17/17	18/17	19/19	22/20	23/24
26.	<i>Lb. casei</i> L	17/18	19/18	21/21	27/24	23/24
27.	<i>Lb. casei</i> 12	17/17	17/17	14/14	22/23	22/23
28.	<i>Lb. acidophilus</i> L	18/18	19/18	16/16	27/25	20/24
29.	<i>Lb. acidophilus</i> 308	14/13	18/16	15/14	27/26	26/24
30.	<i>Lb. acidophilus</i> BL 97	18/15	20/18	24/25	27/26	21/17
31.	<i>Lb. acidophilus</i> 336	14/13	18/16	15/14	30/27	26/24
32.	<i>Lb. acidophilus</i> L41-2B-2v	13/14	15/15	17/16	19/19//11///13	13/14
33.	<i>Lb. acidophilus</i> LM1	17/17	20/19	25/24	26/25	23/22
34.	<i>Lb. helveticus</i> 5	12/12	19/18	13/11	23/23	26/24
35.	<i>Lb. helveticus</i> 19	13/13	19/18	18/16	21/20	27/23
36.	<i>Lb. helveticus</i> 13	17/16	18/18	12/12	21/22	25/23
37.	<i>Lb. helveticus</i> 305	19/18	19/18	15/15	22/22	25/25
38.	<i>Lb. brevis</i> 43	18/15	14/13	20/20	18/21//17///13	17/15
39.■	<i>Lb. acidophilus</i> L 59–30	13/13//10	16/16	17/16	20/20//13///15	14/13//10
40.■	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 14	16/16	18/18	19/21	25/24//10	21/21

Strains selected for future studies are marked by bold font and listed in text

■ Strains of *L. acidophilus* L 59–30 and *L. delbrueckii* subsp. *bulgaricus* 14 which failed in obtaining PCR product. // – ZOI of cell-free neutralisate, mm (if showed), /// – ZOI of cell-free neutralisate after the treatment with catalase mm (if showed)

negative *E. coli* and *S. Typhimurium* reference strains as well as foodborne isolates.

Antifungal activity of LAB

The antifungal activity was induced by a lower number of the tested LAB strains than the antibacterial one (Table 5). The ZOI for all fungal isolates ranged from 11 mm to 30 mm for CC and from 11 mm to 36 mm for CFS, if present. The growth of *C. herbarum*, *S. brevicaulis* and *P. chrysogenum* was inhibited by 44 %, 42 % and 28 % of the tested LAB strains, respectively.

No *L. lactis* subsp. *cremoris* strains revealed antifungal activity, LAB strains representing *L. lactis* subsp. *lactis*, *Lb. casei* and *Lb. helveticus* showed no activity against *P. chrysogenic*, and no *Streptococcus thermophilus* was antagonistic to *S. brevicaulis*. As reported by Magnusson et al. (2003), low amount of LAB strains displayed fungicidal effect against *Aspergillus* spp. and yeasts (Table 5). 12 % of the CFS showed antifungal activity against *A. brasiliensis*. CFS induced more intense suppression of *A. brasiliensis* than CC. The opposite effect was observed against *A. versicolor*. The less inhibitory activity of CC could be observed due to a lower concentration of active compounds in the test material which

Table 5 LAB strains showing antifungal activity against the foodborne isolates of *Debaromyces hansenii*, *Yarrowia lipolytica*, *Aspergillus brasiliensis*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Scopulariopsis brevicaulis*

Lactic acid bacteria strain	Micromycetes susceptible to LAB effect			Micromycetes and yeasts less susceptible to LAB effect	
	<i>C. herbarum</i> SR-11 ZOI of CC/ ZOI of CFS, mm	<i>P. chrysogenum</i> SR-12	<i>S. Brevicaulis</i> Gr-5	ZOI of CC/ ZOI of CFS, mm	Indicator fungi
<i>Lb. plantarum</i> 1	13/15	13/0	0/0	29*/20	<i>A. versicolor</i>
<i>Lb. reuteri</i> 3	16/19	12/15	13/14	0/0	0/0
<i>Lb. reuteri</i> 7	16/18	12/15	13/14	0/0	0/0
<i>S. thermophilus</i> 24-36-13	13*/0	15/15	0/0	0/0	0/0
<i>L. lactis</i> subsp. <i>lactis</i> 140/2	19/20	0/0	19/0	20/0	<i>A. versicolor</i>
<i>L. lactis</i> subsp. <i>lactis</i> 142/21	0/15	0/0	12/11	0/0	0/0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 57	23/23	0/0	0/0	0/0	0/0
<i>L. lactis</i> subsp. <i>lactis</i> biovar. <i>diacetylactis</i> 768/5	16/20	11/12	0/0	18/0	<i>A. versicolor</i>
<i>Lb. casei</i> 12	19/24	0/0	16/18	0/0	0/0
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 3	19/20	0/0	19/19	0/0	0/0
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 148/3	14*/15*	0/0	18/20	0/0	0/0
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 21	30/36	0/0	21/21	18*/0	<i>D. hansenii</i>
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> R	0/18	14/14	0/0	0/0	0/0
<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> 14	24/15	16/14	0/0	0/14*	<i>A. niger</i>
<i>Lb. helveticus</i> 19	12*/0	0/0	12/11	20/0	<i>A. versicolor</i>
<i>Lb. helveticus</i> 13	0/0	0/0	22/21	0/20*	<i>A. niger</i>
<i>Lb. helveticus</i> 305	0/0	0/0	23/16	-	-
<i>Lb. acidophilus</i> L	14/12	13/0	0/0	14/0 16/0	<i>A. versicolor</i> <i>Y. lipolytica</i>
<i>Lb. acidophilus</i> BL 97	18/15	20/18	24/25	0/0	0/0
<i>Lb. acidophilus</i> 336	14/13	18/16	15/14	0/18 12/20 14/14	<i>A. niger</i> <i>A. versicolor</i> <i>Y. lipolytica</i>
<i>Lb. acidophilus</i> L41-2B-2v	17/16	0/0	15/17	16/16	<i>A. niger</i>
<i>Lb. acidophilus</i> LM1	18/20	0/0	16/22	0/0	0/0
<i>Lb. acidophilus</i> L 59–30	0/0	14*/14*	27/28	16/16	<i>A. niger</i>
<i>Lb. brevis</i> 43	23/29	12/14	13/12	0/0	0/0

Only LAB strains with detected antifungal activity are presented in the table. Average of ZOI in mm is given of the cell culture (CC) and cell-free supernatant (CFS). * Fungistatic activity

contained approximately 1/3 of dead cells. While in most cases, the antifungal activity of CC and CFS did not differ significantly. Only 3 strains of *Lactobacillus* spp. were able to inhibit growth of *Y. lipolytica* and *D. hansenii* (Table 5). It was found that the antimicrobial activity of tested reference strains *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404 did not differ from the same foodborne isolates, respectively *C. parapsilopsis* and *A. brasiliensis*. The CC of *Lb. delbrueckii* subsp. *bulgaricus* 21 inhibited growth and showed fungistatic activity against *D. hansenii*. *Lb. acidophilus* L and *Lb. acidophilus* 336 displayed CC acting against *Y. lipolytica*. *Lb. acidophilus* 336 and *Lb. acidophilus* L were found the only strains acting against both molds and yeasts. Strains of *Lb. plantarum*, *Lb. brevis* and *Lb. reuteri* had been previously reported as good inhibitors of *F. graminearum*, *Penicillium* spp. and *A. niger* (Gerez et al. 2009). Our study also revealed good ability of *Lb. brevis* and both *Lb. reuteri* strains to inhibit growth of *C. herbarum*, *S. brevicaulis*, and *P. chrysogenum*. They were not indicating antifungal activity against *Aspergillus* spp. and yeasts.

Just few LAB strains possessed activity against yeast. We detected no inhibition of *Candida parapsilosis*, *Kluyveromyces marxianus* and *Pichia guilliermondii* by LAB. Limitations to inhibit certain yeast species were faced in other studies as well (Cakir 2010; Magnusson et al. 2003). In spite of that, some *Lactobacillus* strains were found to be able to suppress *Candida* spp., *S. cerevisiae*, *K. Marxianus* and *C. parapsilosis* (Cizeikiene et al. 2013; Durlu-Özkaya et al. 2005; Melvydas et al. 2007; Smaoui et al. 2010; Ström et al. 2002).

Conclusions and future work

The study revealed the LAB strains exposing strong inhibiting effect against one or few microorganism. Several strains indicated enhanced antimicrobial activity against a whole complex of the indicator species. The most perspective because of their enhanced inhibiting activity against tested harmful and pathogenic microorganisms were selected *Lactobacillus reuteri* 3 and 7, *Lactobacillus brevis* 43, *Lactobacillus acidophilus* L 59–30, *Lactobacillus acidophilus* L 41-2B 2v, *Lactobacillus delbrueckii* subsp. *bulgaricus* 14, 3, 148/3 and R, *Lactococcus lactis* subsp. *lactis* 140/2, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* 57 and 768/5. All the candidate strains (marked in bold in the Tables 3, 4 and 5) have high potential in practical application as novel strains with enhanced antibacterial and antifungal activity. The frequency of strains superior in food preserving capacity was remarkably higher among the representatives of *Lactobacillus* spp. (2/3). *Lactobacillus* spp. induced the more intense acidification of supernatant, which greatly influenced activity against the tested microorganisms. *Lb. brevis* 43, *Lb. reuteri* 3 and 7, *Lb. acidophilus* L 59–30 7 and 41-2B 2v retained the antagonistic effect against *L. monocytogenes* after CFN was catalase-

treated. No one *S. thermophilus* strain demonstrated enhanced food preservation ability against any of the indicator microorganism. *S. thermophilus* inhibited growth of gram-positive bacteria as reported previously (Mezaini and Bouras 2013). However, here analyzed *S. thermophilus* strains had shown neither high antifungal nor antibacterial activity against gram-negative bacteria. Tadesse et al. (2008) had pointed out that *Lactobacillus* spp. inhibited bacterial growth better than the *Streptococcal* spp. These researchers also reported that *Lactobacillus* spp. formed larger inhibition zones than *Leuconostoc*, *Streptococcus* and *Pediococcus* spp.

The results demonstrated a big influence of genus and species on the potential in selection for LAB with enhanced antibacterial and antifungal activities without any observation of significant impact determined by the intraspecific genotypic relatedness revealed by (GTG)5-PCR fingerprinting on the inhibiting effect against the tested isolates of bacteria and fungi.

Future strategies need to incorporate selected LAB strains posing enhanced antimicrobial activity into in vivo approaches (meat, fish, bread) in order to successfully use of bacteriocinogenic cultures well adapted in the food environment. The changes of the antimicrobial activity of selected LAB under the influence of technological factors will be determined in order to confirm the food preserving capacity. The preliminary studies have already shown that selected LAB strains retain their enhanced antimicrobial activity during the heat and cold treatment. The LAB strains with stable antimicrobial properties will be used for the manufacture of the starters for fermented food products.

Acknowledgments We greatly acknowledge financial support from the Research Council of Lithuania, grant SVE-05/2012 PIENRUGBAKT

References

- Cakir I (2010) Antibacterial and antifungal activities of some lactic acid bacteria isolated from naturally fermented herbs. J Food Agric Environ 8:223–226
- Cizeikiene D, Juodeikiene G, Paskevicius A, Bartkiene E (2013) Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control 31:539–545
- Claesson MJ, van Sinderen D, O'Toole PW (2008) *Lactobacillus* phylogenomics – towards a reclassification of the genus. Int J Syst Evol Microb 58:2945–2954
- Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi. Academic, London
- Durlu-Özkaya F, Karabicak N, Kayali R, Esen B (2005) Inhibition of yeasts isolated from traditional Turkish cheeses by *Lactobacillus* spp. Int J Dairy Technol 58:111–114
- Fhoula I, Najjari A, Turki Y, Jaballah S, Boudabous A, Ouzari H (2013) Diversity and Antimicrobial Properties of Lactic Acid Bacteria Isolated from Rhizosphere of Olive Trees and Desert Truffles of

- Tunisia. Biomed Res Int. <http://dx.doi.org/10.1155/2013/405708> [12 September 2012].
- Garcia-Vallve S, Palau J (1999) Romeu A, Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. *Mol Biol Evol* 16:1125–1134
- Gerez CL, Torino MI, Rollan G, Font de Valdez G (2009) Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food Control* 20:144–148
- Gevers D, Huys G, Swings J (2001) Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* 205:31–36
- Kurtzman CP, Fell JW, Boekhout T, Robert V (2011) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) *The Yeasts, a Taxonomic Study*, 5th edn. Elsevier, Amsterdam, pp 87–110
- Magnusson J, Ström K, Roos S, Sjögren J, Schnürer J (2003) Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiol Lett* 219:129–135
- Melvlydas V, Šakalytė J, Paškevičius A, Gedminienė G (2007) Search for biological control agents against *Candida* yeasts and other dermatomycetes. *Biologija* 18:45–49
- Mezaini A, Bouras AD (2013) Antibacterial activity and probiotic properties of some lactic acid bacteria isolated from dairy products. *Afr J Biotechnol* 12:2949–2956
- Mori S, Mori K, Suzuki I, Kasumi T (2004) Phylogenetic analysis of *Lactococcus lactis* subspecies based on decoding the sequence of the pepT tripeptidase gene, the pepV dipeptidase gene and 16S rRNA. *Syst Appl Microbiol* 27:414–422
- Neyriz-Nagadehi M, Razavi-Rohani SM, Karim G, Zeynali A (2012) The Effects of Monolaurin and Lactic Acid Bacteria Starter Culture on Growth of Vegetative Cells of *Bacillus cereus* in Iranian White Fresh Cheese. *Iran J Sci Technol* 4:75–84
- Petros A, Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E (2006) Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int Dairy J* 16:189–199
- Pitt JI (2007) The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic, London and New York
- Prodalalova J, Spanova A, Rittich B (2005) Application of PCR, rep-PCR and RAPD techniques for typing of *Lactococcus lactis* strains. *Folia Microbiol* 50:150–154
- Rademaker JLW, Herbet H, Starrenburg MJC, Sabri M, Naser SN, Gevers D, Kelly WJ, Hugenholtz J, Swings J, van Hylckama Vlieg JE (2007) Diversity Analysis of Dairy and Nondairy *Lactococcus lactis* Isolates, Using a Novel Multilocus Sequence Analysis Scheme and (GTG)₅-PCR Fingerprinting. *Appl Environ Microbiol* 73:7128–7137
- Rosslund E, Andersen Borge GI, Langsrud T, Sorhaug T (2003) Inhibition of *Bacillus cereus* by strains of *Lactobacillus* and *Lactococcus* in milk. *Int J Food Microbiol* 89:205–212
- Rukure G, Bester BH (2001) Survival and growth of *Bacillus cereus* during Gouda cheese manufacturing. *Food Control* 12:31–36
- Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (2000) Introduction to food- and airborne fungi. CVS, Utrecht
- Scheirlinck I, Van der Meulen R, Van Schoor A, Vancanneyt M, De Vuyst L, Vandamme P, Huys G (2007) Influence of geographical origin and flour type on diversity of lactic acid bacteria in traditional Belgian sourdoughs. *Appl Environ Microbiol* 73:6262–6269
- Sip A, Wieckowicz M, Olejnik-Schmidt A, Grajek W (2012) Antilisteria activity of lactic acid bacteria isolated from gouda, a regional cheese produced in Poland. *Food Control* 26:117–124
- Smaoui S, Elleuch L, Bejar W, Karray-Rehai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L (2010) Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* sp. TN635. *Appl Biochem Biotechnol* 162:1132–1146
- Stoyanova LG, Ustyugova EA, Sultimova TD, Bilanenko EN, Fedorova GB, Khatrukha GS, Netrusov AI (2010) New Antifungal Bacteriocin-Synthesizing Strains of *Lactococcus lactis* ssp. *lactis* as the Perspective Biopreservatives for Protection of Raw Smoked Sausages. *Am J Agric Biol Sci* 5:477–485
- Ström K, Sjögren J, Broberg A, Schnürer J (2002) *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and nL-Phe-trans-4-OH-L-Pro) and 3-henylactic Acid. *Appl Environ Microb* 68:4322–4327
- Suomalainen TH, Mayra-Makinen AM (1999) Propionic acid bacteria as protective cultures in fermented milks and breads. *Lait* 79:165–174
- Svec P, Sedlacek I, Zackova L, Novakova D, Kukletova M (2009) *Lactobacillus* spp. associated with early childhood caries. *Folia Microbiol* 54:53–58
- Svec P, Sedlacek I, Chrapava M, Vandamme P (2011) (GTG)₅-PCR fingerprinting of lactobacilli isolated from cervix of healthy women. *Folia Microbiol* 56:80–83
- Svec P, Novakova D, Zackova L, Kukletova M, Sedlacek I (2008) Evaluation of (GTG)₅-PCR for rapid identification of *Streptococcus mutans*. *Antonie Van Leeuwenhoek* 94:573–579
- Svec P, Sedlacek I (2008) Characterization of *Lactococcus lactis* subsp. *lactis* isolated from surface waters. *Folia Microbiol* 53:53–56
- Tadesse G, Ephraim E, Ashenafi M (2008) Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. *J Food Saf* 5:13–20
- Todorov SD (2008) Bacteriocin production by *Lactobacillus plantarum* AMA-K isolated from Amasi, a Zimbabwean fermented milk product and study of adsorption of bacteriocin AMA-K to *Listeria* spp. *Braz J Microbiol* 38:178–187
- Valerio F, Favilla M, De Bellis P, Sisto A, De Candia S, Lavermicocca P (2009) Antifungal activity of strains of lactic acid bacteria isolated from semolina ecosystem against *Penicillium roqueforti*, *Aspergillus niger* and *Endomyces fibuliger* contaminating bakery products. *Syst Appl Microbiol* 32:438–448
- Vescovo M, Scolari G, Zacconi C (2006) Inhibition of *Listeria innocua* growth by antimicrobial-producing lactic acid cultures in vacuum-packed cold-smoked salmon. *Food Microbiol* 23:689–693
- Watanabe T (2002) Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC Press, Washington DC
- Yang E, Fan L, Jiang Y, Doucette C, Fillmore S (2012) Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express* 2:1–12