JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

LACTOBACILLUS STRAINS FROM APPLE AND FERMENTED MILK AND THEIR PROBIOTIC PROPERTIES

Eteri Tkesheliadze[*1], *Nino Gagelidze*[1], *Tinatin Sadunishvili*[1], *Christian Herzig*[2]

Address(es): Eteri Tkesheliadze,
[1]Agricultural University of Georgia, Sergi Durmishidze Institute of Biochemistry and Biotechnology; Kakha Bendukidze University Campus, David Aghmashenebeli Alley 240, 0159 Tbilisi, Georgia.
[2] University of Kassel, Organic Agricultural Sciences Faculty, Steinstrasse 19, 37213 Witzenhausen, Germany.

*Corresponding author: etkes2015@agruni.edu.ge

https://doi.org/10.55251/jmbfs.5776

ARTICLE INFO

Received 24. 1. 2022
Revised 20. 2. 2023
Accepted 6. 3. 2023
Published 1. 6. 2023

Regular article

OPEN ACCESS

ABSTRACT

In this study, twenty isolates of lactic acid bacteria (LAB) from different apple varieties were obtained. Their probiotic properties together with those of 12 strains, isolated by us earlier from fermented milk, *Levilactobacillus brevis* (10, 15, 18, 49, 51), *Lactiplantibacillus pentosus* (40, 57, 63, 85, 88, 92) and *Lactobacillus fermentum* (44) were studied. Apple *Lactiplantibacillus plantarum* 52, 53, 74, and 76 were found to be pH 2-resistant during incubation for 30, 60 and 90 min. Overall, exposure to bile salts (0.3%; 0.5%; 1%; and 1.5%) had no effect on the growth of *Lactobacillus* sp. Antimicrobial activity and antibiotic resistance/susceptibility studies were performed on ten pathogenic microorganisms and nine antibiotics, respectively. *Lactiplantibacillus* (*Lpb.*) *plantarum* 52, 53, and 74 from apple samples had susceptibility to oxytetracycline, tetracycline, and rifampicin, *Lpb. plantarum* 52, 74 and 76 were susceptible to erythromycin. Almost all strains of *lactobacillus* showed antimicrobial activity to *Enterococcus faecalis* ATCC 29212, except *Lpb. plantarum* 76 and *L. brevis* 51. *Lpb. plantarum* 76 had inhibitory activity against *Bacillus cereus* ATCC 10876 (16.3±0.52), *Klebsiella pneumoniae* ATCC 13833 (16.4±0.4), *Streptococcus pyogenes* ATCC 21059 (16.3±0.3), *Pseudomonas aeruginosa* ATCC 27853 (16.3±0.4), *Escherichia coli* ATCCB322 (16.3±0.5), *Proteus mirabilis* ATCC 12453 (12.4±0.4), and *Shigella flexneri* ATCC 12022 (12.3±0.3). Based on the studied probiotic properties, apple *Lpb. plantarum* 52, *Lpb. plantarum* 53, *Lpb. plantarum* 74, *Lpb. plantarum* 76, identified by MALDI-TOF mass spectrometry and 16S rDNA sequencing, were selected as potential candidates for the fermentation of apple juices. Fermented apple juices could be a new source of probiotics for lactose intolerant and vegetarian consumers.

Keywords: Apple, lactic acid bacteria, molecular identification, acid tolerance, bile tolerance, antibiotic resistance, antimicrobial activity

INTRODUCTION

It is noteworthy that, in the process of evolution, humans have made radical changes in their diet; in particular, a new trend of the XXI century has emerged in the form of probiotics-based foods and beverages **(Biswal *et al.*, 2021)**, which are primarily intended for lactose intolerant and vegetarian consumers **(Samedi and Charles, 2019)**. Probiotic and prebiotic non-dairy products have a great marketing future, as recent studies have shown that strains have adapted well to alternative matrices when incorporated into juices **(Aspri *et al.*, 2020)**. In addition, 65-70% of the world's population faces to lactose intolerance challenge **(Bayless *et al.*, 2017)**. Finally, based on the amount of production, the top three most popular fruits in the world include apples, which underlines their importance from an economic point of view **(Shahbandeh, 2021)**.

Lactic acid bacteria (LAB), especially *Lactobacilli*, are considered to be plant-associated bacteria **(Lamont *et al.*, 2017)**, and LAB are often isolated from fruits and vegetables **(Yu *et al.*, 2019)**. One of the species of lactic acid bacteria, *Lactiplantibacillus (Lpb.) plantarum* (previously named *Lactobacillus plantarum*; **Zheng *et al.*, 2020**) can be used as an interesting probiotic candidate (**Fidanza *et al.*, 2021**). For instance, in one study, *L. plantarum* and *L. casei* were isolated from fruit residues such as banana leaf and stem, pineapple peel, and papaya peel **(Yang *et al.*, 2016)**. There has also been increased interest in autochthonous lactic acid bacteria in table olives, with 197 reports having been published worldwide over the last two decades. Among the LAB, *Lactobacillus* is the most common genus to exist in olives **(Portilha-Cunha *et al.*, 2020)**. Besides, *Leuconostoc mesenteroides* subsp. *mesenteroides* LB7 was found on the surface of the apple **(Ngea *et al.*, 2021)**. In addition, several studies have been conducted on the isolation and characterization of *Lactobacillus* strains from plant leaf surfaces **(Samedi and Charles, 2019)**. Three new species of *Lactobacilli (Lactobacillus micheneri, Lactobacillus timberlakei*, and *Lactobacillus quenuiae*) were isolated from flowers (*Abutilon* sp.) and wild bees **(McFrederick *et al.*, 2017; 2018)**. These bacteria are involved in food digestion, stimulate the immune system, and have the ability to resist pathogenic microorganisms in the gastrointestinal tract **(Lorizzo *et al.*, 2020)**. In general, a common characteristic of both plant-associated and other LABs is the synthesis of large amounts of lactic acid and acetic acid via fermentation **(Tyler *et al.*, 2016)**. From a biochemical point of view, it is important to consider these aspects, as the selection of LAB for the enrichment of apple juice can be based on technological, sensory, or nutritional criteria **(Di Cagno *et al.*, 2013, 2015)**. The survival of LAB while moving through the digestive tract plays an essential role in revealing their probiotic properties **(Yang *et al.*, 2019)**. The probiotic strain should be resistant to small intestinal bile salts at a concentration of approximately 0.3%, as well as resistant to the gastric acid environment, especially its low pH (1.0–3.0) **(Mainville *et al.*, 2005)**. Among the selected criteria of probiotic properties, antimicrobial activity against pathogens is also important (**De Vries *et al.*, 2006**). It should be noted that *Lactiplantibacillus (Lpb.) plantarum* is especially distinguished by these properties **(Garcia-Gonzalez *et al.*, 2021).** According to a study by **Tao *et al.* (2021)**, the use of a plant substrate fermented with *Lactobacillus plantarum* instead of antibiotics may have potential against foodborne infections caused by pathogens. The dependence of lactic acid bacteria on antibiotics is an area of interest as well. Moreover, the significant characteristics of starter or probiotic cultures depend on the strains themselves. Therefore, studies have aimed to discover a new starter culture, which, in turn, is based on screening LAB strains from various sources **(Barbosa *et al.*, 2015; Rzepkowska *et al.*, 2017)**.

There is a great diversity of plant species and cultivars, including the fruits in eastern and western parts of Georgia, suggesting them as an interesting and rich source of LAB. The aim of this study was to isolate *Lactobacillus* sp. from the fruits of different apple varieties from these regions of Georgia to obtain pure cultures, conducting molecular identification, and identify potential probiotic properties. All isolated *Lactobacillus* sp. were selected according to their probiotic characteristics such as resistance to low pH and various concentrations of bile salts; moreover, they were tested based on several parameters, including susceptibility/resistance to antibiotics and antibacterial activity against food pathogens. The selected strains of lactic acid bacteria will be used in the future as starter cultures for the fermentation of apple juices. An important link in this process is the fact that most probiotic foods are based on dairy products because of historical and technological reasons, as well as the nutritional value of milk **(Nguyen *et al.*, 2019)**, but the final product, apple juice enriched with probiotics, will follow the recommendations of the health authority on the one hand focus on plant-based foods, on the other hand, will meet the needs of consumers, in terms of healthy food intake and dietary needs, such as vegetarianism and production of lactose-free beverages **(Pontonio *et al.*, 2020)**.

MATERIAL AND METHODS

Sample Collection

Sixty samples of ripe and raw apples were collected from eastern and western Georgia: specifically, from the villages of Gori Municipality (Rekha, Uplistsikhe, Skra, Tortiza, Qitsnisi, Karaleti, Khidistavi) and Ambrolauri Municipality (Nikortsminda, Bostana, Mukhli, Shaori, Chkvishi, Chrebalo, Akhalsopeli, Khotevi).
Different apple varieties (red/pink and yellow) were manually placed in sterile bags and immediately transported to the laboratory under refrigeration and were analyzed on arrival. All of them were of agreeable sensory quality. The samples used in the study were chosen according to the seasonal fruit picking in September-October 2019 and 2020.

Isolation of lactic acid bacteria

Lactic acid bacteria were isolated from the apples using a serial dilution method. The peels of three to four fruits of each variety (collected from one tree) were crushed. Then, 10 g of the grinded skin of the sample was homogenized in a mortar, transferred into a sterile bag (Whirl-Pak Stand-Up Bags), and was suspended in 90 ml buffered peptone water (Oxoid, Milan, Italy). After thorough mixing under aseptic conditions, 1 mL of the 10^{-1} dilution was serially diluted using nine 9 mL of pre-sterilized physiological saline and this was continued until the required dilution. Additionally, 0.1mL aliquots from each dilution were used as an inoculum and plated onto sterile MRS agar **(De Man *et al*., 1960)** containing cysteine hydrochloride (0.05 g/100 mL) to inhibit fungi, yeast, and Gram-negative microorganisms, also to keep low the redox potential of the medium **(Hartemink *et al*., 1997)**. The Petri plates were incubated anaerobically using a candle jar for 24 h at 37ºC **(De *et al*., 2016)**. The cultures were sub-cultured on MRS agar to obtain pure isolates. The first isolate identification was made based on meeting the characteristic of LAB on agar: a white/cream or yellow color **(Theingi *et al*., 2019)** and round shape. Further Gram staining was done, and the cell morphology of the presumptive *Lactobacillus* sp. was observed using a light microscope and catalase reaction. For these studies, the isolates were grown on MRS agar under appropriate conditions. The Gram staining was conducted using a single colony of each LAB isolate, following the standard protocol for Gram staining while catalase reaction was performed on a sterile microscope glass slide with drops of 3% hydrogen peroxide onto the selected colony, as mentioned by **Nanasombat *et al*. (2012)**.
Only Gram-positive bacterial isolates (cocci or rods) that were catalase-negative were presumptively identified as LAB. The pure cultures were maintained at -20°C in glycerol stocks for further studies.
Except the lactic acid bacteria isolated from apples, in the study were also included the samples previously isolated from the fermented milk for comparison: *Levilactobacillus brevis* (10, 15, 18, 49, 51) *Lactiplantibacillus pentosus* (40, 57, 63, 85, 88, 92), *and Lactobacillus fermentum* (44) **(Bokulich *et al*., 2015)**.

The determination of optimal growth temperature

The selected bacterial isolates were grown in MRS broth at 30, 37, and 40^{0}C for 48-72 hrs. Then, 0.1ml inoculum (10^8 CFU/mL) was transferred to MRS plates by pour plate method and incubated at 37^{0}C for 48hrs. The growth of apple and fermented milk origin LAB on MRS agar plates was used to designate isolates as temperature tolerant (Tambekar and Bhutada, 2010).

Glucose Fermentation

Phenol red broth was used as the medium in this test. Then, 1% of sugar substrate, such as glucose was added into each phenol red broth tubes. Durham tubes were added into each phenol red tubes for the purpose of gas bubbles detection. Sterilized phenol red broth tubes were inoculated (10^8 CFU/mL) with active LAB isolates of apple and fermented milk origin (uninoculated tubes was kept as control) and incubated for 24 hours at 37°C. Changes of phenol red broth from red to yellow indicated positive reaction (Rhaiem et al., 2016).

Molecular Identification of LAB Isolates

DNA was extracted from an overnight culture in 500 µl MRS broth at 37 °C using the OxGEn Gram-positive DNA Purification Kit according to the manufacturer's instructions. DNA concentration and purity were determined via absorbance using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with 260 nm/280 nm absorbance ratios. In the present study, a *Lactobacillu*s genus-specific primer was used to amplify the 16S rRNA gene region of *Lactobacilli* DNA: IDL04F 5'- AGGGTGAAGTCGTAACAAGTAGCC –3', and IDL03R 5'-CCACCTTCCTCCGGTTTGTCA –3' **(Kwon *et al*., 2004)**. All the reaction mixtures were amplified in a GenePro thermocycler PCR system (Bioer, China). The reaction mixture 25 µl 1X Taq Master Mix contained 17 µl of deionized water, 2.5 µL of 10X PCR buffer, 2.5 µl $MgCl_2$ 0.5 µl of primer, 0.5 µl dNTP, 1 U/µl Taq DNA polymerase (Solis BioDyne, Estonia), and an extract containing an equal amount of DNA in 1 µl. The same amount of *Levilactobacillus brevis* 15 DNA isolated from fermented milk was used for positive control **(Bokulich *et al*., 2015)**; the negative control contained only 1 µl deionized water. The cycling conditions were as follows: an initial heating of 95 °C for five minutes, followed by 35 reaction cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for one minute, extension at 72 °C for two minutes, a 10 min final incubation at 72 °C, and a 4 ºC hold. PCR fragment analysis was performed in 1% agarose gel after staining with ethidium bromide in 1 x TAE buffer; visualization was performed with a transilluminator **(Vural and Ozgun, 2011)**.
Initial species identification of the potential probiotic bacteria was done by intact cell MALDI-TOF mass spectrometry (ICMS). ICMS was done by standard procedures recommended for the MALDI Biotyper system (Bruker Daltonics, Bremen, Germany). For analysis, 600 spectra from 2–20 kDa were gathered in 100-shots steps on an Autoflex III system and added up. Results with MALDI Biotyper identification score values ≥2.000 were considered correct.
After the ICMS results which yielded only a group of microbial species, 16S rDNA sequencing was additionally performed. For amplification of a high conserved 899 bp region of the 16SrRNA gene, the following primers were used: 27f: 5'–AGAGTTTGATCMTGGCTCAG–3' and 926r: 5'–CCGTCAATTCCT TTRAGTTT–3'. DNA isolated from samples 52, 53, 74, 76 were used as a matrix for PCR amplification of fragments (The method of DNA extraction is described above). Amplicon purification was performed using the ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems, Waltham, Massachusetts, USA). Asymmetric PCR was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, USA) and amplicon purification was performed using the DyeEx 2.0 Spin Kit (Qiagen, Hilden, Germany) followed by sequencing in a 3130xl Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA). The sequences obtained were analyzed using NCBI BLAST Search.

Resistance to Low pH

Resistance to pH was examined using the method described by **Tambekar and Bhutada (2010)** and **Liong *et al*. (2005)** with certain modifications. The method is used to evaluate the viability of the cells under acidic stress. For this purpose, in sterile tubes, inoculation of MRS broth acidified with 1N HCl to pH 2.0 was performed with 16-18 hour cultures of lactic acid bacteria. After 0-, 30-, 60-, and 90-min incubation periods, 100 µl of each culture from the broth was spread on MRS agar and incubated at 37 °C under anaerobic conditions for 24 h to determine the viable cell. The growth of LAB on MRS agar was used to designate isolates as pH tolerant **(Tambekar and Bhutada, 2010; Liong *et al*., 2005)**. MRS broth without acidification was used as a positive control. pH of MRS broth was 5.7.

Bile Salt Tolerance

The ability of the isolates to tolerate bile salts was studied according to the method described by **Maragkoudakis *et al*. (2006)** and **Tambekar and Bhutada (2010)**, but with additional incubation time periods. 16-18 h grown cultures were added to the MRS broth with varying concentrations of bile salts (0.3%; 0.5%; 1% and 1.5%), and incubation was carried out for 2 h, 4 h, 24 h, and 48 h. Then, 0.1ml inoculums was transferred to MRS agar via the pour plate method and incubated at 37 °C for 24 h under anaerobic conditions. The bile salt tolerance of selected isolates was determined according to LAB culture growth on agar plates. Bile salt-free MRS broth was used as a positive control for this experiment **(Tambekar and Bhutada, 2010; Maragkoudakis *et al*., 2006)**.

Antibiotic Resistance / Susceptibility

An antibiotic susceptibility assay for LAB isolates was carried out via disc diffusion method **(Prabhurajeshwar and Chandrakanth, 2017)**; discs containing different concentrations of nine antibiotics–oxytetracycline (30µg/ml), ciprofloxacin (5µg/ml), bacitracin (10 µg/ml), gentamicin (10 µg/ml), streptomycin (10 µg/ml), neomycin (30 µg/ml), tetracycline (30 µg/ml), erythromycin (15 µg/ml), and rifampicin (5 µg/ml)–were applied.
Overnight cultures of LAB isolates from the MRS broth were swabbed on Mueller-Hinton agar plates, and, after 30 min, different antibiotic discs were placed on them using forceps. The plates were incubated at 37°C for 24 h–48 h. The drug susceptibility of the *Lactobacillus* sp. isolates was evaluated, and the zone of inhibition around the isolate's growth was measured. Based on the inhibition zone around the antibiotic disc, the studied inoculants were classified as susceptible (≥14 mm), intermediate (11–13 mm), and resistant (≤10 mm) **(Kenny *et al*., 1992)**.

Antimicrobial Activity

The antimicrobial activity of the screened LAB isolates was tested against *Salmonella enterica* ATCC 14028; *Klebsiella pneumoniae* ATCC 13833*; Bacillus cereus* ATCC 10876; *Proteus mirabilis* ATCC 12453; *Streptococcus pyogenes* ATCC 21059*; Enterococcus faecalis* ATCC 29212; *Pseudomonas aeruginosa* ATCC 27853*; Staphylococcus aureus* ATCC 25923*; Shigella flexneri* ATCC 12022; *and Escherichia coli* ATCC 25922 using the agar diffusion method (the so-

called method of agar blocks) **(Egorov, 1965).** Mueller-Hinton agar (Biolife, Italy) plates were swabbed with the pathogenic cultures.
Freshly prepared pure cultures of 100 µl LAB isolates were first incubated on MRS agar for 24 h at 37 °C under anaerobic conditions. From the grown isolates, agar blocks were cut and transferred to Mueller-Hinton agar Petri dishes containing the above-mentioned target cultures. The diameter of the agar blocks was 7 mm. The plates were aerobically incubated at 37 °C for 24 h. The inhibition activity of LAB isolates was evaluated by measuring inhibition zone around their blocks. Isolates with an inhibition zone greater than 10 mm are considered to have antimicrobial activity **(Menberu *et al.*, 2021).** The paper disk soaked with sterile distilled water was used as the negative control. In this case, bacteria grow on the agar without any inhibition.

Statistical Analyses

Results were expressed as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was carried out using one-way ANOVA and Tukey's HSD tests. One-way analysis of variance (ANOVA) was done to analyze the variation of the means between the experimental samples. Tukey's HSD test was used to differentiate between the mean values. All the analyses were done using XLSTAT (free trial version 2022, Addinsoft, Inc., Brooklyn, NY, USA). p value < 0.05 was considered statistically significant.

RESULTS

Collection of apple varieties

In the present study, lactic acid bacteria were isolated from both ripe and unripe apple samples collected from two regions (eastern and western) of eco-biodiverse Georgia featuring different soil-climatic zones. Table 1 provides a list of the apple samples collected, and obtained LAB isolates, used in the study.

Table 1 Different types of apple samples collected from various regions of Georgia

Sampling location: Ambrolauri Municipality	**Apple varieties (unripe)**	**Obtained LAB isolates №**
Ambrolauri city	Antonovka (Origin: Russia)	1(1), 1(2)
Village Nikortsminda	Sakartvelos Pioneri (Local) (Aleksidze, 2015)	4 (2), 5 (1)
Village Bostana	Bostana (Local) (Aleksidze, 2015)	7(3)
Village Mukhli	Kitra (Local) (Aleksidze, 2015)	11 (2)
Village Shaori	Wild apple (Mazhalo) (Local) (Aleksidze, 2015)	17 (2)
Village Chkvishi	Winter White Callville (Origin: Europa) (Aleksidze, 2015)	22 (4)
Village Chrebalo	Winter gold Parmain also named as winter Shafrana (origin: England) (Aleksidze, 2015)	47 (2)
Village Akhalsopeli	Lechkhumuri Sinapi (Local) (Aleksidze, 2015)	52 (2) (further identified as *Lactiplantibacillus plantarum* 52)
Ambrolauri city	Royal Short-stemmed (Origin: Europa) (Aleksidze, 2015)	no isolate
Village Khotevi	Rachuli vashli (Local) (Aleksidze, 2015)	no isolate
Sampling location: Gori Municipality	**Apple varieties (ripe)**	
Village Rekha	Jonagold (Origin: USA) (Goginava and Khidesheli, 2019)	no isolate
	Aporte (origin: Ukraine) (Aleksidze, 2015)	no isolate
	Georgian Sinap (Local) (Goginava and Khidesheli, 2019)	53 (1) (further identified as *Lactiplantibacillus plantarum* 53)
Village Uplistsikhe	Kekhura (Local) (Goginava and Khidesheli, 2019)	55 (3)
Village Skra	Antonovka (Origin: Russia)	56 (3)
VillageTortiza	Champagne Renette (Origin: Germany) (Aleksidze, 2015)	62 (1)
Village Qitsnisi	Banan Winter (Origin: USA) (Aleksidze, 2015)	63 (2)
	Jonagold (Origin: USA) (Goginava and Khidesheli, 2019)	65 (3)
	Semerenko (Origin: Russia)	70 (3)
Village Karaleti	Kekhura (Local) (Goginava and Khidesheli, 2019)	74 (3), 76 (1) (further identified as *Lactiplantibacillus plantarum* 74, *Lactiplantibacillus plantarum* 76)
Village Khidistavi	Golden Delicious (Origin: USA) (Aleksidze, 2015)	80 (1)

Isolation of lactic acid bacteria, and determination of their optimal growth temperature and glucose fermentation

Isolates obtained from the aforementioned apple varieties throughout Georgia had both cocci and rod shapes upon microscopy. Four of them with milk isolates were Gram-positive, catalase-negative, and had an optimal growth at 37 °C, indicating that they were lactic acid bacteria. From apple samples, only four isolates (cell shape – short bacilli) had small (1–3 mm) round, white or yellowish colonies on the Petri plates. Along with the general characteristics of the LAB, they expressed the feature of facultative anaerobes with respect to oxygen. According to glucose fermentation, four isolates of apple origin (*Lpb. plantarum* 52, *Lpb. plantarum* 53, *Lpb. plantarum* 74, and *Lpb. plantarum* 76) represent homo fermenter cultures since only lactic acid was produced from glucose and no gas or ethanol production was observed. In the case of milk isolates, all of them were heterofermentative.

Identification of Isolates of Lactic Acid Bacteria

Pure isolates obtained from apple peels were identified by genus-specific PCR. A PCR amplicons electropherogram is represented in Fig. 1.
As is seen in all four isolates, as in the positive control, the 1500 bp fragment was amplified, which indicates that all four experimental isolates belong to the genus *Lactobacillus.*

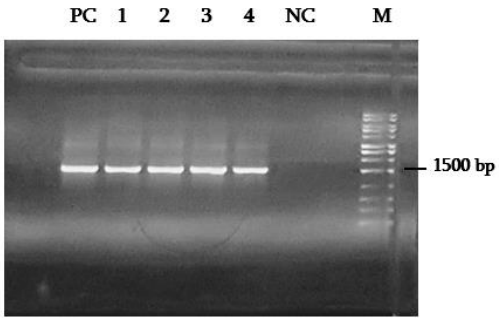

Figure 1 Gel electrophoresis of IDL04F/IDL03R PCR products in 1% agarose gel: PC-positive control (*Levilactobacillus brevis* 15, Bokulich et al., 2015); *Lactobacillus* isolates: 1 - 52, 2 - 53, 3 - 74 and 4 - 76; NC - negative control; M - 1kb DNA Ladder (Solis BioDyne, Estonia).

Intact cell MALDI-TOF mass spectrometry (ICMS) analysis showed that three *Lactobacillus* isolates (52, 53, and 76) belonged to *Lactiplantibacillus pentosus* or *Lpb. plantarum* or *Lpb. paraplantarum* and *Lactobacillus* isolate 74 is identified as *Levilactobacillus brevis*. For specification of the results, 16S rDNA sequencing was additionally done. Based on sequence analysis of the purified amplicons, all of these isolates appeared to be *Lactiplantibacillus plantarum,* which was confirmed by BLAST analysis of the sequence results. As the Table 2 shows, the isolates are 99.88% - 100%, identical to the *Lactiplantibacillus plantarum* strain in the database (Table 2).

Table 2 Results of a BLAST analysis of 16S rRNA gene sequences from four isolates

Isolates	Sequence bp	Description	Max Score	Total score	Query coverage	E value	Per. identity	Accession
16S - 52	813 bp	***Lactiplantibacillus plantarum* strains**	1502	1502	100%	0.0	100%	**OL798083.1** OL719063.1 MZ286591.1 OL589524.1 OL589263.1
16S - 53	802 bp	***Lactobacillus* sp. strain**	1482	**1**482	100%	0.0	100%	MK405704.1 MK397507.1
		***Lactiplantibacillus plantarum* strains**	1482	1482	100%	0.0	100%	MK332083.1 MK332073.1 AB854171.1 AB618817.1 **OL798083.1**
BaSu - 74	839 bp	***Lactiplantibacillus plantarum* strains**	1550	1550	100%	0.0	100%	OM802824.1 OM791722.1 OM760921.1 OM760769.1 OM760745.1 OM758304.1 OM757919.1 OM736085.1 OM721826.1
16S - 76	811 bp	***Lactiplantibacillus plantarum* strains**	1495	1495	100%	0.0	100%	MK332083.1 MK332073.1 AB854171.1 AB618817.1 OL798083.1 OL719063.1

Tolerance of Lactic Acid Bacteria to Acidic Conditions and to Bile Salt Concentrations

In present research, almost all selected LAB isolates were able to grow at pH 2.0 except for two strains of milk origin, *L. brevis* 10 and *L. brevis* 51. *L. brevis* 10 completely lost its resistance after 60-90 min incubation time while *L. brevis* 51 did not show acid tolerance during any of the incubation periods (please see table 3).

Table 3 Tolerance of lactic acid bacteria isolates to pH 2.0 for different incubation periods and viability of *Lactobacillus* sp. in the presence of different concentrations of bile salts

	Incubation time, min				**Incubation time 4 h**					**Incubation time 24 h**			
	pH 2.0				**Varying Bile Salt Concentration (in %)**								
Selected LAB	0 (Control)	30	60	90	0 (Control)	0.3%	0.5%	1%	1.5%	0.3%	0.5%	1%	1.5%
Levilactobacillus brevis 10	+	+	-	-	+	+	+	+	+	+	-	-	-
Levilactobacillus brevis 15	+	+	+	+	+	+	+	+	+	+	-	-	-
Levilactobacillus brevis 18	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 40	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactobacillus fermentum 44	+	+	+	+	+	+	+	+	+	+	-	-	-
Levilactobacillus brevis 49	+	+	+	+	+	+	+	+	+	+	-	-	-
Levilactobacillus brevis 51	+	-	-	-	+	+	+	+	+	+	-	-	-
Lactiplantibacillus plantarum 52	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus plantarum 53	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 57	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 63	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus plantarum 74	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus plantarum 76	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 85	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 88	+	+	+	+	+	+	+	+	+	+	-	-	-
Lactiplantibacillus pentosus 92	+	+	+	+	+	+	+	+	+	+	-	-	-

Legend: "+" Growth and "-" No growth/ No survival

All of the isolates were also able to survive in 0.3, 0.5, 1.0, and 1.5% bile salt after 4h incubation time, while after 24h no growth of any isolate was observed in 0.5-1–1.5% bile salt as shown in Table 3.

Antibiotic Resistance / Susceptibility

Antibiotic Resistance results of the selected *Lactobacillus* sp. isolates are shown in Table 4. All of them were found to be resistant to ciprofloxacin and streptomycin. In the case of erythromycin, all selected *Lactobacillus* strains showed susceptibility except the *Lpb. pentosus* 57, isolated from the fermented milk.

Table 4 Resistance of *Lactobacillus* sp. isolates to antibiotics

Strain	Inhibition zone diameter, mm								
	Name of antibiotic								
	Oxytetracycline	Ciprofloxacin	Bacitracin	Gentamicin	Streptomycin	Neomycin	Tetracycline	Erythromycin	Rifampicin
L. brevis 10	0±0.0 R	0±0.0 R	15±0.2 S	10±0.2 R	0±0.0 R	10±0.6 R	19±0.2 S	22±0.3 S	24±0.7 S
L. brevis 15	0±0.0 R	0±0.0 R	15±0.4 S	11±0.5 I	0±0.0 R	9±0.3 R	15±0.3 S	22±0.2 S	22±0.1 S
L. brevis 18	16±0.4 S	0±0.0 R	18±0.5 S	12±0.5 I	0±0.0 R	11±0.4 I	18±0.3 S	25±0.5 S	27±0.3 S
Lpb. pentosus 40	14±0.5 S	0±0.0 R	14±0.5 S	12±0.7 I	0±0.0 R	10±0.4 R	18±0.7 S	22±0.4 S	15±0.8 S
L. fermentum 44	14±0.3 S	0±0.0 R	14±0.4 S	11±0.4 I	0±0.0 R	11±0.5 I	17±0.5 S	26±0.6 S	24±0.3 S
L. brevis 49	15±0.5 S	0±0.0 R	14±0.6 S	9±0.5 R	0±0.0 R	12±0.5 I	20±0.8 S	23±0.4 S	21±0.4 S
L. brevis 51	13±0.4 I	0±0.0 R	19±0.4 S	9±0.3 R	0±0.0 R	10±0.6 R	19±0.4 S	21±0.5 S	16±0.4 S
Lpb. plantarum 52	15±0.3 S	0±0.0 R	10±0.8 R	9±0.9 R	0±0.0 R	10±0.5 R	16±0.5 S	22±0.2 S	16±0.5 S
Lpb. plantarum 53	16±0.5 S	0±0.0 R	13±0.7 I	9±0.4 R	0±0.0 R	9±0.4 R	15±0.9 S	19±0.3 S	17±0.6 S
Lpb. pentosus 57	16±0.5 S	0±0.0 R	14±0.3 S	10±0.5 R	0±0.0 R	10±0.7 R	21±0.5 S	0±0.0 R	18±0.4 S
Lpb. pentosus 63	15±0.5 S	0±0.0 R	15±0.9 S	9±0.4 R	0±0.0 R	8±0.5 R	18±0.3 S	25±0.4 S	20±0.2 S
Lpb. plantarum 74	15±0.8 S	0±0.0 R	10±1.5 R	0±0.0 R	0±0.0 R	0±0.0 R	16±0.5 S	21±0.4 S	17±0.3 S
Lpb. plantarum 76	0±0.0 R	0±0.0 R	11±0.9 I	8±0.5 R	0±0.0 R	10±0.8 R	18±0.4 S	20±0.5 S	16±0.4 S
Lpb. pentosus 85	0±0.0 R	0±0.0 R	13±0.7 I	9±0.6 R	0±0.0 R	12±0.3 I	0±0.0 R	24±0.3 S	22±0.3 S
Lpb. pentosus 88	12±0.3 I	0±0.0 R	13±0.2 I	10±0.3 R	0±0.0 R	12±0.6 I	15±0.5 S	26±0.4 S	21±0.5 S
Lpb. pentosus 92	0±0.0 R	0±0.0 R	14±0.6 S	10±0.5 R	0±0.0 R	10±0.3 R	15±0.3 S	20±0.6 S	22±0.3 S

Values are presented as mean ± SD.
Legend: S-susceptible (≥14 mm), I-intermediate (11–13 mm), and R-resistant (≤10 mm)

Antagonistic Activity of LAB against Food-Borne Pathogenic Bacteria

The antibacterial activity shown by the selected *Lactobacillus* sp. isolates is presented in Table 5. According to the inhibitory action on pathogens, the results showed that only two *Lactobacillus* sp. isolates, in particular, fermented milk *L. brevis* 51 and apple *Lactiplantibacillus plantarum* 52, had inhibitory activity against *Salmonella enterica* ATCC 14028. Growth of *Proteus mirabilis* ATCC 12453 and *Shigella flexneri* ATCC 12022 were inhibited only by *Lpb. plantarum* 76, isolated from apple sample.
L. brevis 51 and *Lpb. plantarum* 52, *Lpb. plantarum* 53, *Lpb. plantarum* 74 showed low antibacterial activity against *Klebsiella pneumoniae* ATCC 13833. Only *L. brevis* 18 was able to inhibit *Staphylococcus aureus* ATCC 25923, while together with *Lpb. plantarum* 76 it was moderately active against *Klebsiella pneumoniae* ATCC 13833. The *Lactiplantibacillus plantarum* 76 also showed moderate activity as compared to others against *Streptococcus pyogenes* ATCC 21059 and *Escherichia coli* ATCC 25922.
Almost all *Lactobacillus* strains showed significant inhibition against *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853, including 2 *Lactobacillus* strains (*L. brevis* 10, *L. brevis* 15) with strong inhibitory activity.

Table 5 Antimicrobial activity of *Lactobacillus* sp. isolates

Strain	Pathogen									
	Salmonella enterica ATCC 14028	*Klebsiella pneumoniae* ATCC 13833	*Bacillus cereus* ATCC 10876	*Proteus mirabilis* ATCC 12453	*Streptococcus pyogenes* ATCC 21059	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923	*Shigella flexneri* ATCC 12022	*Escherichia coli* ATCC 25922
Negative control	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
L. brevis 10	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	25.3 ± 0.5^{a}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	25.4 ± 0.5^{a}	25.2 ± 0.2^{a}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
L. brevis 15	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	25.1 ± 0.2^{a}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	25.5 ± 0.5^{a}	25.4 ± 0.5^{a}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
L. brevis 18	0.0 ± 0.0^{e}	15.9 ± 0.1^{b}	16.3 ± 0.4^{b}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	25.2 ± 0.3^{a}	16.2 ± 0.2^{b}	12.5 ± 0.5^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
L. brevis 51	15.9 ± 0.9^{b}	11.3 ± 0.6^{d}	12.3 ± 0.4^{cd}	0.0 ± 0.0^{e}	12.3 ± 0.4^{cd}	0.0 ± 0.0^{e}	12.3 ± 0.3^{cd}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	12.5 ± 0.4^{c}
Lpb. plantarum 52	11.9 ± 0.4^{cd}	11.8 ± 1.2^{cd}	16.2 ± 0.3^{b}	0.0 ± 0.0^{e}	12.4 ± 0.4^{cd}	16.4 ± 0.4^{b}	12.4 ± 0.5^{cd}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
Lpb. plantarum 53	0.0 ± 0.0^{e}	12.4 ± 0.4^{cd}	12.4 ± 0.4^{cd}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	16.3 ± 0.4^{b}	12.3 ± 0.3^{cd}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	12.4 ± 0.4^{cd}
Lpb. plantarum 74	0.0 ± 0.0^{e}	12.4 ± 0.4^{c}	12.2 ± 0.3^{cd}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	16.3 ± 0.3^{b}	12.4 ± 0.4^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
Lpb. plantarum 76	0.0 ± 0.0^{e}	16.4 ± 0.4^{b}	16.3 ± 0.52^{b}	12.4 ± 0.4^{c}	16.3 ± 0.3^{b}	0.0 ± 0.0^{e}	16.3 ± 0.4^{b}	0.0 ± 0.0^{e}	12.3 ± 0.3^{cd}	16.3 ± 0.5^{b}

Legend: Means ± standard deviation (SD) in the table with different alphabet letters indicate the significant difference at $p < 0.05$.

DISCUSSION

In the present study, lactic acid bacteria were isolated from both ripe and unripe apple samples collected from two regions (eastern and western) of eco-biodiverse Georgia featuring different soil-climatic zones (Table 1). Although the apple orchards were 150–200 km apart, similar cultures were isolated from the same apple species, namely *Lactiplantibacillus plantarum* 52 from Lechkhumuri Sinapi (Village Akhalsopeli, Ambrolauri Municipality), *Lactiplantibacillus plantarum* 53 from Georgian Sinap (Village Rekha, Gori Municipality) Besides, *Lactiplantibacillus plantarum* 74, 76 were obtained from Kekhura (Village Karaleti). LAB are a natural resident on the surface of fruits; therefore, they represent an important source of probiotics. In recent years, many scientists have studied fruits in this direction. According to **Fessard *et al.* (2019)**, three of the 77 isolates of lactic acid bacteria isolated from fruits and vegetables belonged to *Lactobacillus plantarum*. In addition, *Lactobacillus brevis* was obtained from the mango fruit by **Liao *et al.* (2016)**; a strain of *Lactobacillus plantarum* CM-3 was

isolated from strawberry fruit **(Chen *et al*., 2020)**; **Choi *et al*. (2018)** isolated strains of *Lactobacillus plantarum* from black raspberries and **Tosin and Temitope (2018)** from banana, orange, and watermelon; **Verón *et al*. (2017)** managed to obtain it from Mexican fruits, namely *Opuntia ficus-indica*. A new strain of lactic acid bacterium, *Lactobacillus musae* sp., was obtained from banana fruit **(Chen *et al*., 2017)**. Moreover, a source of *Lactobacilli* is various pickles, with **Prakash *et al*. (2020)** reported in their work about a source of *L. fermentum* in lemon pickles. On the other hand, **Barache *et al*. (2020)** studied blackberries (*Rubus* sp.), Fresh figs (*Ficus carica*), and pickled pears (*Opuntia ficus-indica*), for isolating of lactic acid bacteria such as *Lactobacillus plantarum* and *Lactobacillus paracasei*.

Also noteworthy is the fact that endophytic LABs are promising resources for the biocontrol of post-harvest spoilage caused by fungus **(Chen *et al*., 2020)**. In addition, LAB strains have the ability to inhibit several pathogens, including *L. monocytogenes*, *Salmonella*, and *E. coli* O157: H7 **(Ayala *et al*., 2019)**, which is important in mixed (bacterial) diseases. They can also be used in food production as far as probiotics are non-pathogenic microorganisms, they meet the recommendations of the World Health Organization and EFSA **(Fernández-Pacheco *et al*., 2021)** and generally are recognized as safe (GRAS) microorganisms **(Stiles and Holzapfel, 1997)**.

Until now, there was no available information regarding the microbiota on the surface of apple varieties in Georgia. The main objective of this study was to characterize the important parameters of lactic acid bacteria isolated from apple samples and to select *Lactobacillus* sp. as a starter culture with the purpose of juice enrichment. **Di Cagno *et al*. (2013)** suggested using autochthonous starter cultures of fruits and vegetables, which mainly include lactic acid bacteria. This may be due to the fact that they have the ability to extend the shelf life, as well as to improve the nutritional (synthesis of exo-polysaccharides, increased antioxidant activity) and sensory characteristics (synthesis of aromatic compounds). Due to the great health benefits of probiotics, their use as a way to improve the functional properties of food has an increasing trend **(Afrin *et al*., 2021)**. It should also be noted that a good nutritional rate in turn plays an important role in maintaining the normal functioning of the body and preventing dysfunction caused by internal or external factors **(Muscaritoli, 2021)**.

In the present study, lactic acid bacteria were isolated from both ripe and unripe apple samples collected from two regions (eastern and western) of eco-biodiverse Georgia featuring different soil-climatic zones (Table 1). Although the apple orchards were 150–200 km apart, similar cultures were isolated from the same apple species. According to the characteristics described by **Holt *et al*. (1994)**, lactic acid bacteria belonging to the genus *Lactobacillus* must be Gram-positive, rod-shaped, and catalase negative as well as have the ability to break down sugars and produce acid. LAB should also be tolerant of pH, temperature, and bile salts. Based on these parameters, as well as on the amplification fragment of 1500 bp detected by gel electrophoresis, and comparison with the positive control (*Levilactobacillus brevis*) we can summarize that four of the 20 isolates belong to the genus *Lactobacillus.* Moreover, based on sequence analysis of the purified amplicons, all of these isolates appeared to be *Lactiplantibacillus plantarum*. This indicates the presence of *Lpb. plantarum* on the surface of apple varieties cultivated in Georgia.

First, an important step in the selection of probiotics is the ability to survive in the gastrointestinal tract, as gastric juice is generally considered to be one of the strongest barriers to probiotics, followed by bile acids in the small intestine **(Piano *et al*., 2011)**, after which probiotics reach their destination and are able to be metabolized in the human body and have a beneficial effect on the "host" **(Charteris *et al*., 1998; Argyri *et al*., 2013)**. It should be noted, however, that not all probiotic strains can survive in sufficient numbers to reach the gut microbiome, and even if they do, they can leave the body soon after **(Hughes, 2020)**.

Cell viability at different concentrations of pH and bile salts was determined by increasing the intensity of growth on the MRS agar during a 24-hour incubation period at 37 °C **(De *et al*., 2016)**. According to the results, all *Lactobacillus* isolates except for *L. brevis* 10, and 51, were found to have growth at pH 2 (Table 3). Milk and apple *Lactobacillus* samples were distinguished based on their tolerance to bile salts. All isolates were found to have the ability to grow on MRS agar containing a 0.3% concentration of bile salts during 4 h incubation period. *Lactobacillus* sp. were considered resistant to this concentration. As Table 3 shows, according to these two parameters, isolates obtained from similar species had similar results, in the example of *Lactiplantibacillus plantarum* 52 from Lechkhumuri Sinapi and *Lactiplantibacillus plantarum* 53 from Georgian Sinap. This characteristic play one of the most important roles in probiotic properties since it is known that the retention time of food in the small intestine is four hours and the value of intestinal bile concentration on average is considered to be 0.3% (0.3 kg per 100 ml) **(Prasad *et al*., 1998)**. It is also noteworthy that similar findings have been obtained by other researchers. **Abouloifa *et al*. (2020)** studied *Lactobacillus* strains isolated from naturally fermented Moroccan green olive brine. All of them showed high resistance up to 2% of bile salts.

In addition to pH and bile salt tolerance, in this work, we also studied various characteristics of probiotic bacteria, in particular resistance to antibiotics and antimicrobial activity. The safety of using strains as probiotics should be evaluated according to their antibiotic susceptibility profile and antibiotic resistance. The resistance of probiotic strains to some antibiotics can be used for both prophylactic and therapeutic purposes in the control of intestinal infections. Moreover, resistance of the *Lactobacillus sp.* to antibiotics underscores their potential to reduce the negative effects of antibiotic therapy on the bacterial ecosystem of the host organism **(El-Naggar, 2004)**.

A study of antibiotic dependence showed a diverse picture. Based on the inhibition zone, *Lactobacillus* sp. showed susceptibility to the following antibiotics: bacitracin– *L. brevis* 51 (19±0.4 mm), tetracycline – *L. brevis* 10, *L. brevis* 51 (19±0.2, 19±0.4 mm), *L. brevis* 49 (20±0.8 mm), and *Lpb. pentosus* 57 (21±0.5 mm), rifampicin–by *L. brevis* 10 and *L. fermentum* 44 (24±0.7, 24±0.3 mm), *L. brevis* 15, *Lpb. pentosus* 85, and *Lpb. pentosus* 92 (22±0.1, 22±0.3, 22±0.3 mm), *L. brevis* 18 (27±0.3 mm), *L. brevis* 49 and *Lpb. pentosus* 88 (21±0.4, 21±0.5 mm), and *Lpb. pentosus* 63 (20±0.2 mm). As for the classification according to resistance (R), all *Lactobacillus* sp. were found to be resistant to ciprofloxacin and streptomycin (Table 4). *Lpb. plantarum* 52, *Lpb. plantarum* 53, and *Lpb. plantarum* 74 isolated from the surface of the apple (presented in the table), had a susceptibility to oxytetracycline (15±0.3 mm, 16±0.5 mm, 15±0.8 mm), tetracycline (16±0.5 mm, 15±0.9, 16±0.5 mm), and rifampicin (16±0.5 mm, 17±0.6 mm, 17±0.3 mm), all four apple samples also showed a susceptibility to erythromycin (22±0.2 mm, 19±0.3 mm, 21±0.4 mm, 20±0.5 mm). The diameter of the tetracycline inhibitions was higher than that obtained for *Lpb. plantarum* 76 (18±0.4 mm). Moreover, *Lactobacillus* sp. isolated from apple varieties had both intermediate susceptibilities to some antibiotics. Comparing results to other scientists' experimental data, **Mohkam *et al*. (2021)** obtained that all bacterial samples isolated from native Iranian food were susceptible to erythromycin, our work showed an almost similar picture as well, except for one sample all of them were sensitive to this antibiotic. Approximately similar picture of erythromycin was shown by our samples with respect to gentamicin; Some isolates were intermediate susceptible. Like to an experiment conducted by **Mohkam *et al*. (2021)**, where three samples of *Lactobacillus, L. hilgardii, L. fermentum*, and *L. plantarum* were susceptible to gentamicin, while *L. casei* was resistant. According to the antibiotic susceptibility, *Lpb. plantarum* 52 showed a bit strong susceptibility (22±0.2) to erythromycin as compared to *Lpb. plantarum* 53 (19±0.3). Also, isolates belonging to *Lactiplantibacillus plantarum* obtained from the same apple varieties (Kekhura) showed a different picture. In particular, *Lpb. plantarum* 74 had susceptibility (15±0.8) to oxytetracycline, while *Lpb. plantarum* 76 was resistant to it (0±0.0).

A study by **Trias *et al*. (2008)** showed that lactic acid bacteria isolated from fruits and vegetables had the ability to inhibit food pathogens. Most of the LAB belonged to *Leuconostoc* spp. and *Lactobacillus plantarum*. Among the studying probiotic properties of *Lpb. plantarum*, antimicrobial potential played an important role. It is shown that these species have a wide range of antibacterial activity against many food spoilage bacteria (**Dinev *et al*., 2018**). They can inhibit both gram-positive and negative bacteria such as *Listeria monocytogenes, Staphylococcus aureus, Enterococcus, Bacillus, Clostridium, Pseudomonas aeruginosa, Helicobacter pylori, Yersinia enterocolitica, Campylobacter jejuni, Klebsiella, Salmonella, Shigella and Escherichia coli* (including E. coli 0157:H7) (**Garcia-Gonzalez *et al*., 2021).** According to a study conducted by **Kariyawasam *et al*. (2020)**, *L. brevis* had a high rate of survival in gastric conditions, as well as activity against selected pathogens, including *L. monocytogenes* ATCC 15313, *E. coli* O157: H4 FRIK 125, *S. aureus* KCCM 4051, *Salmonella Enteritidis* ATCC 13076. The present work aimed to study the antimicrobial activity of isolates, which was established using pre-selected pathogens via a diffusion method in agar. The study of the samples isolated from the apples showed different antibacterial activity (Table 5). In particular, *Lactiplantibacillus plantarum* 52, 53, 74 were found to have relatively good resistance to *Enterococcus faecalis* ATCC 29212 (16.4±0.4, 16.3±0.4, 16.3±0.3). *Lpb. plantarum* 52 and *Lpb. plantarum* 76 showed antibacterial activity against *Bacillus cereus* ATCC 10876 (16.2±0.3, 16.3±0.52). *Lpb. plantarum* 76 exhibited the ability to inhibit *Klebsiella pneumoniae* ATCC 13833 (16.4±0.4), *Streptococcus pyogenes* ATCC 21059 (16.3±0.3), *Pseudomonas aeruginosa* ATCC 27853 (16.3±0.4), and *Escherichia coli* ATCC 25922 (16.3±0.5). It should also be noted that only *Lpb. plantarum* 76 was found to inhibit the growth of *Proteus mirabilis* ATCC 12453 (12.4±0.4) and *Shigella flexneri* ATCC 12022 (12.3±0.3). In addition, except for *L. brevis* 51, *Lactiplantibacillus plantarum* 52 from Lechkhumuri Sinapi also showed inhibitory activity against *Salmonella enterica* ATCC 14028 (11.9±0.4).

This study also examined the relationship between apple *Lpb. plantarum* 52, *Lpb. plantarum* 53, *Lpb. plantarum* 74, and *Lpb. plantarum* 76. The purpose of this was to determine whether the samples of *Lactobacillus* sp. inhibit each other's activity. Finally, four isolates of the *Lactobacilli* were selected based on their probiotic properties making them a potential “candidate” for the fermentation of apple juices **(Tkesheliadze *et al*., 2022).**

CONCLUSION

The current study aimed to isolate, identify, and study some probiotic properties of lactic acid bacteria from the surface of both local and imported apple varieties throughout Georgia. Of the 20 isolates, four met the selected characteristics of probiotics. A comparison of the cultures isolated from the apple and fermented milk samples showed a similar picture. In particular, *Lpb. plantarum* 52, *Lpb. plantarum* 53, *Lpb. plantarum* 74, and *Lpb. plantarum* 76 and all milk isolates

except for *L. brevis* 10, and *L. brevis* 51 were distinguished by their ability to withstand the pH 2 of the gastric acid environment. All samples were also characterized by their tolerance to bile salts. In our study of antibiotic resistance/susceptibility, all *Lactobacillus* sp. were found to be susceptible to ciprofloxacin and streptomycin, and approximately the same results could be seen in regard to antimicrobial activity. In particular, the ability to inhibit the growth of *Salmonella enterica* ATCC 14028 was found in both milk *L. brevis* 51 and *Lactiplantibacillus plantarum* 52 of apple. Almost none of the isolated *Lactobacillus* sp. were characterized by the inhibition of growth against *Proteus mirabilis* ATCC 12453 and *Shigella flexneri* ATCC 12022–except for *Lactiplantibacillus plantarum* 76.

Thus, LAB autochthonous apple strains, like the cultures obtained from the milk samples, were distinguished by their tolerance to pH and bile salts and also had a well-defined resistance to selected antibiotics. They can be characterized by their different activity in regard to food pathogens. Therefore, it can be concluded that apples can be considered as source of lactic acid bacteria, suggesting significant technological potential for the production of apple juices with probiotic properties. The selected LAB isolates will be used for future studies, which include investigating the relationship of the apple juice fermentation at different periods and LAB isolates' cell viability. Future research should focus also on the optimization of probiotic cell use, considering key factors such as the effect of fermentation on total phenolic content (TPC), and the total antioxidant capacity, observation of the metabolism of sugars, and organic acids during fermentation. pH and titratable acidity should be studied before and after fermentation as well. Evaluating these properties will be used for future industrial applications of the selected probiotic microorganisms.

Acknowledgment: The article was developed within the Sustainable Agricultural and Food Systems (SAFS) Structured Doctoral Project, jointly offered by the University of Kassel (Germany) and the Agricultural University of Georgia. The research was funded by the Volkswagen Foundation and Shota Rustaveli National Science Foundation of Georgia (SRNSFG)–Contract No. 04/47.

REFERENCES

Abouloifa, H., Rokni, Y., Bellaouchi, R., Hasnaoui, I., Gaamouche, S., Ghabbour, N., ... & Asehraou, A. (2020). Technological properties of potential probiotic *Lactobacillus* strains isolated from traditional fermenting green olive. *Journal of microbiology, biotechnology and food sciences*, *9*(5), 884-889. https://doi.org/10.15414/jmbfs.2020.9.5.884-889

Afrin, S., Akter, S., Begum, S. and Hossain, M.N. (2021). The Prospects of *Lactobacillus oris* as a Potential Probiotic With Cholesterol-Reducing Property From Mother's Milk. *Front. Nutr*, 8, 619506. https://doi.org/10.3389/fnut.2021.619506

Aleksidze, G. (2015). Agrobiodiversity of Georgia. edited by Keshelashvili O, Magradze D, Giorgadze A, Epitashvili T. *Tbilisi: Georgia Academy of Agricultural sciences Exhibition catalogue*. pp.61-65 *https://www.gaas.dsl.ge/pdf/catalogue.pdf*

Argyri, A.A., Zoumpopoulou, G., Karatzas, K.A., Tsakalidou, E., Nychas, G.J.E., Paganou, E.Z., et al. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbio*, 33, 282–291. https://doi.org/10.1016/j.fm.2012.10.005

Aspri, M., Papademas, P. and Tsaltas, D. (2020). Review on Non-Dairy Probiotics and Their Use in Non-Dairy Based Products. *Fermentation*, 6 (1), 30. https://doi.org/10.3390/fermentation6010030

Bayless, T.M., Brown, E., & Paige, D.M. (2017). Lactase Non-persistence and Lactose Intolerance. *Curr Gastroenterol Rep*, 19 (5), 23. https://doi.org/10.1007/s11894-017-0558-9

Ayala, D.I., Cook, P.W., Franco, J.G., Bugarel, M., Kottapalli, K.R., Loneragan, G.H., Brashears, M.M., Nightingale, K.K. (2019). A Systematic Approach to Identify and Characterize the Effectiveness and Safety of Novel Probiotic Strains to Control Foodborne Pathogens. *Front Microbiol*, 10, 1108. https://doi.org/10.3389/fmicb.2019.01108

Barache, N., Ladjouzi, R., Belguesmia, Y., Bendali, F., Drider, D. (2020). Abundance of *Lactobacillus plantarum* strains with beneficial attributes in blackberries (Rubus sp.), fresh figs (Ficus carica), and prickly pears (Opuntia ficus-indica) grown and harvested in Algeria. *Probiotics and Antimicrobial Proteins*, pp. 1-10. https://doi.org/10.1007/s12602-020-09632-z

Barbosa, J., Borges, S., & Teixeira, P. (2015). *Pediococcus acidilactici* as a potential probiotic to be used food industry. *International Journal of Food Science & Technology*, 50, 1151-1157. https://doi.org/10.1111/ijfs.12768

Biswal, P., Pal, A., Das, A.P. (2021). Screening for Probiotic Potential of *Lactobacillus Rhamnosus* Strain CRD4. *Biointerface Research in Applied Chemistry*, 11 (3), 10174 – 10184. https://doi.org/10.33263/BRIAC113.1017410184

Bokulich, N., Amiranashvili, L., Chitchyan, K., Ghazanchyan, N., Gagelidze, N., Sadunishvili, T., et al. (2015). Microbial biogeography of the transnational fermented milk Matsoni. *Food Microbiology*, 50, 12-19. https://doi.org/10.1016/j.fm.2015.01.018

Charteris, W.P., Kelly, P.M., Morelli, L., Collins, J.K. (1998). Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *J. Food Prot*, 61, 1636–1643. https://doi.org/10.4315/0362-028X-61.12.1636

Chen, C., Cao, Z., Li, J., Tao, C., Feng, Y., Han, Y. (2020). A novel endophytic strain of *Lactobacillus plantarum* CM-3 with antagonistic activity against Botrytis cinerea on strawberry fruit. *Biol. Control*, 148, 104306. https://doi.org/10.1016/j.biocontrol.2020.104306

Chen, Y.S., Wang, L.T., Liao, Y.J., Lan, Y.S., Chang, C.H., Chang, Y.C., et al. (2017) . *Lactobacillus musae* sp. nov., a novel lactic acid bacterium isolated from banana fruits. *Int. J. Syst. Evol. Microbio*, 67, 5144–5149. https://doi.org/10.1099/ijsem.0.002429

Choi, H.R., Chung, Y.H., Yuk, H.G., Lee, H., Jang, H.S., Kim, Y., Shin, D. (2018). Characterization of *Lactobacillus plantarum* strains isolated from black raspberry and their effect on BALB/c mice gut microbiota. *Food Sci. Biotechnol*, 27, 1747–1754. https://doi.org/10.1007/s10068-018-0420-3

De Man, J.C., Rogosa, M. and Sharpe, M.T. (1960). A medium for the cultivation of *lactobacilli*. *J. Appl. Bacteriol*, 23, 130–135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

De, S., Sena, S., Bhowmik, I., Maity, S., Bhowmik, S. (2016). Isolation, Characterisation and Identification of *Lactobacilli* spp. And Study of Its Pharmacological Activity in Vitro. *Int J Recent Sci Res*, 7(11), 14296-14298.

De Vries, M.C., Vaughan, E.E., Kleerebezem, M., & De Vos, W.M. (2006). *Lactobacillus plantarum*-Survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, 16, 1018-1028. https://doi.org/10.1016/j.idairyj.2005.09.003

Di Cagno, R., Coda, R., De Angelis, M., Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol*, 33, 1–10. https://doi.org/10.1016/j.fm.2012.09.003

Di Cagno, R., Filannino, P., Gobbetti, M. (2015). Vegetable and fruit fermentation by lactic acid bacteria. In: Mozzi F, Raya RR, Vignolo GM, editors. *Biotechnology of Lactic Acid Bacteria: Novel Applications*. 2nd ed. Chichester, United Kingdom: John Wiley & Sons Ltd pp. 216–230. https://doi.org/10.1002/9781118868386.ch14

Dinev, T., Beev, G., Tzanova, M., Denev, S., Dermendzhieva, D., & Stoyanova, A. (2018). Antimicrobial activity of *Lactobacillus plantarum* against pathogenic and food spoilage microorganisms: a review. Bulgarian Journal of Veterinary Medicine, 21(3).

Egorov, N. S. (1965). Microbe antagonists and biological methods of determining antibiotic activity. *Microbe antagonists and biological methods of determining antibiotic activity.*

El-Naggar, M.Y.M. (2004). Comparative study of probiotic cultures to control the growth of *Escherichia coli* O157:H7 and *Salmonella typhimurium*. *Asian Network for Scientific Information Biotechnol*, 3, 173-180. https://doi.org/10.3923/biotech.2004.173.180

Fernández-Pacheco, P., Ramos Monge, I.M., Fernández-González, M., Poveda Colado, J.M. and Arévalo-Villena, M. (2021). Safety Evaluation of Yeasts With Probiotic Potential. *Front Nutr*, 8, 659328. https://doi.org/10.3389/fnut.2021.659328

Fessard, A., Remize, F. (2019). Genetic and technological characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables. *Int. J. Food Microbiol*, 301, 61–72. https://doi.org/10.1016/j.ijfoodmicro.2019.05.003

Fidanza, M., Panigrahi, P., & Kollmann, T. R. (2021). *Lactiplantibacillus plantarum*–Nomad and Ideal Probiotic. *Frontiers in Microbiology*, 2911. https://doi.org/10.3389/fmicb.2021.712236

Garcia-Gonzalez, N., Battista, N., Prete, R., & Corsetti, A. (2021). Health-promoting role of *lactiplantibacillus plantarum* isolated from fermented foods. *Microorganisms*, 9(2), 349.

Goginava L, Khidesheli Z. (2019). Fruit orchard planting and maintenance. edited by Gigauri M. *Tbilisi*: pp. 6-7. *https://elkana.org.ge/uploads/page/217/pdf/eng/publication/Fruit.pdf*

Hartemink, R., Domenech, V.R., Rombouts, F.M. (1997). LAMVAB - A new selective medium for the isolation of *lactobacilli* from faeces. *J Microbiol Meth*, 29(2), 77-84. https://doi.org/10.1016/S0167-7012(97)00025-0

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams ST. (1994). Bergey's manual of determinative bacteriology. Baltimore, Williams and Wilkins. 786-788.

Hughes, R.L. (2020). A Review of the Role of the Gut Microbiome in Personalized Sports Nutrition. *Front. Nutr*, 6, 191. https://doi.org/10.3389/fnut.2019.00191

Iorizzo, M., Lombardi, S.J., Ganassi, S., Testa, B., Ianiro, M., Letizia, F., Succi, M., Tremonte, P., Vergalito, F., Cozzolino, A., et al. (2020). Antagonistic Activity against *Ascosphaera apis* and Functional Properties of *Lactobacillus kunkeei* Strains. *Antibiotics*, 9, 262. https://doi.org/10.3390/antibiotics9050262

Kariyawasam, K. M. G. M. M., Yang, S. J., Lee, N. K., & Paik, H. D. (2020). Probiotic properties of *Lactobacillus brevis* KU200019 and synergistic activity with fructooligosaccharides in antagonistic activity against foodborne pathogens. Food science of animal resources, 40(2), 297.

Kenny, M.T., Mayer, G.D., Dulworth, J.K., Brackman, M.A., Farrar, K. (1992). Evaluation of the teicoplanin broth microdilution and disk diffusion susceptibility tests and recommended interpretive criteria. *Diagn Microbiol Infect Dis.* Sep-Oct;15(7):609-12. doi: 10.1016/0732-8893(90)90038-w.

Kwon, H.S., Yang, E.H., Yeon, S.W., Kang, B.H., and Kim, T.Y. (2004). Rapid identification of probiotic *Lactobacillus* species by multiplex PCR using species-specific primers based on the region extending from 16S rRNA through 23S rRNA. *FEMS Microbiol Lett*, 239, 267-275.

Lamont, J.R., Wilkins, O., Bywater-Ekegard, M., and Smith, D.L. (2017). From yogurt to yield: potential applications of lactic acid bacteria in plant production. *Soil Biol.Biochem*, 111, 1–9. https://doi.org/10.1016/j.soilbio.2017.03.015

Liao, X., Guo, L., Ye, Z., Qiu, L., Gu, F., Lin, J. (2016). Use of autochthonous lactic acid bacteria starters to ferment mango juice for promoting its probiotic roles. *Prep. Biochem. Biotechnol*, 46, 399–405. https://doi.org/10.1080/10826068.2015.1045615

Liong, M.T., Shah, N.P. (2005). Acid and Bile Tolerance and Cholesterol Removal Ability of *Lactobacilli* Strains. J. Dairy Sci, 88, 55–66. https://doi.org/10.3168/jds.S0022-0302(05)72662-X

Mainville, I., Arcand, Y., Farnworth, E.R. (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *Int J Food Microbiol*, 99, 287-296. https://doi.org/10.1016/j.ijfoodmicro.2004.08.020

Maragkoudakis, P.A., Zoumpopouloua, G., Miarisa, C., Kalantzopoulosa, G., Potb, B. and Tsakalidoua , E. (2006). Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal*, 16, 189– 199.

McFrederick, Q.S., Thomas, J.M., Neff, J.L., Vuong, H.Q., Russell, K.A., Hale, A,R., Mueller, U.G. (2017). Flowers and wild megachilid bees share microbes. *Microb Ecol*, 73, 188– 200. https://doi.org/10.1007/s00248-016-0838-1

McFrederick, Q.S., Vuong H.Q. (2018). Rothman, J.A. *Lactobacillus micheneri* sp. nov., *Lactobacillus timberlakei* sp. nov. and *Lactobacillus quenuiae* sp. nov., lactic acid bacteria isolated from wild bees and flowers. *Int J Syst Evol Microbiol*, 68, 1879 – 1884. https://doi.org/10.1099/ijsem.0.002758

Menberu, M. A., Liu, S., Cooksley, C., Hayes, A. J., Psaltis, A. J., Wormald, P. J., & Vreugde, S. (2021). *Corynebacterium accolens* has antimicrobial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* pathogens isolated from the sinonasal niche of chronic rhinosinusitis patients. *Pathogens*, 10(2), 207.

Mohkam, M., Firoozabadi, S. G., Ghasemi, Y., & Gholami, A. (2021). THE PROBIOTIC AND NUTRITIONAL CONTENT OF SOURO, AN IRANIAN TRADITIONAL FOOD. *Journal of microbiology, biotechnology and food sciences*, e2627-e2627.

Muscaritoli, M. (2021). The Impact of Nutrients on Mental Health and Well-Being: Insights From the Literature. *Front. Nutr*, 8, 656290. https://doi.org/10.3389/fnut.2021.656290

Nanasombat, S., Phunpruch, S. and Jaichalad T. (2012). Screening and identification of lactic acid bacteria from raw seafoods and Thai fermented seafood products for their potential use as starter cultures. *Songklanakarin J. Sci. Technol*, 34, 255–62.

Ngea, G.L.N., Yang, Q., Tchabo, W., Castoria, R., Zhang, X., Zhang, H. (2021). *Leuconostoc mesenteroides* subsp. mesenteroides LB7 isolated from apple surface inhibits *P. expansum* in vitro and reduces patulin in fruit juices. *International Journal of Food Microbiology*, 339. https://doi.org/10.1016/j.ijfoodmicro.2020.109025

Nguyen, B.T., Bujna, E., Fekete, N., Tran, A.T.M., Rezessy-Szabo, J.M., Prasad, R. and Nguyen, Q.D. (2019). Probiotic Beverage From Pineapple Juice Fermented With *Lactobacillus* and *Bifidobacterium* Strains. *Front. Nutr*, 6, 54. https://doi.org/10.3389/fnut.2019.00054

Piano, M.D., Carmagnola, S., Ballarè, M., Sartori, M., Orsello, M., Balzarini, M., et al. (2011). Is microencapsulation the future of probiotic preparations? The increased efficacy of gastro-protected probiotics. *Gut Microbes*, 2(2), 120-123. https://doi.org/10.4161/gmic.2.2.15784

Pontonio, E., Raho, S., Dingeo, C., Centrone, D., Carofiglio, V.E. and Rizzello, C.G. (2020). Nutritional, Functional, and Technological Characterization of a Novel Gluten- and Lactose-Free Yogurt-Style Snack Produced With Selected Lactic Acid Bacteria and Leguminosae Flours. *Front. Microbiol*, 11, 1664. https://doi.org/10.3389/fmicb.2020.01664

Portilha-Cunha, M.F., Macedo, A. and Malcata, F.X. (2020). A Review on Adventitious Lactic Acid Bacteria from Table Olives. *Foods*, 9(7), 948. https://doi.org/10.3390/foods9070948

Prabhurajeshwar, C., Chandrakanth, R.K. (2017). Probiotic Potential of *Lactobacilli* with Antagonistic Activity against Pathogenic Strains: An in Vitro Validation for the Production of Inhibitory Substances. *Biomed. J*, 40, 270–283. https://doi.org/10.1016/j.bj.2017.06.008

Prakash, V., Veedu, A.P., Babu, P., Jothish, A., Nair, S.S., Suhail, A., et al. (2020). *Lactobacillus fermentum* strains from rice water and lemon pickle with potential probiotic properties and wastewater treatment applications. https://doi.org/10.21203/rs.3.rs-37101/v1

Prasad, J., Gill, H., Smart, J., Gopal, P.K. (1998). Selection and characterisation of *Lactobacillus and Bifidobacterium* strains for use as probiotics. *Int. Dairy J*, 8, 993-1002. https://doi.org/10.1016/S0958-6946(99)00024-2

Rhaiem, N., Chahboun, N., Inekach, S., & Ouhssine, M. (2016). Identification and characterisation of lactic acid bacteria isolated from cow milk and olives brine. *Journal of Materials and Environmental Science*, 7(5), 1504-1509.

Rzepkowska, A., Zielińska, D., Ołdak, A., & Kołozyn-Krajewska, D. (2017). Organic whey as a source of Lactobacillus strains with selected technological and antimicrobial properties. *International Journal of Food Science & Technology*, 52, 1983-1994. https://doi.org/10.1111/ijfs.13471

Samedi, L., & Charles, A. L. (2019). Evaluation of Technological and Probiotic Abilities of Local Lactic Acid Bacteria. *Journal of Applied & Environmental Microbiology*, *7*(1), 9-19. https://doi.org/10.12691/jaem-7-1-3

Samedi, L., & Charles, A.L. (2019). Isolation and characterization of potential probiotic *Lactobacilli* from leaves of food plants for possible additives in pellet feeding. *Annals of Agricultural Sciences*, 64(1), 55-62. https://doi.org/10.1016/j.aoas.2019.05.004

Shahbandeh, M. Data from: Global production of fruit by variety selected 2019. Available online at: https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety/ (accessed 12, January 2021)

Stiles, M.E., Holzapfel, W.H. (1997). Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol*, 36, 1-29. https://doi.org/10.1016/S0168-1605(96)01233-0

Tyler, C.A., Kopit, L., Doyle, C., Yu, A.O., Hugenholtz, J., & Marco, M.L. (2016). Polyol production during heterofermentative growth of the plant isolate *Lactobacillus florum* 2F. *Journal of applied microbiology*, *120*(5), 1336-1345. https://doi.org/10.1111/jam.13108

Tambekar, D. H., & Bhutada, S. A. (2010). An evaluation of probiotic potential of *Lactobacillus* sp. from milk of domestic animals and commercial available probiotic preparations in prevention of enteric bacterial infections. *Recent Research in Science and Technology*, *2*(10), 82-88.

Tao, X., Tian, L., Zhan, H., He, Y., Zhong, C., & Wei, H. (2021). In vitro and in vivo assessments of Artemisia argyi fermented with *Lactobacillus plantarum* WLPL01 as an alternative anti-*Salmonella* agent. *Food Control*, *126*, 108079. https://doi.org/10.1016/j.foodcont.2021.108079

Theingi, P.P., Aye, S.M., Win, H.H. Efficacy of lactic acid bacteria from edible fruits against antibiotics and pathogenic bacteria. J. Myanmar Acad. Arts Sci. (2019) Vol. XVII. No.4

Tkesheliadze, E., Gagelidze, N., Sadunishvili, T., & Herzig, C. (2022). Fermentation of apple juice using selected autochthonous lactic acid bacteria. *Ukrainian Food Journal*, 11(1). https://doi.org/10.24263/2304-974x-2022-11-1-7

Tosin, O.C., & Temitope, O. S. (2018). Molecular Characterisation and Antagonistic Activity of Fructophilic Lactic Acid Bacteria Isolated from Selected Fruits. *Microbiology Research Journal International*, 1-17. https://doi.org/10.9734/MRJI/2018/44484

Trias, R., Baneras, L., Badosa, E., Montesinos, E. (2008). Bioprotection of golden delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *Int. J. Food Microbiol*, 123, 50–60. https://doi.org/10.1016/j.ijfoodmicro.2007.11.065

Verón, H.E., Di Risio, H.D., Isla, M.I., Torres, S. (2017). Isolation and selection of potential probiotic lactic acid bacteria from *Opuntia ficus*-indica fruits that grow in Northwest Argentina. *LWT Food Sci. Technol*, 84, pp. 231-240. https://doi.org/10.1016/j.lwt.2017.05.058

Vural, H.C., Ozgun, D. (2011). An improving DNA isolation method for identification of anaerobic bacteria in human colostrum and faeces samples. *J. Med. Genet. Genom*, 3, 95-100.

Yang, Y., Liu, Y., Zhou, S., Huang, L., Chen, Y., Huan, H. (2019). Bile salt hydrolase can improve Lactobacillus plantarum survival in gastrointestinal tract by enhancing their adhesion ability. *FEMS Microbiol. Lett*, 366(8), fnz100. https://doi.org/10.1093/femsle/fnz100

Yang, J., Tan, H., Cai, Y. (2016). Characteristics of lactic acid bacteria isolates and their effect on silage fermentation of fruit residues. *J. Dairy Sci*, 99, 5325-5334. https://doi.org/10.3168/jds.2016-10952

Yu, A.O., Leveau, J.H.J., Marco, M.L. (2019). Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria. *Environmental microbiology reports*, 12, 16-29. https://doi.org/10.1111/1758-2229.12794

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H., Mattarelli, P., ... & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus Beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. https://doi.org/10.1099/ijsem.0.004107