Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.24821

Probiotic lactobacilli in faeces of breastfed babies

Nazan Tokatli DEMIROK[1]*, Muhammed Zeki DURAK[2], Muhammet ARICI[2]

Abstract

Balanced colonization of the gastrointestinal tract in the newborn is very important for the acquisition of an immune system in infancy. *Lactobacillus* spp. is useful in human nutrition because of its potential probiotic and functional features. In this research, 27 strains were identified representing 104 species of *Lactobacillus* isolated from baby feces. The probiotic and functional properties of isolates were investigated. In this study, the sample consisted of 14 children aged 3-47 weeks who were breastfed. Strains were determined phenotypically by testing arginine hydrolysis, salt tolerance, production of gas from glucose, and growth at 15 and 45 °C. Isolated strains were genotypically characterized as *Lactobacillus paracase*i subsp. *paracasei* (41), *L. casei* (17), *L. fermentum* (24), *Lactobacillus* spp. (11), and *L. rhamnosus* (11) using 16S rDNA sequence analysis. Several strains of *L. fermentum,* and a majority of the strains of *L. rhamnosus* and *L. casei*/*L. paracasei* subsp. *paracasei* were able to produce hydrogen sulfide. Almost all strains showed antibacterial activity against the enteric pathogens *Escherichia coli* O157:H7, *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Enteritidis. In this research, lactobacilli isolated from babies had probiotic properties.

Keywords: Lactobacilli; baby feces; probiotic.

Practical Application: The isolates' contribution to the formation of the basis of a healthy life was investigated.

1 Introduction

The intestinal microbiota is an active ecosystem with high complexity, which is composed of more than 400 bacterial species and an average of 10^{14} microorganisms (Pinto et al., 2006). This microbiota plays crucial roles in preventing colonization from potential pathogenic microorganisms and developing the immune system. The microbiota is associated with allergy and infection risks in early life stages and even with later risks of obesity. The FAO of the UN and the WHO (WHO/FAO) describe 'probiotics' as living microorganisms that provide a health benefit to the host when administered in adequate amounts (Munoz-Quezada et al., 2013). Dairy products, cereal products, juices, processed meat, and vegetable products, nutritional supplements, and drugs have been considered as a source of probiotic (Zendeboodi et al., 2020). Different terms about probiotics have also been proposed in recent years. postbiotics (healthful metabolites of probiotics), probioactives (probitic bacterial lysates that eliciting immune response), paraprobiotics (inactivated/dead cells of probiotics) psycobiotic (mental healthful probiotics) (Champagne et al., 2018; Zendeboodi et al., 2020).

To be classified as probiotic, a microorganism must be resistant to bile and gastric acid and non-pathogenic. It should have technological processes that lead to antimicrobial effects through the potential for adhesion to intestinal epithelial tissues (Kirtzalidou et al., 2011). The genus *Lactobacillus* is generally confirmed as safe for human consumption and exhibits probiotic properties (Jovanović et al., 2015). *Lactobacillus* bacteria are a species of lactic acid bacteria that are anaerobic or microaerophilic bacteria, non-spore-forming, catalase-negative, and Gram-positive (Davoodabadi et al., 2015). Most *Lactobacillus* species are tenants of the animal and human intestine, non-pathogenic, and support the intestinal microbiota (Verdenelli et al., 2009). Studies on the gut microbiome show that *Lactobacillus* are an invariable content of the intestinal microbiota (Archer & Halami, 2015). Grom et al. (2020), reported that *lactobacillus* spp. can contribute to the reduction of postprandial glycemia in healthy individuals. Balthazar et al. (2021a) indicated that *L.casei* (especially with inulin), can reduce chemically induced mouse colon carcinogenesis. Vasconcelos et al. (2019) suggested that some probiotic lactobacillus strain was reduced oxidative stress in the liver lungs, and gut. Lactobacilli have an ability to adhere to mucosal layers and intestinal epithelium, which is presumptively necessary to provide health benefits. Probiotics must have antibacterial activities against some pathogenic bacteria (Jovanović et al., 2015).

The main aim of this research was to use phenotypic (biochemical tests) and genotypic (sequencing of PCR products) methods to identify *Lactobacillus* strains isolated from healthy infant stools in Turkey. In addition, the assessment of tolerance of each probiotic strain to bile salts and acidity were undertaken to show their survivability in the small intestine and colon Moreover, their antibiotic susceptibility, antimicrobial activity against pathogens, and hydrophobicity were investigated to screen for potential probiotic isolates. The probiotic properties were investigated with in vitro assays.

Received 20 Apr., 2021
Accepted 22 May, 2021
[1]*Department of Nutrition and Dietetics, Health School, Namık Kemal University, Süleymanpaşa, Tekirdağ, Turkey*
[2]*Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, Davutpaşa Campus, Esenler, Istanbul, Turkey*
*Corresponding author: ntokatli@nku.edu.tr

2 Materials and methods

2.1 Sample collection

In this study, the sample consisted of 14 children aged 3-47 weeks who were breastfed. Samples were stored at -20 ºC and processed within 5 h.

2.2 Bacterial isolation

For isolation of *Lactobacillus* spp., 1 g of feces from a healthy child was blended and diluted in a saline solution (0.1% peptone and 0.85% NaCl), plated on MRS agar (pH 5.7, Merck), and incubated at 37 ºC for 48-72 h under anaerobic conditions. Isolates with characteristic morphology were randomly picked from distinct colonies, sub-cultured, and isolated three times on the MRS agar medium. The colonies picked were white, medium-small, catalase-negative, and Gram-positive. Pure culture spots were cryopreserved in MRS with 15% (w/v) glycerol at -40 ºC.

2.3 Phenotypic characterization of isolates

Strains were characterized phenotypically by trial production of gas from glucose, salt tolerance, growth at 15 and 45 °C on MRS, the configuration of the lactic acid enantiomers, and hydrolysis of arginine (Merck) (Megazyme International Ireland, 2014).

2.4 Genotypic characterization of isolates

Isolates were genotypically characterized by sequence analysis of the 16S rDNA gene. After DNA extraction, DNA purity was measured using a Nano Drop ND 1000 UV spectrophotometer (Shimadzu, Japan) and was stored at −20 °C until PCR analysis. The reaction mixture consisted of 3 pmol primers, 2 µL DNA lysate, 21 µL H_2O (nuclease-free water), and 25 µL 2X Taq Master Mix (Vivantis, Malaysia) in a final volume of 50 µL. Amplification was performed in a programmable thermal cycler (Applied Biosystem, ABD). 16S rDNA gene sequences were also amplified using the forward primer, 5'GCA AAC AGG ATT AGA TAC CC-3' and reverse primer, 5'AGG AGG TGA TCC AAC CGC A-3' [12,13], or 27f, 5'CCG AAT TCG TCG ACA ACA GAG TTT GAT CMT GGA-3', and 1492r, 5'CCC GGG ATC CAG CTT TAC CTT GTT ACG ACT T-3' (Lane, 1991). Each reaction was incubated for 2 min at 94 °C (initial denaturation), subjected to 30 cycles of 1 min at 94 °C (denaturation), 1 min at 55 °C (annealing), and 1 min at 72 °C (extension), and a final extension for 5 min at 72 °C. The PCR products were electrophoresed in 1% agarose gels (Meyers et al., 1976) for 30-60 min in 0.5 X TBE (Tris-borate-EDTA) at 100 V-325 mA (using Thermo Scientific Electrophoresis Systems) followed by ethidium bromide staining (0.5 g/mL). The resulting image was captured using a gel imaging system (BIO-RAD, France). Alignment of the 16S-rRNA sequence was directed using the ABI 3130 genetic analyzer in the BLASTN program from the NCBI web site (http://www.ncbi.nlm.nih.gov).

2.5 Characterization of potential probiotic strains

Resistance to low pH

Resistance to low pH (acid tolerance) of isolates was defined as reported previously by Charteris et al. (1998) with some changes. The effect of acidity was evaluated in MRS broth (Merck) adjusted to pH 3.0 with 0.1 M HCl. Overnight *Lactobacillus* cultures were inoculated in acidified MRS broth and at the beginning and after 3 h of incubation, samples were serially diluted. After plating on MRS agar, they were incubated under anaerobic conditions for 48 h at 37 °C for the definition of viability.

Bile tolerance

The bile tolerance assay of isolates was tested as described by Kotsou et al. (2008). Tolerance to bile salts was tested by inoculation in MRS broth enriched with 0.3% (w/v) Oxbile. Samples (0.1 mL at 0 h and 24 h) were removed and incubated at 37 °C. Later, bacterial cells were measured by colony counts (cfu/mL) on the plates.

Detection of antibacterial activity

For the determination of antimicrobial activity, the agar spot test method described by Arici et al. (2004) was used with some modifications. The following pathogens were used: *Salmonella* Enteritidis (ATCC 13076), *Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC 25922), *Escherichia coli* O157:H7 (NCTC 12900), and *Staphylococcus aureus* (ATCC 25923). Activated *Lactobacillus* spp. cultures were transplanted onto MRS agar (containing 0.2% glucose) plates. Test bacteria were incubated in nutrient broth at a suitable temperature for 24 h. Later, the culture was centrifuged and filtered and the supernatants were plated in diameter (mm) wells.

Antibiotic susceptibility

The antibiotic sensitivity of the isolated *Lactobacillus* strains was determined using antibiotic discs (CHL; Chloramphenicol 30 µg, TET; Tetracycline 30 µg, PEN; Penicillin G 10U, KAN; Kanamycin 30 µg, and STR; Streptomycin 10 µg) (Bioanalyse) on MRS agar plates. Plates were inoculated with 100 µL of active culture and incubated for 24 h. The plates were kept at room temperature for 1 hour and then incubated at 37 °C for 36 h. After incubation, the diameters of the free zones were measured using a caliper gage (Sadrani et al., 2014).

Cell surface hydrophobicity

The hydrophobicity assays as described by Pérez et al. (1998) were used to detect the ability of *Lactobacillus* isolates to adhere to hydrocarbons. First, 0.4 mL xylene and 2 mL of the bacterial suspension were vortexed for 120 s (A_0). After waiting a few minutes, the xylene phase (aqueous phase) was collected to measure its absorbance at 600 nm (A).

The percentage of hydrophobicity (%) was calculated using the Formula 1

$$H\% = \left[\left(Ao - A \right) / Ao \right] \times 100 \quad (1)$$

H_2S production

The ability to produce H_2S was tested according to Lee & Simard (1984). A loop full of activated *Lactobacillus* spp. culture was streaked onto slant Triple Sugar Iron Agar and incubated

for two weeks at 30 °C. The H_2S production was determined based on the darkening of the colonies.

Detection of H_2O_2 production

H_2O_2 production was determined using a spectrophotometric method described by Toksoy et al. (1999). Activated *Lactobacillus* cultures were centrifuged at 5000 rpm for 15 min and 1 mL 1 N sulfuric acid, 1 mL 0.01 M ammonium molybdate, and 1 mL 1 M potassium iodide was added and the absorbance at 400 nm was measured using a spectrophotometer.

Statistical analysis

Tests were performed in triplicate and results elaborated as the mean±standard error of the mean of three experiments. Data of each assay were analyzed by one-way analysis of variance ANOVA (at log10 transformation of the viable counts acid and bile tolerance were examined). The statistical significance for antibacterial activity analysis was assessed by Student's t test. Statistical analysis was performed using the program PASW Statistics version 18. Differences were considered significant at p value <0.05.

3 Results and discussion

3.1 Phenotypic characterization of bacterial isolates

The strains of catalase-negative, Gram-positive rods were differentiated into species, according to the production of CO_2 from glucose, hydrolysis of arginine, growth in MRS broth at 15 and 45 °C, lactic acid isomer production, and salt tolerance.

3.2 Genotypic characterization of bacterial isolates

Identification of 104 *Lactobacillus* species isolated from baby feces was determined on the basis of 16S rDNA gene sequencing. The isolated strains identified were *Lactobacillus paracase*i subsp. *paracasei* (41), *L. casei* (17), *L. fermentum* (24), *Lactobacillus* spp. (11), and *L. rhamnosus* (11) based on 16S rDNA sequence analysis. In addition, 16s rRNA gene sequences of *L. casei* (GenBank accession numbers: MG551255.1, MF108641.1, KT291151.1, KF673503.1, and MF108648.1), *L. paracasei* (GenBank accession numbers: MG770031.1, CP016355.1, and CP025582.1), *Lactobacillus* spp. (GenBank accession numbers: MF424343.1, KY283156.1, KY283155.1, MG754429.1, HQ177095.1, MF108804.1, and MG757514.1), *L. fermentum* (GenBank accession numbers: MG708112.1 KY364812.1, CP025592.1, MF575842.1, CP016803.1, MF164138.2, and MF575843.1), and *L. rhamnosus* (GenBank accession number: CP021426.1, LC333198.1, CP006804.1, MG685875.1, and AP011548.1) were identified in the study. *Lactobacillus* isolates were classified mainly as *L. rhamnosus* with 95-100% similarity (five strains), *L. paracasei* ssp. *paracasei* with 99-100% similarity (three strains), *L. fermentum* with 89-100% similarity (seven strains), *L. casei* with 99-100% similarity (five strains), and *Lactobacillus* ssp. with 99-100% similarity (seven strains). Some researchers isolated *L. fermentum*, *L. rhamnosus*, and *L. paracasei* ssp. *paracasei* from infant feces (Arici et al., 2004; Pérez et al., 1998). Wall et al. (2007) identified *L. casei*/*paracasei*, *L. rhamnosus*, *L. gasseri*, *L. reuteri*, *L. salivarius*, and *L. brevis* isolates using 16S rDNA from infants (ages ranging from 3 days to 3 months). Khalil et al. (2007) identified *L. plantarum*, *L. acidophilus*, *L. brevis*, *L. paracasei* subsp. *paracasei*, *L. pentosus*, *L. fermentum*, *E. faecalis, and E. faecium* isolates from infants (ages ranging from 3 to 6 months).

3.4 Characterization of potential probiotic isolates

Acid and bile tolerance

Acid and bile tolerances are desired properties of probiotics. After digestion, probiotics must overcome the bile salts and gastrointestinal tract and at the same time have a beneficial effect on health (Papadimitriou et al. 2015). As shown in Table 1, *Lactobacillus* was resistant to the simulated gastrointestinal system conditions because there was no significant decrease in cell counts. The survival rates of twenty-seven isolates at pH 3 are indicated in Table 1. Most isolates showed high tolerance to high acid conditions after 3 h of incubation. The results demonstrate that all strains showed high resistance to pH 3. Similarly, 22 isolates had a survival rate of 79% or higher in the presence of 0.3% bile salt during a 3 h incubation, suggesting the majority of isolates tolerated up to 0.3% bile salt. These results indicate that the isolates were able to tolerate gastrointestinal system conditions.

Only twelve isolates out of the twenty-seven could grow at pH 3 after 48 h of incubation. IF199 showed the best survival rate over the 3 h at pH 3.0 (increase of 6.54%), while IF2 was the most acid-sensitive strain (decrease of 5.19%) after the 3 h incubation. These results are similar to several previous studies (Kotsou et al., 2008; Kirtzalidou et al., 2011). Prezzi et al. (2020), found that *L. rhamnosus* strains had high survival rate (> 74.6–86.4%) in Minas Frescal cheeses from the 7th day of storage, after gastrointestinal conditions (gastric solution at pH 2.0 for 30 min.) Balthazar et al. (2019) indicated that lactic acid bacteria could survive the storage period in fermented semi-skimmed sheep milk strawberry beverages, but only *L. plantarum* maintained good viability after simulated digestion.

The results of bile salt tolerance showed that most of the isolates (fifteen) could grow in the presence of bile, five isolates (IF2, IF70, IF73, IF97, IF160) could not survive, and the remaining isolates had slightly decreased survival. This suggests that most isolates can tolerate high bile concentrations with minimum cell count loss. Špano vá et al. (2015) studied 30 *Lactobacillus* isolates from fecal samples and found that all *L. fermentum* isolates showed good tolerance to bile (1% bile; 82.7% surviving cells) with the exception of *L. rhamnosus* isolates, which showed a high susceptibility to bile salts. Kirtzalidou et al. (2011) noticed that *L. paracasei*, *L. rhamnosus,* and *L. fermentum* isolates could grow in 0.3% bile. Similarly, Fuochi et al. (2015) reported that *L. fermentum* and *L. rhamnosus* could survive in 0.5% bile. Archer & Halami (2015) also reported the tolerance of *L. fermentum* strains to 0.3% bile. *L. fermentum* 650 and *L. frementum* 511, which were isolated from human feces, survived after 24 h of incubation (98.72% and 89.24%) in bile. Bao et al. (2010) found that eleven strains of ninety *L. fermentum* isolates grew in the presence of 0.3% oxgall. Delgado et al. (2015) reported that the *L. casei* strain grew despite the presence of 4% bile. Jovanović et al. (2015) found that

Table 1. pH and bile tolerance of isolates.

Strain	pH 3		Bile		Control	
	0 h	3 h	0 h	24 h	0 h	24 h
IF2	5.78 ± 0.10	5.48[a] ± 0.02	5.76 ± 0.09	-	5.71 ± 0.10	6.88 ± 0.03
IF7	5.43 ± 0.01	5.46[a] ± 0.01	5.32 ± 0.03	5.43[b] ± 0.07	5.60 ± 0.03	7.03 ± 0.06
IF10	5.76 ± 0.08	5.95[a] ± 0.08	6.13 ± 0.03	6.69[b] ± 0.06	6.13 ± 0.06	8.29 ± 0.09
IF14	6.24 ± 0.02	6.28[a] ± 0.10	5.18 ± 0.07	5.25[b] ± 0.08	5.38 ± 0.11	7.45 ± 0.05
IF24	5.25 ± 0.05	5.47[a] ± 0.07	6.15 ± 0.03	6.00[b] ± 0.04	6.01 ± 0.09	7.26 ± 0.01
IF32	5.85 ± 0.01	5.92[a] ± 0.02	6.09 ± 0.05	6.36[b] ± 0.10	6.10 ± 0.02	8.50 ± 0.01
IF37	6.98 ± 0.07	6.96[a] ± 0.03	5.23 ± 0.06	5.49[b] ± 0.03	5.27 ± 0.02	7.10 ± 0.02
IF41	5.66 ± 0.06	5.59[a] ± 0.01	5.96 ± 0.07	6.28[b] ± 0.09	6.04 ± 0.05	8.02 ± 0.06
IF57	6.13 ± 0.06	6.17[a] ± 0.02	6.33 ± 0.06	6.51[b] ± 0.02	6.21 ± 0.03	7.87 ± 0.03
IF70	5.87 ± 0.01	5.80[a] ± 0.03	6.03 ± 0.07	-	6.00 ± 0.07	6.90 ± 0.07
IF73	6.12 ± 0.05	5.96[a] ± 0.01	6.07 ± 0.07	-	6.55 ± 0.10	8.16 ± 0.14
IF74	6.39 ± 0.03	6.26[a] ± 0.02	5.84 ± 0.04	5.11[b] ± 0.07	5.88 ± 0.01	7.28 ± 0.01
IF86	5.77 ± 0.01	5.59[a] ± 0.05	5.68 ± 0.02	5.16[b] ± 0.07	5.67 ± 0.08	6.82 ± 0.08
IF96	5.33 ± 0.02	5.41[a] ± 0.01	6.45 ± 0.11	6.65[b] ± 0.09	6.14 ± 0.08	8.33 ± 0.02
IF97	5.11 ± 0.02	4.93[a] ± 0.04	5.37 ± 0.03	-	5.30 ± 0.15	7.29 ± 0.04
IF105	5.66 ± 0.03	5.43[a] ± 0.01	5.16 ± 0.07	5.66[b] ± 0.35	6.16 ± 0.04	8.71 ± 0.05
IF111	5.86 ± 0.02	5.82[a] ± 0.02	6.12 ± 0.05	5.84[b] ± 0.07	6.21 ± 0.02	7.74 ± 0.03
IF120	5.34 ± 0.03	5.29[a] ± 0.08	6.34 ± 0.03	6.20[b] ± 0.09	6.42 ± 0.06	7.50 ± 0.02
IF132	5.37 ± 0.01	5.45[a] ± 0.05	5.32 ± 0.07	5.73[b] ± 0.08	5.35 ± 0.05	7.06 ± 0.06
IF160	5.42 ± 0.02	5.38[a] ± 0.04	6.13 ± 0.11	-	6.06 ± 0.09	7.65 ± 1.02
IF164	5.60 ± 0.02	5.52[a] ± 0.01	5.26 ± 0.02	5.03[b] ± 0.08	5.87 ± 0.13	6.77 ± 0.04
IF169	5.38 ± 0.01	5.42[a] ± 0.01	7.11 ± 0.22	7.15[b] ± 0.14	7.12 ± 0.02	8.76 ± 0.01
IF174	5.41 ± 0.01	5.47[a] ± 0.03	5.48 ± 0.03	4.33[b] ± 0.04	5.43 ± 0.08	6.59 ± 0.03
IF199	6.42 ± 0.02	6.84[a] ± 0.06	5.63 ± 0.03	6.86[b] ± 0.07	5.59 ± 0.01	8.84 ± 0.02
IF205	7.12 ± 0.09	7.05[a] ± 0.14	5.65 ± 0.04	5.88[b] ± 0.13	5.63 ± 0.03	6.78 ± 0.07
IF214	7.28 ± 0.03	7.18[a] ± 0.09	6.12 ± 0.06	7.08[b] ± 0.03	6.44 ± 0.05	8.61 ± 0.04
IF217	5.49 ± 0.07	5.51[a] ± 0.01	7.17 ± 0.10	7.39[b] ± 0.06	7.08 ± 0.05	8.46 ± 0.01

values represent the $\log_{10}$ transformation of viable counts; ± Indicates standard deviation from the mean (n = 3).; One-way repeated measures ANOVA at $\log_{10}$ cfu ml^{-1}; [a]No significant decrease of the viable counts of strain was observed after 3 h at pH 3 of incubation compared to the initial inoculum ($p > 0.05$) ; [b]Significant differences ($p < 0.05$) between $\log_{10}$ cfu measurements in MRS + 0.3% oxbile and control group.

L. paracasei NRIC 1942 was resistant to concentrations of 0.5% - 2.0% bile salts. Presti et al. (2015) indicated that *L. fermentum* was less resistant whereas *L. rhamnosus* showed a high resistance level to bile salts. Davoodabadi et al. (2015) previously reported that *L. rhamnosus*, *L. paracasei*, and *L. fermentum* strains isolated from 95 healthy infant feces survived in 0.3% bile. Shokryazdan et al. (2014) reported that *L. casei* (isolated from infant feces) and *L. fermentum* (isolated from human milk) strains showed good bile tolerance. Kotsou et al. (2008) reported similar results for *Lactobacillus* strains resistance to bile. Verdenelli et al. (2009) and Munoz-Quezada et al. (2013) noticed that *L. paracasei* and *L. rhamnosus* resisted high bile salt concentrations.

Antimicrobial activity

The antagonism ability of the 27 probiotic candidate bacterial isolates was ordered according to the size of the zones of inhibition against five pathogenic bacteria (Table 2).

The majority of isolates expressed a clear inhibition zone against the *E. coli* ATCC 25922, *E. coli* O157:H7 NCTC 12900, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 25923, and *S.* Enteritidis ATCC 13076 indicator strains. Many of the *Lactobacillus* strains showed higher antagonistic effects against the test pathogen bacteria than the reference strain *L. delbrueckii* subsp. *bulgaricus* (ATCC 11842). Only three isolates showed no effect against three different strains. In this test, the inhibition zones from the agar spot method were between 6 mm and 20 mm. All *Lactobacillus* cultures showed antimicrobial effects to all the enteropathogenic bacteria except IF7, IF10, and IF32. But, the degree of inhibition was different among the *Lactobacillus* strains. IF32 showed the highest antimicrobial activity against *L. monocytogenes* ATCC 7644, *S.* Enteritidis ATCC 13076, and *E. coli* O157:H7 NCTC 12900. On the other hand, IF41 and IF199 showed the highest antimicrobial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. *S.* Enteritidis ATCC 13076 and *E. coli* O157:H7 NCTC 12900 were susceptible to all of the lactobacilli strains. Balthazar et al. (2021b) reported that, the addition of probiotic *L. casei* culture; decreased the lipolysis, promoted the proteolysis, and increased the volatile compounds. Davoodabadi et al. (2015) previously determined that *L. rhamnosus GG*, *L. fermentum 89-1*, and *L. paracasei 6-4b* isolated from healthy infant feces inhibits the growth of *S.* Enteritidis H7. Sadrani et al. (2014) found that strains of *L. paracasei* and *L. rhamnosus* were able to exhibit high antimicrobial activity against *S. aureus* and *E. coli*. Ren et al. (2014) and Presti et al. (2015) also screened overnight cultures of lactobacilli strains demonstrating their antibacterial

effects against *S. aureus* and *E. coli*. Some researchers reported that *L. fermentum* isolated from human feces could have antagonistic activity to *L. monocytogenes* (Kirtzalidou et al., 2011; Muhammad et al., 2011). In accordance with previous reports, this study exhibited that *Lactobacillus* strains have inhibitory activity against enteropathogenic bacteria.

Antibiotic susceptibility

To further investigate probiotic characteristics, the antibiotic sensitivity of 27 *Lactobacillus* strains was assessed. For this aim, the inhibition ability of five antibiotics were measured for isolates (Table 3). All isolates were susceptible to chloramphenicol, tetracycline, and penicillin whereas most isolates were resistant to kanamycin and streptomycin.

When considering the size of the inhibition zone, the isolates were less susceptible to kanamycin and streptomycin while highly susceptible to the antibiotics chloramphenicol, penicillin, and tetracycline. Among the strains, only IF41, IF70, IF73, IF74, and IF111 were sensitive to kanamycin and IF7, IF41, IF57, IF70, IF73, IF96, and IF111 were sensitive to streptomycin. Verdenelli et al. (2009) found that *L. rhamnosus IMC 501* and *L. paracasei IMC 502* were susceptible to tetracycline and Penicillin G and resistant to kanamycin. Several previous studies determined that all examined lactobacilli show high resistance to streptomycin (Arici et al., 2004; Zhou et al., 2005). Some researchers also found that certain *Lactobacillus* strains can be resistant to kanamycin (Birri et al. 2013). Previous studies report a lower resistance of the lactobacilli to chloramphenicol and tetracycline (Birri et al., 2013; Kirtzalidou et al., 2011; Pithva et al., 2014). Our findings on the susceptibility of the lactobacilli to antibiotics are in agreement with previous studies, as described above.

Cell surface hydrophobicity

The hydrophobicity potential of 27 *Lactobacillus* strains was examined with xylene. IF74 showed the lowest hydrophobicity with a hydrophobicity of 4.20% However, IF199, IF169, IF7, and IF96 had higher percentages of hydrophobicity (34.86%, 29.12%, 29.10%, and 28.74%) compared to other isolates.

Table 2. Degree of inhibition of tested potential human pathogens by isolate.

Antibacterial activity of *Lactobacillus* strains determined by agar spot assay					
Isolate Number	Zone of inhibition (mm) ± SD				
	E. coli ATCC 25922	*E. coli* O157:H7 NCTC 12900	*L. monocytogenes* ATCC 7644	*S. aureus* ATCC 25923	*S.* Enteritidis ATCC 13076
IF2	$14.4^{a} \pm 0.3$	10.0 ± 0.9	$6.7^{c} \pm 0.6$	$16.3^{d} \pm 0.8$	10.9 ± 1.1
IF7	-	10.7 ± 0.7	10.5 ± 0.6	11.5 ± 0.2	$12.3^{e} \pm 0.6$
IF10	8.3 ± 0.3	$8.8^{b} \pm 0.3$	-	13.6 ± 0.5	$14.0^{e} \pm 0.3$
IF14	$15.2^{a} \pm 0.3$	10.7 ± 0.3	$7.9^{c} \pm 0.5$	$17.1^{d} \pm 0.8$	$14.0^{e} \pm 1.1$
IF24	$15.2^{a} \pm 0.6$	10.7 ± 0.6	$8.1^{c} \pm 0.3$	$17.8^{d} \pm 0.3$	$13.5^{e} \pm 0.9$
IF32	$17.3^{a} \pm 0.8$	$24.0^{b} \pm 0.5$	$15.4^{c} \pm 0.6$	-	$21.6^{e} \pm 0.9$
IF37	10.2 ± 0.6	$9.4^{b} \pm 0.6$	10.3 ± 0.7	8.1 ± 0.4	9.3 ± 1.2
IF41	12.6 ± 0.5	$9.0^{b} \pm 0.5$	$11.3^{c} \pm 0.9$	$21.5^{d} \pm 0.5$	7.9 ± 0.4
IF57	$15.1^{a} \pm 0.5$	10.3 ± 0.6	$8.0^{c} \pm 0.8$	$18.3^{d} \pm 0.5$	$14.5^{e} \pm 0.5$
IF70	$13.4^{a} \pm 0.4$	$8.7^{b} \pm 0.3$	$13.1^{c} \pm 0.3$	14.2 ± 0.8	11.2 ± 0.7
IF73	$13.6^{a} \pm 0.9$	$8.3^{b} \pm 0.3$	$13.5^{c} \pm 0.7$	$14.3^{d} \pm 0.6$	11.4 ± 0.3
IF74	$16.3^{a} \pm 0.3$	11.3 ± 0.7	9.5 ± 0.6	$17.7^{d} \pm 0.9$	$6.8^{e} \pm 1.4$
IF86	12.3 ± 0.9	$10.0^{b} \pm 0.5$	$8.6^{c} \pm 0.5$	$14.7^{d} \pm 0.9$	10.7 ± 0.5
IF96	$17.4^{a} \pm 0.7$	10.9 ± 1.0	$12.6^{c} \pm 0.7$	12.1 ± 0.7	10.5 ± 0.4
IF97	$14.1^{a} \pm 0.9$	$10.0^{b} \pm 0.7$	$6.3^{c} \pm 0.8$	$16.0^{d} \pm 1.2$	10.5 ± 0.6
IF105	$13.7^{a} \pm 0.5$	$7.3^{b} \pm 0.9$	$13.5^{c} \pm 0.4$	13.2 ± 0.6	$13.9^{e} \pm 0.4$
IF111	$13.7^{a} \pm 0.4$	$8.6^{b} \pm 1.0$	$13.5^{c} \pm 0.8$	$14.8^{d} \pm 1.0$	11.1 ± 0.3
IF120	$14.3^{a} \pm 1.1$	$10.5^{b} \pm 0.8$	$6.5^{c} \pm 0.7$	$16.8^{d} \pm 0.6$	10.3 ± 0.6
IF132	11.2 ± 0.9	11.5 ± 1.0	$12.3^{c} \pm 0.3$	13.0 ± 1.6	$13.1^{e} \pm 0.3$
IF160	$14.6^{a} \pm 0.7$	$10.2^{b} \pm 0.2$	$6.6^{c} \pm 0.7$	$16.0^{d} \pm 0.3$	10.9 ± 0.7
IF164	13.1 ± 1.1	$9.5^{b} \pm 0.9$	$12.6^{c} \pm 0.4$	12.5 ± 0.5	9.7 ± 0.3
IF169	$17.0^{a} \pm 0.9$	$15.6^{b} \pm 0.3$	$14.8^{c} \pm 0.9$	$19.3^{d} \pm 0.6$	$17.1^{e} \pm 0.7$
IF174	$14.1^{a} \pm 0.2$	10.8 ± 0.4	$7.7^{c} \pm 0.9$	$15.9^{d} \pm 0.6$	$13.5^{e} \pm 0.9$
IF199	$20.1^{a} \pm 0.7$	$9.2^{b} \pm 0.7$	10.1 ± 0.7	11.3 ± 0.8	11.2 ± 1.0
IF205	11.2 ± 1.0	11.9 ± 0.9	$12.5^{c} \pm 0.7$	13.4 ± 0.3	$12.1^{e} \pm 0.6$
IF214	8.5 ± 0.3	$8.6^{b} \pm 0.3$	$9.5^{c} \pm 0.3$	9.9 ± 0.5	9.8 ± 0.6
IF217	11.3 ± 0.8	11.6 ± 0.3	$12.4^{c} \pm 1.2$	13.2 ± 0.5	$12.7^{e} \pm 0.9$
R.S.*	**10.6 ± 0.7**	**13.1 ± 0.4**	**9.8 ± 1.1**	**7.7 ± 0.5**	**9.6 ± 0.5**

*Reference strain: *L. delbrueckii* subsp. *bulgaricus* (ATCC 11842); ± Indicates standard deviation from the mean (n = 3); $^{a-e}$: Significant differences ($p < 0.05$) between the reference strain and the tested strains.

Table 3. Antibiotic susceptibility test of isolates.

	Diameter (mm) of inhibition zone				
	Antibiotic				
Isolate	Chloramphenicol	Penicillin	Tetracycline	Kanamycin	Streptomycin
IF2	24.5 ± 0.8	23.0 ± 0.9	27.3 ± 1.6	-	-
IF7	20.2 ± 1.3	21.6 ± 0.9	22.3 ± 0.4	-	7.3 ± 0.6
IF10	21.2 ± 0.6	24.8 ± 0.8	22.2 ± 0.9	-	-
IF14	21.3 ± 0.5	20.1 ± 1.3	17.1 ± 0.3	-	-
IF24	22.3 ± 0.9	18.6 ± 0.8	15.4 ± 0.8	-	-
IF32	21.2 ± 0.7	24.9 ± 1.2	22.5 ± 0.7	-	-
IF37	21.4 ± 1.3	24.9 ± 0.8	23.1 ± 0.3	-	-
IF41	21.1 ± 0.7	21.1 ± 0.7	19.2 ± 0.6	7.6 ± 0.5	9.2 ± 0.7
IF57	20.3 ± 0.6	24.4 ± 0.9	16.8 ± 1.2	-	9.5 ± 0.4
IF70	20.2 ± 0.6	22.4 ± 0.5	21.3 ± 1.3	7.2 ± 0.6	7.1 ± 0.7
IF73	20.8 ± 0.8	22.7 ± 0.8	22.0 ± 1.3	7.3 ± 0.3	7.5 ± 1.1
IF74	18.5 ± 1.1	17.8 ± 0.2	14.1 ± 0.4	7.9 ± 1.1	-
IF86	25.4 ± 0.5	22.6 ± 1.9	27.9 ± 0.8	-	-
IF96	20.7 ± 0.9	21.4 ± 1.4	18.7 ± 0.8	-	7.1 ± 0.6
IF97	25.0 ± 1.0	22.4 ± 0.6	27.8 ± 0.9	-	-
IF105	21.7 ± 0.2	24.5 ± 0.2	23.3 ± 1.7	-	-
IF111	21.4 ± 0.8	23.0 ± 0.7	21.7 ± 0.9	7.5 ± 0.4	7.8 ± 0.8
IF120	24.9 ± 0.9	22.7 ± 1.3	27.6 ± 1.4	-	-
IF132	20.3 ± 1.5	18.1 ± 0.2	17.5 ± 0.7	-	-
IF160	25.2 ± 0.9	22.9 ± 0.7	27.5 ± 1.4	-	-
IF164	24.7 ± 1.7	22.4 ± 1.0	21.3 ± 0.8	-	-
IF169	18.4 ± 0.3	17.2 ± 0.6	14.2 ± 0.9	-	-
IF174	18.8 ± 0.8	17.9 ± 0.7	17.1 ± 0.1	-	-
IF199	19.8 ± 0.5	20.5 ± 0.7	17.2 ± 0.1	-	-
IF205	19.9 ± 0.1	18.6 ± 0.3	17.2 ± 0.7	-	-
IF214	21.6 ± 0.9	24.9 ± 1.2	23.1 ± 1.5	-	-
IF217	20.3 ± 1.6	19.7 ± 0.7	17.2 ± 1.0	-	-

± Indicates standard deviation from the mean (n = 3).

Cell surface hydrophobicity of some strains of lactic acid bacteria is as high as 60.85% (Marin et al., 1997). Archer & Halami (2015) report that the percentage of hydrophobicity for lactobacilli isolated from infant feces was in the range of 11.23–57.69% for xylene. Kotzamanidis et al. (2010) found the hydrophobicity for *L. paracasei* strains was 3.4%, while that of *L. rhamnosus* ranged from 1.8 to 3.4%. According to the results of Kotzamanidis et al. (2010), adhesion ability in our study was also higher among lactobacilli. Also, some *L. casei* strains exhibit 5.81-42.52% cell surface hydrophobicity (Mishra & Prasad, 2005).

Production of H_2O_2 and H_2S

It was found that 16 out of 27 *Lactobacillus* isolates produced between 0.39 and 1.01 g/mL H_2O_2 and 14 produced H_2S.

The H_2S producing isolates *L. paracasei ssp. paracasei* (IF24), *L. fermentum* (IF105 and IF132), *L. rhamnosus* (IF70, IF73, IF120, and IF199), and all *L. casei* (IF86, IF96, IF164, IF169, and IF174) strains were detected on TSI agar. In a previous study, Arici et al. (2004) found that *L. fermentum, L. paracasei* subsp. *paracasei,* and *L. buchneri* strains had H_2S production ability and Lee & Simard (1984) also reported that *L. casei*, *L. rhamnosus*, *L. plantarum*, and *L. fermentum* produced hydrogen sulfide.

Sixteen isolates were able to produce H_2O_2 and the amount of production varied between 0.39 and 1.01 µg/mL. IF199 produced the highest amount of hydrogen peroxide. In a previous study by Song et al. (1999), strains of *L. rhamnosus* and *L. fermentum* produced hydrogen peroxide, but *L. paracasei* subsp. *paracasei* and *L. plantarum* were unable to do so. Similarly, Pinto et al. (2006) indicated that *L. paracasei* subsp. *paracasei* did not possess H_2O_2 production ability. Ren et al. (2014) highlighted that *L. fermentum*, *L. plantarum*, and *L. delbrueckii* subsp. *lactis* were strong producers of hydrogen peroxide. Angeles-López et al. (2001) found that four out of ten *L. fermentum* strains and one *L. paracasei* strain were H_2O_2 producers. Furthermore, Ocaña et al. (1999) reported the H_2O_2 production ability of the *Lactobacillus* spp. that were isolated from humans such as *L. casei* and *L. paracasei* produced H_2O_2 in the range of 0.51-0.77 mmol/L and 0.06-2.17 mmol/L, respectively. It was also observed that *L. paracasei* subsp. *paracasei* F2 and *L. paracasei* subsp. *paracasei* F28 were strong producers and they could inhibit *S. aureus*.

In conclusion, our in vitro results screened 12 lactobacilli strains (IF14, IF24, IF37, IF86, IF105, IF132, IF164, IF169, IF174, IF199, IF205, and IF217) which were isolated from infant feces. Results indicated that these isolates have the potential to be utilized as probiotics. Based on degrees of survival under

artificial gastrointestinal conditions (pH 3 and 0.3% bile), adhesion abilities (higher than 10%), antimicrobial activities, and antibiotic susceptibilities, these strains can be considered as good candidates for further study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Angeles-López, M., García-Cano Ramos, E., & Aquino Santiago, C. (2001). Hydrogen peroxide production and resistance to nonoxinol-9 in Lactobacillus spp. isolated from the vagina of reproductive age women. *Revista Latinoamericana de Microbiologia*, 43(4), 171-176. PMid:17061505.

Archer, A. C., & Halami, P. M. (2015). Probiotic attributes of Lactobacillus fermentum isolated from human feces and dairy products. *Applied Microbiology and Biotechnology*, 99(19), 8113-8123. http://dx.doi.org/10.1007/s00253-015-6679-x. PMid:26004804.

Arici, M., Bilgin, B., Sagdic, O., & Ozdemir, C. (2004). Some characteristics of Lactobacillus isolates from infant faeces. *Food Microbiology*, 21(1), 19-24. http://dx.doi.org/10.1016/S0740-0020(03)00044-3.

Balthazar, C. F., Santillo, A., Guimarães, J. T., Capozzi, V., Russo, P., Caroprese, M., Marino, R., Esmerino, E. A., Raices, R. S. L., Silva, M. C., Silva, H. L. A., Freitas, M. Q., Granato, D., Cruz, A. G., & Albenzio, M. (2019). Novel milk–juice beverage with fermented sheep milk and strawberry (Fragaria× ananassa): Nutritional and functional characterization. *Journal of Dairy Science*, 102(12), 10724-10736. http://dx.doi.org/10.3168/jds.2019-16909. PMid:31521367.

Balthazar, C. F., de Moura, N. A., Romualdo, G. R., Rocha, R. S., Pimentel, T. C., Esmerino, E. A., Freitas, M. Q., Santillo, A., Silva, M. C., Barbisan, L. F., Cruz, A. G., & Albenzio, M. (2021a). Synbiotic sheep milk ice cream reduces chemically induced mouse colon carcinogenesis. *Journal of Dairy Science*, S0022-0302(21), 00567-1. http://dx.doi.org/10.3168/jds.2020-19979. PMid:33934866.

Balthazar, C. F., Guimarães, J. T., Silva, R., Filho, E. G. A., Brito, E. S., Pimentel, T. C., Rodrigues, S., Esmerino, E. A., Silva, M. C., Raices, R. S. L., Granato, D., Duarte, M. C. K. H., Freitas, M. Q., & Cruz, A. G.. (2021b). Effect of probiotic Minas Frescal cheese on the volatile compound and metabolic profiles assessed by nuclear magnetic resonance spectroscopy and chemometric tools. *Journal of Dairy Science*, 104(5), 5133-5140. http://dx.doi.org/10.3168/jds.2020-19172. PMid:33663866.

Bao, Y., Zhang, Y., Zhang, Y., Liu, Y., Wang, S., Dong, X., Wang, Y., & Zhang, H. (2010). Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. *Food Control*, 21(5), 695-701. http://dx.doi.org/10.1016/j.foodcont.2009.10.010.

Birri, D. J., Brede, D. A., Tessema, G. T., & Nes, I. F. (2013). Bacteriocin production, antibiotic susceptibility and prevalence of haemolytic and gelatinase activity in faecal lactic acid bacteria isolated from healthy Ethiopian infants. *Microbial Ecology*, 65(2), 504-516. http://dx.doi.org/10.1007/s00248-012-0134-7. PMid:23184155.

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, 22, 160-166. http://dx.doi.org/10.1016/j.cofs.2018.04.008.

Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (1998). Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *Journal of Applied Microbiology*, 84(5), 759-768. http://dx.doi.org/10.1046/j.1365-2672.1998.00407.x. PMid:9674129.

Davoodabadi, A., Soltan Dallal, M. M., Rahimi Foroushani, A., Douraghi, M., Sharifi Yazdi, M. K., & Amin Harati, F. (2015). Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Anaerobe*, 34, 53-58. http://dx.doi.org/10.1016/j.anaerobe.2015.04.014. PMid:25930687.

Delgado, S., Leite, A. M., Ruas-Madiedo, P., & Mayo, B. (2015). Probiotic and technological properties of Lactobacillus spp. strains from the human stomach in the search for potential candidates against gastric microbial dysbiosis. *Frontiers in Microbiology*, 5, 766. http://dx.doi.org/10.3389/fmicb.2014.00766. PMid:25642213.

Fuochi, V., Petronio, G. P., Lissandrello, E., & Furneri, P. M. (2015). Evaluation of resistance to low pH and bile salts of human Lactobacillus spp. isolates. *International Journal of Immunopathology and Pharmacology*, 28(3), 426-433. http://dx.doi.org/10.1177/0394632015590948. PMid:26216909.

Grom, L. C., Rocha, R. S., Balthazar, C. F., Guimarães, J. T., Coutinho, N. M., Barros, C. P., Pimentel, T. C., Venâncio, E. L., Collopy, I. Jr., Maciel, P. M. C., Silva, P. H. F., Granato, D., Freitas, M. Q., Esmerino, E. A., Silva, M. C., & Cruz, A. G. (2020). Postprandial glycemia in healthy subjects: Which probiotic dairy food is more adequate? *Journal of Dairy Science*, 103(2), 1110-1119. http://dx.doi.org/10.3168/jds.2019-17401. PMid:31785881.

Jovanović, J. N., Nikolić, B., Šeatović, S., Zavišić, G., Mitić-Ćulafić, D., Vuković-Gačić, B., & Knežević-Vukčević, J. (2015). Characterization of some potentially probiotic Lactobacillus strains of human origin. *Food Science and Biotechnology*, 24(5), 1781-1788. http://dx.doi.org/10.1007/s10068-015-0232-7.

Khalil, R., El-Halafawy, K., Mahrous, H., Kamaly, K., Frank, J., & El Soda, M. (2007). Evaluation of the probiotic potential of lactic acid bacteria isolated from faeces of breast-fed infants in Egypt. *African Journal of Biotechnology*, 6(7)

Kirtzalidou, E., Pramateftaki, P., Kotsou, M., & Kyriacou, A. (2011). Screening for lactobacilli with probiotic properties in the infant gut microbiota. *Anaerobe*, 17(6), 440-443. http://dx.doi.org/10.1016/j.anaerobe.2011.05.007. PMid:21621627.

Kotsou, M. G., Mitsou, E. K., Oikonomou, I. G., & Kyriacou, A. A. (2008). In vitro assessment of probiotic properties of Lactobacillus strains from infant gut microflora. *Food Biotechnology*, 22(1), 1-17. http://dx.doi.org/10.1080/08905430701707844.

Kotzamanidis, C., Kourelis, A., Litopoulou-Tzanetaki, E., Tzanetakis, N., & Yiangou, M. (2010). Evaluation of adhesion capacity, cell surface traits and immunomodulatory activity of presumptive probiotic Lactobacillus strains. *International Journal of Food Microbiology*, 140(2-3), 154-163. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.04.004. PMid:20452079.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In E. Stackebrandt & M. Goodfellow (Eds.), *Nucleic acid techniques in bacterial systematics* (pp. 115-175). New York: John Wiley & Sons, Inc.

Lee, B. H., & Simard, R. E. (1984). Evaluation of methods for detecting the production of H2S, volatile sulfides, and greening by lactobacilli. *Journal of Food Science*, 49(4), 981-983. http://dx.doi.org/10.1111/j.1365-2621.1984.tb10374.x.

Marin, M. L., Benito, Y., Pin, C., Fernandez, M. F., Garcia, M. L., Selgas, M. D., & Casas, C. (1997). Lactic acid bacteria: hydrophobicity and strength of attachment to meat surfaces. *Letters in Applied Microbiology*, 24(1), 14-18. http://dx.doi.org/10.1046/j.1472-765X.1997.00334.x. PMid:9023999.

Megazyme International Ireland. (2014). *D-Lactic acid (D-lactate) and L-lactic acid (L-lactate) Assay procedures* (pp. 1-14). Bray, Co Wicklow: Megazyme International Ireland.

Meyers, J. A., Sanchez, D., Elwell, L. P., & Falkow, S. (1976). Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *Journal of Bacteriology*, 127(3), 1529-1537. http://dx.doi.org/10.1128/jb.127.3.1529-1537.1976. PMid:821935.

Mishra, V., & Prasad, D. N. (2005). Application of in vitro methods for selection of Lactobacillus casei strains as potential probiotics. *International Journal of Food Microbiology*, 103(1), 109-115. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.10.047. PMid:16040148.

Muhammad, N., Juan, W., Aiping, Z., Chaofeng, M., Xiaokang, W., & Jiru, X. (2011). Screening and characterization of new potentially probiotic lactobacilli from breast-fed healthy babies in Pakistan. *African Journal of Microbiological Research*, 5(12), 1428-1436. http://dx.doi.org/10.5897/AJMR10.737.

Munoz-Quezada, S., Chenoll, E., Vieites, J. M., Genovés, S., Maldonado, J., Bermúdez-Brito, M., Gomez-Llorente, C., Matencio, E., Bernal, M. J., Romero, F., Suárez, A., Ramón, D., & Gil, A. (2013). Isolation, identification and characterisation of three novel probiotic strains (Lactobacillus paracasei CNCM I-4034, Bifidobacterium breve CNCM I-4035 and Lactobacillus rhamnosus CNCM I-4036) from the faeces of exclusively breast-fed infants. *The British Journal of Nutrition*, 109(Suppl 2), S51-S62. http://dx.doi.org/10.1017/S0007114512005211. PMid:23360881.

Ocaña, V. S., de Ruiz Holgado, A. A., & Nader-Macías, M. E. (1999). Growth inhibition of Staphylococcus aureus by H2O2-producing Lactobacillus paracasei subsp. paracasei isolated from the human vagina. *FEMS Immunology and Medical Microbiology*, 23(2), 87-92. http://dx.doi.org/10.1016/S0928-8244(98)00116-3. PMid:10076905.

Papadimitriou, K., Zoumpopoulou, G., FolignÃ©, B., Alexandraki, V., Kazou, M., Pot, B., & Tsakalidou, E. (2015). Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. *Frontiers in Microbiology*, 6, 58. http://dx.doi.org/10.3389/fmicb.2015.00058. PMid:25741323.

Pérez, P. F., Minnaard, Y., Disalvo, E. A., & De Antoni, G. L. (1998). Surface properties of bifidobacterial strains of human origin. *Applied and Environmental Microbiology*, 64(1), 21-26. http://dx.doi.org/10.1128/AEM.64.1.21-26.1998. PMid:9435057.

Pinto, M. G. V., Franz, C. M., Schillinger, U., & Holzapfel, W. H. (2006). Lactobacillus spp. with in vitro probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology*, 109(3), 205-214. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.01.029. PMid:16503361.

Pithva, S., Shekh, S., Dave, J., & Vyas, B. R. M. (2014). Probiotic attributes of autochthonous Lactobacillus rhamnosus strains of human origin. *Applied Biochemistry and Biotechnology*, 173(1), 259-277. http://dx.doi.org/10.1007/s12010-014-0839-9. PMid:24682879.

Presti, I., D'Orazio, G., Labra, M., La Ferla, B., Mezzasalma, V., Bizzaro, G., Giardina, S., Michelotti, A., Tursi, F., Vassallo, M., & Di Gennaro, P. (2015). Evaluation of the probiotic properties of new Lactobacillus and Bifidobacterium strains and their in vitro effect. *Applied Microbiology and Biotechnology*, 99(13), 5613-5626. http://dx.doi.org/10.1007/s00253-015-6482-8. PMid:25744647.

Prezzi, L. E., Lee, S. H., Nunes, V. M., Corassin, C. H., Pimentel, T. C., Rocha, R. S., Ramos, G. L. P. A., Guimarães, J. T., Balthazar, C. F., Duarte, M. C. K. H., Freitas, M. Q., Esmerino, E. A., Silva, M. C., Cruz, A. G., & Oliveira, C. A. F. (2020). Effect of *Lactobacillus rhamnosus* on growth of *Listeria monocytogenes* and *Staphylococcus aureus* in a probiotic Minas Frescal cheese. *Food Microbiology*, 92, 103557. http://dx.doi.org/10.1016/j.fm.2020.103557. PMid:32950151.

Ren, D., Li, C., Qin, Y., Yin, R., Du, S., Ye, F., Liu, C., Liu, H., Wang, M., Li, Y., Sun, Y., Li, X., Tian, M., & Jin, N. (2014). In vitro evaluation of the probiotic and functional potential of Lactobacillus strains isolated from fermented food and human intestine. *Anaerobe*, 30, 1-10. http://dx.doi.org/10.1016/j.anaerobe.2014.07.004. PMid:25046742.

Sadrani, H., Dave, J., & Vyas, B. R. M. (2014). Screening of potential probiotic Lactobacillus strains isolated from fermented foods, fruits and of human origin. *Asian Journal of Pharmaceutical and Clinical Research*, 7(2), 216-225.

Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Faseleh Jahromi, M., & Ho, Y. W. (2014). Probiotic potential of Lactobacillus strains with antimicrobial activity against some human pathogenic strains. *BioMed Research International*, 2014, 927268. http://dx.doi.org/10.1155/2014/927268. PMid:25105147.

Song, Y. L., Kato, N., Matsumiya, Y., Liu, C. X., Kato, H., & Watanabe, K. (1999). Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. *Journal of Clinical Microbiology*, 37(9), 3062-3064. http://dx.doi.org/10.1128/JCM.37.9.3062-3064.1999. PMid:10449509.

Španová, A., Dráb, V., Turková, K., Špano, M., Burdychová, R., Šedo, O., Šrůtková, D., Rada, V., & Rittich, B. (2015). Selection of potential probiotic Lactobacillus strains of human origin for use in dairy industry. *European Food Research and Technology*, 241(6), 861-869. http://dx.doi.org/10.1007/s00217-015-2511-1.

Toksoy, A., Beyatli, Y., & Aslim, B. (1999). Studingon metabolic and antimicrobial activities of some L. plantarum strains isolated from sausages. *Turkish Journal of Veterinary and Animal Sciences*, 23(6), 533-540.

Vasconcelos, F. M., Silva, H. L., Poso, S. M., Barroso, M. V., Lanzetti, M., Rocha, R. S., Graça, J. S., Esmerino, E. A., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Granato, D., Pimentel, T. C., Sant'Ana, A. S., Cruz, A. G., & Valença, S. S. (2019). Probiotic Prato cheese attenuates cigarette smoke-induced injuries in mice. *Food Research International*, 123, 697-703. http://dx.doi.org/10.1016/j.foodres.2019.06.001. PMid:31285019.

Verdenelli, M. C., Ghelfi, F., Silvi, S., Orpianesi, C., Cecchini, C., & Cresci, A. (2009). Probiotic properties of Lactobacillus rhamnosus and Lactobacillus paracasei isolated from human faeces. *European Journal of Nutrition*, 48(6), 355-363. http://dx.doi.org/10.1007/s00394-009-0021-2. PMid:19365593.

Wall, R., Fitzgerald, G., Hussey, S., Ryan, T., Murphy, B., Ross, P., & Stanton, C. (2007). Genomic diversity of cultivable Lactobacillus populations residing in the neonatal and adult gastrointestinal tract. *FEMS Microbiology Ecology*, 59(1), 127-137. http://dx.doi.org/10.1111/j.1574-6941.2006.00202.x. PMid:16978242.

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, 32, 103-123. http://dx.doi.org/10.1016/j.cofs.2020.03.009.

Zhou, J. S., Pillidge, C. J., Gopal, P. K., & Gill, H. S. (2005). Antibiotic susceptibility profiles of new probiotic Lactobacillus and Bifidobacterium strains. *International Journal of Food Microbiology*, 98(2), 211-217. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.011. PMid:15681048.