

Article

Not peer-reviewed version

Isolation, Identification and Characterization of Some Probiotic Properties of Lactic Acid Bacteria Strains Isolated from Rose Blossom of *Rosa damascena* Mill.

[Zapryana Denkova](#) , [Polina Zapryanova](#) , [Yordanka Gaytanska](#) , Bogdan Goranov , [Vesela Shopska](#) , [Rositsa Denkova_Kostova](#) * , [Georgi Kostov](#)

Posted Date: 29 April 2025

doi: 10.20944/preprints202504.2473.v1

Keywords: lactic acid bacteria; *Rosa damascena* Mill.; isolation; identification; sequencing; antimicrobial activity; pathogen; antibiotic; probiotic



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Article

Isolation, Identification and Characterization of Some Probiotic Properties of Lactic Acid Bacteria Strains Isolated from Rose Blossom of *Rosa damascena* Mill.

Zapryana Denkova ¹, Polina Zapryanova ², Yordanka Gaytanska ¹, Bogdan Goranov ²,
Vesela Shopska ², Rositsa Denkova_Kostova ^{3,*} and Georgi Kostov ²

¹ University of Food Technologies, Department "Microbiology and biotechnology"

² University of Food Technologies, Department "Technology of wine and beer"

³ University of Food Technologies, Department "Biochemistry and nutrition"

* Correspondence: rositsa_denkova@uft-plovdiv.bg

Abstract: The study on the isolation, identification, and characterization of probiotic properties of lactic acid bacteria strains from the rose blossom of *Rosa damascena* Mill. is crucial for discovering novel, plant-derived probiotics with potential health benefits and applications in food, medicine, and cosmetics. Nine lactic acid bacteria (LAB) strains were isolated from rose blossom of *Rosa damascena* Mill. and they were identified to the species level by applying physiological-biochemical (API 50 CHL) and molecular-genetic (16S rRNA gene sequencing). The isolates were identified as representatives of the species *Lactobacillus helveticus*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum*. Some probiotic properties of the newly isolated and identified LAB strains were examined: antimicrobial activity against pathogens by the agar-diffusion method with wells and antibiotic resistance profile by the agar-diffusion method with paper discs. The studied LAB strains demonstrated high antimicrobial activity against the pathogenic bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella abony* NTCC 6017, *Proteus vulgaris* J, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 29212 and were resistant to most of the antibiotics used in clinical practice, which in turn opens up the possibility of their joint inclusion in complex therapy in the composition of probiotics. A batch fermentation process was carried out with *Lactiplantibacillus plantarum* 5/20 and the kinetic parameters of the fermentation process to obtain a concentrate with a high content of viable cells (10^{13} cfu/cm³) were determined. The obtained concentrate was freeze-dried and lyophilic preparations with a high content of viable cells (over 10^{12} cfu/g) were obtained. This research on *Rosa damascena* Mill.-isolated LAB strains could uncover valuable LAB strains with significant probiotic properties. These strains would be suitable for various applications in the composition of starters for functional foods and beverages as well as probiotic preparations, showcasing the untapped potential of plant-associated microbiota.

Keywords: lactic acid bacteria; *Rosa damascena* Mill.; isolation; identification; sequencing; antimicrobial activity; pathogen; antibiotic; probiotic

1. Introduction

Lactic acid bacteria (LAB) are recognized for their numerous probiotic properties, which can significantly enhance the health benefits of fermented foods. The isolation of LAB strains from diverse natural environments, such as rose blossom of *Rosa damascena* Mill., opens up new perspectives for comprehending their probiotic potential and applications [1].

LAB antimicrobial properties are particularly noteworthy [2]. The role of LAB in metabolic processes, particularly in carbohydrate fermentation, further emphasizes their probiotic benefits. LAB convert fermentable sugars into lactic acid and other metabolites, which can lower the gut pH, thus creating an inhospitable environment for pathogens and consequently inhibiting their growth,

and enhancing nutrient absorption due to improving digestibility and nutrient bioavailability for the host [3]. Besides lactic acid, LAB also produce various metabolites, such as bacteriocins, hydrogen peroxide, and other organic acids, which can also inhibit the growth of pathogenic microorganisms [2,4,5]. The unique production of these antimicrobial compounds allows LAB to outcompete harmful microorganisms for nutrients and colonization sites within the gut [4,6]. Thus, they not only enhance food safety by preventing spoilage but also contribute to overall health by maintaining a balanced gut microbiota.

The isolation of probiotic LAB strains from *Rosa damascena* Mill. may present novel opportunities for developing functional foods with enhanced protective effects against spoilage microorganisms and foodborne pathogens. The ability of these strains to produce lactic acid and other antimicrobial agents while demonstrating resistance to adverse conditions suggests their potential utility in the development of novel probiotic-rich foods, such as fortified beverages, fermented vegetables, and even non-dairy alternatives [7]. Therefore, the exploration of LAB from such a unique source as rose blossom not only broadens the scope of probiotic research but also paves the way for innovative applications in functional food industries.

The significance of microbial interactions, particularly between LAB and other microorganisms, can amplify the health benefits provided by these probiotics. The use of LAB as starters in fermentation processes has been associated with improved sensory profiles and biological activity in food products [8].

Furthermore, laboratory screening of various LAB strains for specific probiotic properties can reflect a more directed approach to developing functional foods tailored to consumer health needs. For instance, selecting strains with pronounced antimicrobial activity against common gut pathogens while maintaining gut epithelial adherence can produce more effective probiotics [9,10].

Research into the probiotic potential of natural and indigenous LAB strains is essential as they bear unique genetic makeups that may enhance their functional properties. Cultivating and utilizing strains specifically adapted to local environments could lead to the development of regionally significant functional foods that cater to the specific health needs of local populations [11]. This aligns with broader efforts toward sustainability and resilience in functional food production.

Last but not least, as consumer interest in functional foods continues to rise, the exploration of traditional sources such as *Rosa damascena* Mill. for LAB isolation could serve both health and economic purposes. Combining ancient ethnobotanical knowledge with modern microbiological techniques may yield new probiotic sources that not only benefit health but also honor cultural practices surrounding food production and fermentation. The exploration of LAB from such unique sources can indeed stimulate innovation in the field while preserving sustainable practices. Continued research is necessary to isolate and identify new LAB strains from plant sources and to explore their probiotic properties in order to identify specific applications in the development of both probiotic formulations and food bio-preservation strategies.

The objectives of present study were to isolate and identify LAB strains from the rose blossom of *Rosa damascena* Mill. and to examine some of their probiotic properties, namely their antimicrobial activity against spoilage microorganisms and pathogens and their antibiotic resistance profile.

2. Materials and Methods

2.1. Microorganisms

1. Lactic acid bacteria. The research in the present work was carried out with 11 LAB strains, designated as: LAB 4/20, LAB 12/20, LAB 5/20, LAB 6/20, LAB 8/20, LAB 9/20, LAB 10/20, LAB 13/20, LAB 16/20, LAB 19/20, LAB 22/20, isolated from rose blossom of *Rosa damascena* Mill.
2. Pathogenic test-microorganisms for the determination of the antimicrobial activity against pathogens. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella abony* NTCC 6017, *Proteus vulgaris* J, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 29212.

2.2. Nutrient Media:

1. MRS-broth (Merck); MRS-agar (Merck); 0.5 % NaCl solution (Merck); LAPTg10-broth; LAPTg10-agar; LBG-broth; LBG-agar
2. LAPTg10-broth. Composition (g/dm³): peptone – 15; yeast extract – 10; tryptone – 10; glucose – 10. The pH was adjusted to 6.6 – 6.8 and Tween 80 - 1cm³/dm³ was added. Sterilization - 20 min at 121 °C.
3. LAPTg10-agar. Composition (g/dm³): LAPTg10-broth, agar - 20. Sterilization - 20 min at 121 °C.
4. LBG-broth. Composition (g/dm³): tryptone - 10; yeast extract - 5; NaCl - 10; glucose - 10. Sterilization - 20 min at 121 °C.
5. LBG-agar. Composition (g/dm³): tryptone - 10; yeast extract - 5; NaCl - 10; glucose - 10 agar - 15. Sterilization - 20 min at 121 °C

2.3. Physiological and Biochemical Methods

2.3.1. Isolation of Lactic Acid Bacteria

Lactic acid bacteria were isolated from the rose blossoms of *Rosa damascena* Mill. Isolation was carried out using the combined microbiological approach and the use of various selective conditions and nutrient media (MRS-broth and MRS-agar). Isolation of LAB strains was carried out by morphological characteristics and by classical phenotypic methods.

2.3.2. Determination of the Titratable Acidity

The Thörner method was used to determine the acid-forming ability of microorganisms. One °T is equal to 1 cm³ of 0.1 N NaOH, used to neutralize an equivalent amount of organic acid contained in 100 cm³ of culture medium. The method is based on the titration of the sample with 0.1 N NaOH. For this purpose, 10 cm³ of each sample was taken and 20 cm³ of distilled water were added. The mixture was titrated with 0.1 N NaOH using phenolphthalein as an indicator until the appearance of a pale pink color that persists for one minute.

2.3.3. Determination of the Number of Viable Microorganisms

Appropriate tenfold dilutions of the sample for analysis in saline were prepared. 0,1 cm³ from the last three were spread plated on the corresponding solid nutrient medium. The inoculated Petri dishes were cultured for 48 to 72 hours at the optimum temperature for the growth of the corresponding microorganism until the appearance of countable single colonies appear. The number of the single colonies was used to estimate the number of viable microorganisms.

2.3.4. Determination of the Biochemical Profile

The API 50 CHL system (BioMerieux SA, France) was used according to the manufacturer's instructions. The results obtained were processed with apiweb® identification software.

2.3.5. Determination of the Antibiotic Susceptibility Profile

The antibiotic susceptibility profile was determined by the disk diffusion method [12]. A fresh 24-hour culture of each strain in MRS-broth was adjusted to a 0.5 McFarland standard and inoculated onto MRS-agar plates.

The paper discs were impregnated with antibiotics targeting:

- **Cell wall synthesis:** Penicillin, Ampicillin, Oxacillin, Piperacillin, Amoxicillin, Bacitracin, Vancomycin
- **Protein synthesis:** Tetracycline, Doxycycline, Tobramycin, Amikacin, Gentamicin, Erythromycin, Chloramphenicol, Lincomycin
- **DNA synthesis and cell division:** Nalidixic acid, Ciprofloxacin, Novobiocin, Rifampin

The Petri dishes were incubated for 48 hours at 37 ± 1 °C. The diameter (in mm) of the formed inhibition zones around each of the antibiotic discs was recorded. The following designations were used when reporting the results: R – resistant ($d_{iz} < 8$ mm), SR – intermediately sensitive ($d_{iz} 8 - 16$ mm), S – sensitive ($d_{iz} > 16$ mm).

2.3.6. Determination of the Antimicrobial Activity Against Pathogens – Agar-Diffusion Method with Wells

To determine the antimicrobial activity of the studied LAB strains against pathogenic microorganisms, 3 samples for each LAB strain were prepared: culture liquid (CL), biomass in saline solution (BSS), cell-free supernatant (CFSN) and neutralized cell-free supernatant (NCFSN) (pH=6.5), obtained from a 24-hour culture of the respective LAB strains. The antimicrobial activity was tested against the following test microorganisms: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella abony* NTCC 6017, *Proteus vulgaris* J, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 29212.

Each of the test-microorganisms (suspension in saline solution with a concentration of 10^7 cfu/cm³) was inoculated into Petri dishes with LBG-agar medium and after solidification of the agar, wells ($d_{well}=7$ mm) were prepared. 0.1 cm³ of CL, BSS and NCFSN were pipetted into the wells and the Petri dishes were incubated for 1 h at 4 ± 2 °C for the pipetted samples to diffuse into the medium. Then the Petri dishes with the test-microorganisms were incubated at 37 ± 1 °C for 24 h, after which the diameter of the inhibition zones in mm were recorded.

2.4. Molecular-Genetic Methods

2.4.1. Sequencing of the 16S rRNA Gene

The sequencing of the 16S rRNA gene was performed by the Sanger method by the “Macrogen Europe Laboratory”, the Netherlands [13]. The forward and reverse partial sequences obtained during the sequencing of the 16S rRNA gene were assembled using the *CLC Sequence Viewer* software. The entire sequences of the 16S rRNA gene were compared with the available sequences in the online GenBank database using the online BLASTn software and the species affiliation of the strains was determined with the corresponding percentage of coincidence between the sequence of the LAB strain and the reference strain from the online database.

2.5. Batch Cultivation

The cultivation of *Lactiplantibacillus plantarum* 5/20 was carried out in a laboratory bioreactor with a geometric volume of 2 dm³ and a working volume of 1.5 dm³. The control and monitoring of the main fermentation parameters was carried out by a “Sartorius A2” control device, which included control loops for the stirring rate, temperature, pH, etc. The cultivation of lactic acid bacteria was carried out in the following order: the apparatus was washed, then filled with 0.3% neomycin solution for cold sterilization for 24 h. After the sterilization time had elapsed, the apparatus was washed several times with sterile saline solution or distilled water, after which it was ready to carry out the fermentation process. The MRS-broth medium was loaded into the apparatus sterilely, using a peristaltic pump. The process of batch cultivation was carried out in MRS-broth medium. The nutrient medium was sterilized at 121 °C for 20 min. After cooling to 39-40 °C, the prepared medium in the bioreactor was inoculated with 5% (v/v) inoculum from a fresh 24-hour culture of the LAB strain in MRS-broth medium. The cultivation process was carried out at 37 °C, stirring rate of 150 rpm, without aeration. The duration of the cultivation was 24 h, with periodic samples of the culture fluid being taken for analysis of the total number of viable cells of the respective strain (cfu/cm³) and the titratable acidity [14,15].

2.6. Modeling of the Process Kinetics and Identification of the Model Parameters

Since the main target product of the fermentation process is the amount of biomass, the logistic curve equation (equation (1)), which contains a clear biological meaning, was used to model the growth kinetics. The duration of the induction period and the rate constant of adaptation were determined by equation (2).

$$\frac{dX}{d\tau} = \mu_m X - \beta X^2 = \mu_m X - \frac{\mu_m}{X_f} X^2 = \mu_m \left(1 - \frac{X}{X_f}\right) X \quad (1)$$

$$\ln \frac{M}{N_0} = \mu_m \tau + \ln \left\{ \frac{k_0}{k_0 + \mu_m} \left[1 + \frac{\mu_m}{k_0} e^{[-(k_0 + \mu_m)\tau]} \right] \right\} \quad (2)$$

where: μ_m is the maximum specific growth rate, h^{-1} ; X_{in} and X_f are the initial and final concentration of viable cells, cfu/cm^3 ; β – coefficient of intrapopulation competition $cfu/cm^3 \cdot h$; M – current biomass concentration, cfu/cm^3 ; N_0 – initial biomass concentration, cfu/cm^3 ; τ_a – induction period, h ; k_0 – rate constant of cell adaptation to the environment and cultivation conditions, h^{-1} ;

The logistic curve model (1) was solved numerically using the 4th order Runge-Kutta method. For the identification of the model parameters, the Solver function in Excel was used. The model parameters were determined by minimizing the square of the difference between the experimental data and the data obtained from the corresponding model. The parametric identification of the model (2) was performed in the *Curve Expert Professional* software product through nonlinear regression [16].

2.7. Freeze-Drying

The freeze-dried product was technologically obtained by a combined method of technological treatment - immobilization and freeze-drying, at the following stages of the process - primary treatment, freezing and freeze-drying. The immobilization matrix used was hydrocolloid and contained a 4% solution of highly esterified apple pectin and a 1.2% solution of sodium alginate in a ratio of 1:1, respectively. The mechanical method of immobilization of microbial cells was applied, i.e., cell attachment to the carrier was carried out by mixing the cell suspension with the carrier (hydrocolloid matrix) at the following sequence of technological stages: 1. Obtaining the cell suspension by culturing of the strain.; 2. Mixing of the obtained cultural suspension with the polymer solution at a temperature of 40 – 45 °C and homogenization for 1 hour 30 minutes in a reactor with a stirring speed of 500 rpm [17]. The material thus prepared was subjected to lyophilization in a sublimation installation of the company “Hochvakuum-TG – 16.50” with contact heating of the plates at the Institute of Cryobiology and Food Technology – Sofia at the following parameters: layer thickness – 11 mm; freezing temperature – -30 °C to – 35 °C; freezing by slow method – 0.05 – 0.06 °C/sec; eutectic temperature – - 37 °C; solidification temperature – - 40.00 °C; load on the supporting surface – 10.60 kg/m²; temperature in the desublimator – from – 55 °C to – 60 °C before drying and from - 65.0 °C to – 70 °C during post-drying; partial pressure – 30 Pa to 34 Pa; chamber pressure – 20 Pa to 27 Pa; post-drying temperature – 30 °C; maximum product temperature – 30 °C; residual moisture content – from 1.37 % to 3.09 %; process duration – 14 to 16 hours; storage conditions – in bags of three-layer aluminum foil, sealed under vacuum; stored at a relative humidity of no more than 35 % at 20 – 22 °C.

Before drying, the installation was prepared for loading by cooling the plates to a minimum temperature of –20 °C to –45 °C. The desublimator of the installation was cooled to a minimum temperature of –50 °C to –60 °C, which ensured a sufficiently low partial pressure of water vapor above the desublimator in the initial period and created favorable conditions for mass transfer.

After lyophilization, the lyophilizate was ground in an “Erveka” granulator. Thus processed, the lyophilized bioproduct was packed in a package made of three-layer aluminum foil, hermetically sealed under vacuum.

2.8. Microbiological Studies of the Lyophilized Concentrate

2.8.1. Microbiological Status of Native and Lyophilized Samples

According to, BDS EN ISO 4833: 2004 [18].

Indicators:

- lactic acid bacteria, CFU/g [19];
- total number of mesophilic aerobic and facultative anaerobic bacteria – CFU/g [20];
- *Escherichia coli* in 0.1 g of the product [21];
- pathogenic microorganisms, including *Salmonella* sp. in 25.0 g of the product [22];
- coagulase-positive staphylococci in 1.0 g of the product [23];
- sulfite-reducing clostridia in 0.1 g of product [24];
- microscopic mold spores, CFU/g [25];
- yeast, CFU/g [25];

2.9. Processing of the Results

The data from the triplicate experiments were processed using MS Office Excel 2013 software products, using statistical functions to determine the standard deviation and the maximum error of the estimate at significance levels of $\alpha < 0.05$.

3. Results and Discussion

3.1. Isolation, Identification and Selection of LAB Strains from the Rose Blossom of *Rosa damascena* Mill L.

3.1.1. Isolation and Identification of LAB Strains

A. Phenotypic characteristics of the newly isolated LAB strains

The characterization of the newly isolated strains began with an assessment of the purity of the culture, macroscopic and microscopic morphological control. The micro - and macromorphological characteristics of the 11 LAB isolates from rose blossom of *Rosa damascena* Mill. are presented in Table. 1. When cultivated on MRS-agar medium, the isolated strains grew in the form of small, milky-white colonies with a star-shaped or biconvex lenticular shape, which were easily separated from the medium. The isolated strains are Gram-positive (Gr (+), non-spore-forming rods.

B. Physiological and biochemical characteristics of the isolated strains

After characterization according to the main morphological criteria (Table 1), the newly isolated strains were subjected to determination of their biochemical profile with the rapid identification system API 50 CHL, and their ability to assimilate 49 carbon sources included in the system was determined (Table 2a and Table 2b). Based on the studies conducted with the API 50 CHL kits, the isolated strains were assigned to different LAB species with the corresponding percentage of confidence (Table 3).

The increasing use of molecular methods allows for more accurate, rapid, and reproducible differentiation between closely related species, which are difficult to distinguish based on phenotypic characteristics alone [26,27]. The similarity in the biochemical profiles of phylogenetically close species, as well as the influence of some factors on the metabolic properties of lactic acid bacteria, necessitate additional characterization by applying molecular genetic methods [26,28].

Table 1. Colonial characteristics and cell morphology of the isolated strains.


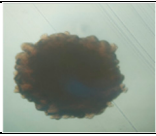

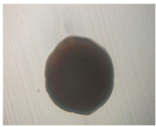
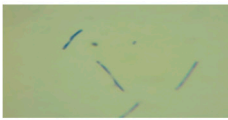
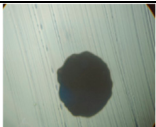


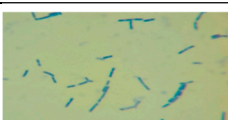
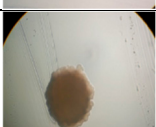

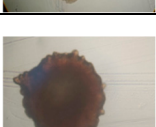
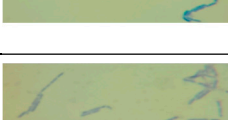
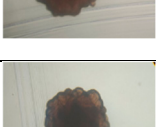


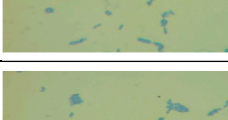
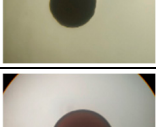
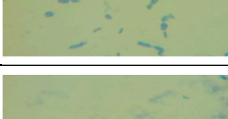
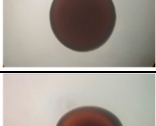
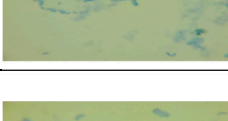
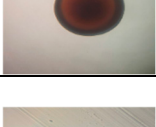
Strain	Cellular morphology		Colonial characteristics	
	Description	Visualization	Description	Visualization
LAB 4/20	Long, thickened rods with rounded ends, arranged singly, in pairs and in short chains		snowflake-shaped colonies, serrated ends, 2-3 mm in diameter	
LAB 5/20	Fine, short rods with rounded ends, arranged in pairs and in short chains		round colonies, with wavy ends, soft consistency, 2-3 mm in diameter	
LAB 6/20	Long, thin rods with rounded ends, arranged singly and in long chains		round colonies with wavy ends, soft consistency, 2-3 mm in diameter	
LAB 8/20	Long, thin rods with rounded ends, arranged singly and in long chains		round colonies with wavy ends, soft consistency, 2-3 mm in diameter	
LAB 9/20	Short, thickened rods with rounded ends, arranged singly and in clusters		round colonies with wavy ends, soft consistency, 2-3 mm in diameter	
LAB 10/20	Long rods with rounded ends, arranged singly and in short chains		snowflake-shaped colonies with wavy ends, soft consistency, 2-3 mm in diameter	
LAB 12/20	Long thin rods with rounded ends, arranged singly and in short chains		snowflake-shaped colonies with serrated ends, 2-3 mm in diameter	
LAB 13/20	Short thickened rods with rounded ends, arranged singly, in pairs and in clusters		round colonies with even ends, soft consistency, 2-3 mm in diameter	
LAB 16/20	Cocci, arranged in pairs and in groups		round colonies with even ends, soft consistency, 2-3 mm in diameter	
LAB 19/20	Very short rods with rounded ends, arranged singly and in groups		round colonies with even ends, soft consistency, 2-3 mm in diameter	
LAB 22/20	Short rods with rounded ends, arranged singly and in groups round, flat ends, soft consistency, 2-3 mm		round colonies with even ends, soft consistency, 2-3 mm in diameter	

Table 2a. Ability of strains LAB 4/20, LAB 5/20, LAB 6/20, LAB 8/20, LAB 9/20, LAB 10/20 to assimilate 49 carbon sources included in the API 50 CHL identification system.

#	Carbohydrates	LAB 4/20	LAB 5/20	LAB 6/20	LAB 8/20	LAB 9/20	LAB 10/20
1	Glycerol	-	-	-	-	-	-
2	Erythriol	-	-	-	-	-	-
3	D-arabinose	-	-	-	-	-	-
4	L-arabinose	-	+ (90-100%)	-	-	-	+ (90-100%)
5	Ribose	-	+ (90-100%)	+ (90-100%)	-	-	+ (90-100%)
6	D-xylose	-	-	-	-	-	+ (90-100%)
7	L-xylose	-	-	-	-	-	-
8	Adonitol	-	-	-	-	-	-
9	β -metil-D-xyloside	-	-	-	-	-	-
10	Galactose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
11	D-glucose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
12	D-fructose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
13	D-mannose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
14	L-sorbose	-	-	-	+ (90-100%)	-	-
15	Rhamnose	-	-	-	+ (90-100%)	-	-
16	Dulcitol	-	-	-	-	-	-
17	Inositol	-	-	-	-	-	-
18	Manitol	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	-	+ (90-100%)
19	Sorbitol	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	-	+ (90-100%)
20	α -methyl-D-mannoside	-	+ (90-100%)	+ (90-100%)	-	-	+ (90-100%)
21	α -methyl-D-glucoside	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
22	N-acetyl-glucosamine	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
23	Amigdalín	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
24	Arbutin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
25	Esculin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
26	Salicin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
27	Cellobiose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
28	Maltose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)

29	Lactose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
30	Melibiose	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	-	+ (90-100%)
31	Saccharose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
32	Trehalose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
33	Inulin	-	-	+ (90-100%)	-	-	-
34	Melezitose	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	-	+ (90-100%)
35	D-raffinose	-	-	+ (90-100%)	-	-	+ (90-100%)
36	Amidon	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	-
37	Glycogen	-	-	-	-	-	-
38	Xylitol	-	-	-	-	-	-
39	β -gentiobiose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
40	D-turanose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
41	D-lyxose	-	-	-	-	-	-
42	D-tagarose	-	-	-	-	-	-
43	D-fucose	-	-	-	-	-	-
44	L-fucose	-	-	-	-	-	-
45	D-arabitol	-	-	-	-	-	-
46	L-arabitol	-	-	-	-	-	-
47	Gluconate	-	+ (90-100%)	+ (90-100%)	-	-	+ (90-100%)
48	2-keto-gluconate	-	-	-	-	-	-
49	5-keto-gluconate	-	-	-	-	-	-

Table 2b. Ability of strains LAB 12/20, LAB 13/20, LAB 16/20, LAB 19/20, LAB 22/20 to assimilate 49 carbon sources included in the API 50 CHL identification system.

#	Sugars	LAB 12/20	LAB 13/20	LAB 16/20	LAB 19/20	LAB 22/20
1	Glycerol	-	-	-	-	-
2	Erythriol	-	-	-	-	-
3	D-arabinose	-	-	-	-	-
4	L-arabinose	-	+ (90-100%)	+ (90-100%)	-	-
5	Ribose	-	+ (90-100%)	+ (90-100%)	-	-
6	D-xylose	-	-	+ (90-100%)	-	-
7	L-xylose	-	-	-	-	-
8	Adonitol	-	-	-	-	-
9	β -metil-D-xyloside	-	-	-	-	-
10	Galactose	+ (90-100%)	+ (90-100%)	+ (90-100%)	-	+ (90-100%)
11	D-glucose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
12	D-fructose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)

13	D-mannose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
14	L-sorbose	-	-	-	-	-
15	Rhamnose	-	-	-	-	-
16	Dulcitol	-	-	-	-	-
17	Inositol	-	-	-	-	-
18	Manitol	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
19	Sorbitol	-	+ (90-100%)	-	+ (90-100%)	+ (90-100%)
20	α -methyl-D-mannoside	-	+ (90-100%)	+ (90-100%)	-	-
21	α -methyl-D-glucoside	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
22	N-acetyl-glucosamine	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
23	Amigdaline	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
24	Arbutin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
25	Esculin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
26	Salicin	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
27	Cellobiose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
28	Maltose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
29	Lactose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
30	Melibiose	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
31	Saccharose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
32	Trehalose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
33	Inulin	-	-	+ (90-100%)	-	-
34	Melezitose	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
35	D-raffinose	-	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
36	Amidon	+ (90-100%)	-	-	-	-
37	Glycogen	-	-	-	-	-
38	Xylitol	-	-	-	-	-
39	β -gentiobiose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	-
40	D-turanose	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)	+ (90-100%)
41	D-lyxose	-	-	-	-	-
42	D-tagarose	-	-	-	-	-
43	D-fucose	-	-	-	-	-
44	L-fucose	-	-	-	-	-
45	D-arabitol	-	-	-	-	-
46	L-arabitol	-	-	-	-	-
47	Gluconate	-	+ (90-100%)	-	+ (90-100%)	-
48	2-keto-gluconate	-	-	-	-	-
49	5-keto-gluconate	-	-	-	-	-

Table 3. Species identification of the newly isolated LAB strains after processing the API 50 CHL results with apiweb®.

Strain	Species affiliation	Reliability, %
LAB 4/20	<i>Lactobacillus crispatus</i>	65.3
LAB 5/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 6/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 8/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 9/20	<i>Lactobacillus crispatus</i>	65.3
LAB 10/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 12/20	<i>Lactobacillus crispatus</i>	65.3
LAB 13/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 16/20	<i>Lactiplantibacillus plantarum</i>	94.1
LAB 19/20	<i>Lactiplantibacillus plantarum</i>	99.9
LAB 22/20	<i>Lactiplantibacillus plantarum</i>	99.9

C. Molecular - taxonomic characterization. Genotypic characteristics of the studied LAB strains. Sequencing of the 16S rRNA gene

For the complete species identification of the newly isolated LAB strains, a genetic method for genotyping was used - sequencing of the 16S rRNA gene. The results of the 16S rDNA sequence analysis refer LAB 4/20 to *Lactobacillus helveticus* with 99% similarity between the 16S rDNA sequence of LAB 4/20 and the partial 16S rDNA sequence of *Lactobacillus helveticus* NBRC 15019 (Figure 1, Table 4), LAB 5/20 to *Lactiplantibacillus plantarum* with 99% similarity between the 16S rDNA sequence of LAB 5/20 and the partial 16S rDNA sequence of *Lactiplantibacillus plantarum* NBRC 15891 (Figure 2, Table 4), LAB 6/20 to *Lactobacillus helveticus* with 99% similarity between the 16S rDNA sequence of LAB 6/20 and the partial 16S rDNA sequence of *Lactobacillus helveticus* NBRC 15019 (Figure 3, Table 4), LAB 8/20 to *Lactobacillus helveticus* with 99% similarity between the 16S rDNA sequence of LAB 8/20 and the partial 16S rDNA sequence of *Lactobacillus helveticus* NBRC 15019 (Figure 4, Table 4), LAB 9/20 – to *Lactobacillus helveticus* with 99% similarity between the 16S rDNA sequence of LAB 9/20 and the partial 16S rDNA sequence of *Lactobacillus helveticus* NBRC 15019 (Figure 5, Table 4), LAB 10/20 – to *Lactobacillus acidophilus* with 99% similarity between the 16S rDNA sequence of LAB 10/20 and the partial 16S rDNA sequence of *Lactobacillus acidophilus* NBRC 13951 (Figure 6, Table 4), LAB 12/20 – to *Lactobacillus helveticus* with 99% similarity between the 16S rDNA sequence of LAB 12/20 and the partial 16S rDNA sequence of *Lactobacillus helveticus* NBRC 15019 (Figure 7, Table 4), LAB 13/20 – to *Lactiplantibacillus plantarum* with 99% similarity between the 16S rDNA sequence of LAB 13/20 and the partial 16S rDNA sequence of *Lactiplantibacillus plantarum* NBRC 15891 (Figure 8, Table 4), LAB 16/20 – to *Leuconostoc mesenteroides* with 99% similarity between the 16S rDNA sequence of LAB 16/20 and the partial 16S rDNA sequence of *Leuconostoc mesenteroides* ATCC 8293 (Figure 9, Table 4); LAB 19/20 – to *Lactiplantibacillus plantarum* with 99% similarity between the 16S rDNA sequence of LAB 19/20 and the partial 16S rDNA sequence of *Lactiplantibacillus plantarum* NBRC 15891 (Figure 10, Table 4), LAB 22/20 – to *Leuconostoc mesenteroides* with 99% similarity between the 16S rDNA sequence of LAB 22/20 and the partial 16S rDNA sequence of *Leuconostoc mesenteroides* ATCC 8293 (Figure 11, Table 4).

Table 4. Species identification of the newly isolated LAB strains, determined as a result of the 16S rRNA gene sequence analysis.

Strain	Species affiliation	Similarity, %
LAB 4/20	<i>Lactobacillus helveticus</i>	99%
LAB 5/20	<i>Lactiplantibacillus plantarum</i>	99%
LAB 6/20	<i>Lactobacillus helveticus</i>	99%
LAB 8/20	<i>Lactobacillus helveticus</i>	99%
LAB 9/20	<i>Lactobacillus helveticus</i>	99%
LAB 10/20	<i>Lactobacillus acidophilus</i>	99%
LAB 12/20	<i>Lactobacillus helveticus</i>	99%
LAB 13/20	<i>Lactiplantibacillus plantarum</i>	99%
LAB 16/20	<i>Leuconostoc mesenteroides</i>	99%
LAB 19/20	<i>Lactiplantibacillus plantarum</i>	99%
LAB 22/20	<i>Leuconostoc mesenteroides</i>	99%

3.2. Characterization of the Probiotic Potential of the Isolated Lactobacilli

3.2.1. Antimicrobial Activity of the Isolated LAB Strains Against Pathogenic Microorganisms

In a series of experiments, the antimicrobial activity against pathogenic microorganisms of the newly isolated LAB strains – *Lactobacillus helveticus* 4/20, *Lactiplantibacillus plantarum* 5/20, *Lactobacillus helveticus* 6/20, *Lactobacillus helveticus* 8/20, *Lactobacillus helveticus* 9/20, *Lactobacillus acidophilus* 10/20, *Lactobacillus helveticus* 12/20, *Lactiplantibacillus plantarum* 13/20 and *Lactiplantibacillus plantarum* 19/20, isolated from rose blossom of *Rosa damascena* Mill. by the agar diffusion method with wells was studied (Table. 5.1 and Table. 5.2). All of the studied LAB strains had pronounced antimicrobial activity against the pathogenic bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella abony* NTCC 6017, *Proteus vulgaris* J, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 29212 (Table 5.1 and Table 5.2). When neutralizing the organic acids released into the medium by the LAB strains, no antimicrobial activity was found on the growth of pathogenic bacteria that cause foodborne infections and intoxications (Table 5.1 and Table 5.2) indicating that the studied LAB strains did not produce any other substances besides organic acids that inhibit the growth of the test-pathogenic bacteria.

Query	8	GATGAGTGCTA-GTGTGGGAGGTTTCCGCCTCTCAGTGTGCAGCTAACGCATTAAGCA	66
Sbjct	812	GATGAGTGCTAAGTGTGGGAGGTTTCCGCCTCTCAGTGTGCAGCTAACGCATTAAGCA	871
Query	67	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCGCAC	126
Sbjct	872	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCGCAC	931
Query	127	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	186
Sbjct	932	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	991
Query	187	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGACGCTAAGACAGGTGGTGCA	246
Sbjct	992	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGACGCTAAGACAGGTGGTGCA	1051
Query	247	GGCTGTCGTCTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCT	306
Sbjct	1052	GGCTGTCGTCTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCT	1111
Query	307	ATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAG	366
Sbjct	1112	GTTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACCGGAG	1171
Query	367	GAAGGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACACGTGCTAC	426
Sbjct	1172	GAAGGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACACGTGCTAC	1231
Query	427	AATGGGCAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	486
Sbjct	1232	AATGGACAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	1291
Query	487	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	546
Sbjct	1292	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	1351
Query	547	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTACACACGCCCGTACACCATGGA	606
Sbjct	1352	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTACACACGCCCGTACACCATGGA	1411
Query	607	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	666
Sbjct	1412	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	1471
Query	667	AGATGACTGGGGTGAA	682
Sbjct	1472	AGATGACTGGGGTGAA	1487

Figure 1. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 4/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus helveticus* NBRC 15019.

Query	11	TCTCCAGGCGGA-TGCTTA-TGCGTTAGCTGCAGACTGAAGGGCGGAAACCTCCAACA	68
Sbjct	887	TCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGACTGAAGGGCGGAAACCTCCAACA	828
Query	69	CTTAGCATTTCATCGTTTACGGTATGACTACAGGGTATCTAATCCTGTTTGCTACCCAT	128
Sbjct	827	CTTAGCATTTCATCGTTTACGGTATGACTACAGGGTATCTAATCCTGTTTGCTACCCAT	768
Query	129	ACTTTCGAGCCTCAGCGTCAGTTACAGACAGACAGCGCCTTCGCACTGGTGTCTTC	188
Sbjct	767	ACTTTCGAGCCTCAGCGTCAGTTACAGACAGACAGCGCCTTCGCACTGGTGTCTTC	708
Query	189	CATATATCTACGCATTTACCGCTACACATGGAGTTCACCTGTCTCTTCTGCACTCAAG	248
Sbjct	707	CATATATCTACGCATTTACCGCTACACATGGAGTTCACCTGTCTCTTCTGCACTCAAG	648
Query	249	TTTCCAGTTTCGATGCACCTCTTCGTTGAGCGGAGGCTTTCACATCAGACTTAAAA	308
Sbjct	647	TTTCCAGTTTCGATGCACCTCTTCGTTGAGCGGAGGCTTTCACATCAGACTTAAAA	588
Query	309	AACCGCCTGCGCTGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA	368
Sbjct	587	AACCGCCTGCGCTGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA	528
Query	369	CCGCGCTGCTGGCAGTAGTTAGCCGTGGCTTCTGGTTAATACCGTCAATACCTGAA	428
Sbjct	527	CCGCGCTGCTGGCAGTAGTTAGCCGTGGCTTCTGGTTAATACCGTCAATACCTGAA	468
Query	429	CAGTTACTCTCAGATATGTTCTTCTTAAACAACAGAGTTTACGAGCCGAAACCTTCTT	488
Sbjct	467	CAGTTACTCTCAGATATGTTCTTCTTAAACAACAGAGTTTACGAGCCGAAACCTTCTT	408
Query	489	CACTCAGCGCGCTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGC	548
Sbjct	407	CACTCAGCGCGCTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGC	348
Query	549	CTCCCGTAGGAGTTTGGGCGTGCTCAGTCCCAATGTGGCGATTACCTCTCAGGTCG	608
Sbjct	347	CTCCCGTAGGAGTTTGGGCGTGCTCAGTCCCAATGTGGCGATTACCTCTCAGGTCG	288
Query	609	GCTACGTATCATTGCCATGGTGAGCCGTTACCTCACCATCTAGCTAATACGCCGCGGGAC	668
Sbjct	287	GCTACGTATCATTGCCATGGTGAGCCGTTACCTCACCATCTAGCTAATACGCCGCGGGAC	228
Query	669	CATCCAAAAGTGATAGCGAAGCCATCTTTCAAACCTCGGACCATGCGGTCCAGTTGTTA	728
Sbjct	227	CATCCAAAAGTGATAGCGAAGCCATCTTTCAANCTCGGACCATGCGGTCCAGTTGTTA	168
Query	729	TGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCGCTTCTGGGCGAGGTTTCCACGTG	788
Sbjct	167	TGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCGCTTCTGGGCGAGGTTTCCACGTG	108
Query	789	TTACTCACCAGTTGCGCACTCACTCAAATGTAAATCATGATGCAAGCAACAATCAATACC	848
Sbjct	107	TTACTCACCAGTTGCGCACTCACTCAAATGTAAATCATGATGCAAGCAACAATCAATACC	48
Query	849	AGAGTTGTTGACTTGCAATGATTAGGCAGCGCCGAGCGTTGCTC	895
Sbjct	47	AGAGTTGTTGACTTGCAATGATTAGGCAGCGCCGAGCGTTGCTC	1

Figure 2. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 5/20 and the partial nucleotide sequence of the 16S rDNA of *Lactiplantibacillus plantarum* NBRC 15891.

Figure 3. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 6/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus helveticus* NBRC 15019.

Query	9	GATGAGTGCTA-GTGTGGGAGGTTTCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	67
Sbjct	812	GATGAGTGCTAAGTGTTGGGAGGTTTCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	871
Query	68	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCAC	127
Sbjct	872	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCAC	931
Query	128	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	187
Sbjct	932	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	991
Query	188	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGTGCAT	247
Sbjct	992	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGTGCAT	1051
Query	248	GGCTGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	307
Sbjct	1052	GGCTGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	1111
Query	308	ATTATTAGTTGCCAGCATTAAAGTTGGGCACCTCTAATGAGACTGCCGGTGACAAACCGGAG	367
Sbjct	1112	GTTATTAGTTGCCAGCATTAAAGTTGGGCACCTCTAATGAGACTGCCGGTGATAAACCGGAG	1171
Query	368	GAAGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACACGTGCTAC	427
Sbjct	1172	GAAGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACACGTGCTAC	1231
Query	428	AATGGGCAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	487
Sbjct	1232	AATGGACAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	1291
Query	488	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	547
Sbjct	1292	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	1351
Query	548	TCAGAACGCCGCGTGAATACGTTCCCGGGCCTTGATACACACCGCCCGTCACACCATGGA	607
Sbjct	1352	TCAGAACGCCGCGTGAATACGTTCCCGGGCCTTGATACACACCGCCCGTCACACCATGGA	1411
Query	608	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	667
Sbjct	1412	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	1471
Query	668	AGATGACTGGGGTGAA	683
Sbjct	1472	AGATGACTGGGGTGAA	1487

Figure 4. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 8/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus helveticus* NBRC 15019.

Query	14	GATGAGTGCTA-GTGTGGGAGGTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	72
Sbjct	812	GATGAGTGCTAAGTGTTGGGAGGTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	871
Query	73	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCGCAC	132
Sbjct	872	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCGCAC	931
Query	133	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	192
Sbjct	932	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	991
Query	193	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGAT	252
Sbjct	992	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCCTTCGGGGACGCTAAGACAGGTGGTGAT	1051
Query	253	GGCTGTCGTGCTCAGTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	312
Sbjct	1052	GGCTGTCGTGCTCAGTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	1111
Query	313	ATTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAG	372
Sbjct	1112	GTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACCGGAG	1171
Query	373	GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACAGTGTCTAC	432
Sbjct	1172	GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACAGTGTCTAC	1231
Query	433	AATGGGCAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	492
Sbjct	1232	AATGGACAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	1291
Query	493	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	552
Sbjct	1292	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	1351
Query	553	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGA	612
Sbjct	1352	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGA	1411
Query	613	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	672
Sbjct	1412	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	1471
Query	673	AGATGACTGGGGTGAA	688
Sbjct	1472	AGATGACTGGGGTGAA	1487

Figure 5. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 9/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus helveticus* NBRC 15019.

Query	15	CCAGGCGGAGTGCTTA-TGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTT	73
Sbjct	882	CCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGAGGCGGAAACCTCCCAACACTT	823
Query	74	AGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTGCTACCCATGCT	133
Sbjct	822	AGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTGCTACCCATGCT	763
Query	134	TTCGAGCCTCAGCGTCAGTTGCGAGACCAGAGAGCGCCTTCGCCACTGGTGTCTTCCAT	193
Sbjct	762	TTCGAGCCTCAGCGTCAGTTGCGAGACCAGAGAGCGCCTTCGCCACTGGTGTCTTCCAT	703
Query	194	ATATCTACGCATTCCACCGCTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAA	253
Sbjct	702	ATATCTACGCATTCCACCGCTACACATGGAGTTCCACTCTCCTCTTCTGCACTCAAGAAA	643
Query	254	AACAGTTTCCGATGCAGTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTC	313
Sbjct	642	AACAGTTTCCGATGCAGTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTATTCTTC	583
Query	314	CGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCG	373
Sbjct	582	CGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCG	523
Query	374	CGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAG	433
Sbjct	522	CGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTGATTACCGTCAAATAAAGGCCAG	463
Query	434	TTACTACCTCTATCCTTCTTACCAACAACAGAGCTTTACGATCCGAAAACCTTCTTAC	493
Sbjct	462	TTACTACCTCTATCCTTCTTACCAACAACAGAGCTTTACGATCCGAAAACCTTCTTAC	403
Query	494	TCACGCGGCGTTGCTCCATCAGACTTTTCGTCATTGTGGAAGATTCCCTACTGCTGCCTC	553
Sbjct	402	TCACGCGGCGTTGCTCCATCAGACTTTTCGTCATTGTGGAAGATTCCCTACTGCTGCCTC	343
Query	554	CCGTAGGAGTTTGGGCGGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCT	613
Sbjct	342	CCGTAGGAGTTTGGGCGGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCT	283
Query	614	ATGCATCATTGCCCTTGTTAGGCCGTTACCCTACCAACTAGCTAATGCACCGCGGGCCAT	673
Sbjct	282	ATGCATCATTGCCCTTGTTAGGCCGTTACCCTACCAACTAGCTAATGCACCGCGGGCCAT	223
Query	674	CCCATAGCGACAGCTTACGCCGCCTTTTATAAGCTGATCATGCGATCTGCTTTCTTATCC	733
Sbjct	222	CCCATAGCGACAGCTTACGCCGCCTTTTATAAGCTGATCATGCGATCTGCTTTCTTATCC	163
Query	734	GGTATTAGCACCTGTTTCCAAGTGGTATCCAGACTATGGGGCAGGTTCCCCACGTGTTA	793
Sbjct	162	GGTATTAGCACCTGTTTCCAAGTGGTATCCAGACTATGGGGCAGGTTCCCCACGTGTTA	103
Query	794	CTCACCACATCCGCGCTCGCGTTCCCAACGTCATCACCGAAGTGAATCTGTTGGTTCAGC	853
Sbjct	102	CTCACCACATCCGCGCTCGCGTTCCCAACGTCATCACCGAAGTGAATCTGTTGGTTCAGC	43
Query	854	TCGCTCGACTTGCAATGATTAGGCACGCCGCGAGCGTTCGTC	895
Sbjct	42	TCGCTCGACTTGCAATGATTAGGCACGCCGCGAGCGTTCGTC	1

Figure 6. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 10/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus acidophilus* NBRC 13951.

Query	8	GATGAGTGCTA-GTGTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	66
Sbjct	812	GATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCA	871
Query	67	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCGCAC	126
Sbjct	872	CTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGCCCGCAC	931
Query	127	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	186
Sbjct	932	AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA	991
Query	187	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGTGCA	246
Sbjct	992	TCTAGTGCCATCCTAAGAGATTAGGAGTTCCTTCGGGGACGCTAAGACAGGTGGTGCA	1051
Query	247	GGCTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	306
Sbjct	1052	GGCTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT	1111
Query	307	ATTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAG	366
Sbjct	1112	GTTATTAGTTGCCAGCATTAAAGTTGGGCACTCTAATGAGACTGCCGGTGATAAACCGGAG	1171
Query	367	GAAGGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACAGTGCTAC	426
Sbjct	1172	GAAGGTGGGGATGACGTCAAGTCATCATGCCCTTATGACCTGGGCTACACAGTGCTAC	1231
Query	427	AATGGGCAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	486
Sbjct	1232	AATGGGCAGTACAACGAGAAGCGAGCCTGCGAAGGCAAGCGAATCTCTGAAAGCTGTTCT	1291
Query	487	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	546
Sbjct	1292	CAGTTCGGACTGCAGTCTGCAACTCGACTGCACGAAGCTGGAATCGCTAGTAATCGCGGA	1351
Query	547	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTTACACACCGCCCGTCACACCATGGA	606
Sbjct	1352	TCAGAACGCCCGGTGAATACGTTCCCGGGCCTTGTTACACACCGCCCGTCACACCATGGA	1411
Query	607	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	666
Sbjct	1412	AGTCTGCAATGCCCAAAGCCGGTGGCCTAACCTTCGGGAAGGAGCCGTCTAAGGCAGGGC	1471
Query	667	AGATGACTGGGGTGAA	682
Sbjct	1472	AGATGACTGGGGTGAA	1487

Figure 7. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 12/20 and the partial nucleotide sequence of the 16S rDNA of *Lactobacillus helveticus* NBRC 15019.

Query	10	TCTCCAGGCGGA-TGCTTA-TGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCCTCCAACA	67
Sbjct	887	TCCCCAGGCGGAATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCCTCCAACA	828
Query	68	CTTAGCATTTCATCGTTTACGGTATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCAT	127
Sbjct	827	CTTAGCATTTCATCGTTTACGGTATGGACTACCAGGGTATCTAATCCTGTTTGCTACCCAT	768
Query	128	ACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCTC	187
Sbjct	767	ACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCTTCGCCACTGGTGTCTTCTC	708
Query	188	CATATATCTACGCATTTACCGCTACACATGGAGTTCCTCTCTTCTGCACTCAAG	247
Sbjct	707	CATATATCTACGCATTTACCGCTACACATGGAGTTCCTCTCTTCTGCACTCAAG	648
Query	248	TTTCCAGTTTCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAA	307
Sbjct	647	TTTCCAGTTTCGATGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAA	588
Query	308	AACCGCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA	367
Sbjct	587	AACCGCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA	528
Query	368	CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATACCTGAA	427
Sbjct	527	CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATACCTGAA	468
Query	428	CAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTACGAGCCGAAACCCCTTCTT	487
Sbjct	467	CAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTACGAGCCGAAACCCCTTCTT	408
Query	488	CCTCACGCGCGCTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGC	547
Sbjct	407	CCTCACGCGCGCTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTGC	348
Query	548	CTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCTCTCAGGTCG	607
Sbjct	347	CTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCTCTCAGGTCG	288
Query	608	GCTACGTATCATTGCCATGGTGAGCCGTTACCCACCATCTAGCTAATACGCCCGGGAC	667
Sbjct	287	GCTACGTATCATTGCCATGGTGAGCCGTTACCCACCATCTAGCTAATACGCCCGGGAC	228
Query	668	CATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTGTTA	727
Sbjct	227	CATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTGTTA	168
Query	728	TGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCACGTG	787
Sbjct	167	TGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCACGTG	108
Query	788	TTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCATCAATACC	847
Sbjct	107	TTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCATCAATACC	48
Query	848	AGAGTTCGTTTCGACTTGCAATGATTAGGCACGCCGCCAGCGTTCGTC	894
Sbjct	47	AGAGTTCGTTTCGACTTGCAATGATTAGGCACGCCGCCAGCGTTCGTC	1

Figure 8. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 13/20 and the partial nucleotide sequence of the 16S rDNA of *Lactiplantibacillus plantarum* NBRC 15891.

Query	15	CCCAGGCGGA-CACTTAATGCGTTAGCTTCGGCACTAAGAGGCGGAAACCTCCTAACACC	73
Sbjct	891	CCCAGGCGGAACACTTAATGCGTTAGCTTCGGCACTAAGAGGCGGAAACCTCCTAACACC	832
Query	74	TAGTGTTTCATCGTTTACGGGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCACAC	133
Sbjct	831	TAGTGTTTCATCGTTTACGGGTGGACTACCAGGGTATCTAATCCTGTTTGCTACCCACAC	772
Query	134	TTTCGAGCCTCAACGTCAGTTGCAGTCCAGTAAGCCGCCTTCGCCACTGGTGTCTTCCCA	193
Sbjct	771	TTTCGAGCCTCAACGTCAGTTGCAGTCCAGTAAGCCGCCTTCGCCACTGGTGTCTTCCCA	712
Query	194	TATATCTACGCATTCCACCGCTACACATGGAGTTCCACTACCTCTACTGCACTCAAGTT	253
Sbjct	711	TATATCTACGCATTCCACCGCTACACATGGAGTTCCACTACCTCTACTGCACTCAAGTT	652
Query	254	AACCAGTTTCCAATGCCATTCCGGAGTTGAGCTCCGGGCTTTCACATCAGACTTAATAAA	313
Sbjct	651	AACCAGTTTCCAATGCCATTCCGGAGTTGAGCTCCGGGCTTTCACATCAGACTTAATAAA	592
Query	314	CCGTCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTCGGGACATACGTATTACC	373
Sbjct	591	CCGTCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTCGGGACATACGTATTACC	532
Query	374	GCGGCTGCTGGCACGTATTTAGCCGTCCCTTTCTGGTATGGTACCGTCAAACTAAAATCA	433
Sbjct	531	GCGGCTGCTGGCACGTATTTAGCCGTCCCTTTCTGGTATGGTACCGTCAAACTAAAATCA	472
Query	434	TTTCCTATTCTAGCTGTTCTTCCCATACAACAGTGCTTTACGACCCGAAAGCCTTCATCA	493
Sbjct	471	TTTCCTATTCTAGCTGTTCTTCCCATACAACAGTGCTTTACGACCCGAAAGCCTTCATCA	412
Query	494	CACACGCGCGTTTGCTCCATCAGGCTTTTCGCCCATGTGGAAGATTCCCTACTGCAGCCT	553
Sbjct	411	CACACGCGCGTTTGCTCCATCAGGCTTTTCGCCCATGTGGAAGATTCCCTACTGCAGCCT	352
Query	554	CCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGC	613
Sbjct	351	CCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGC	292
Query	614	TATGCATCATTTGTCTTGGTAGGCCTTTACCCCACTAACTAATGCACCGCGGATCCA	673
Sbjct	291	TATGCATCATTTGTCTTGGTAGGCCTTTACCCCACTAACTAATGCACCGCGGATCCA	232
Query	674	TCTCTAGGTGACGCCGAAGCGCCTTTTAACTTTGTGTTCATGCGACACTAAGTTTATTTCG	733
Sbjct	231	TCTCTAGGTGACGCCGAAGCGCCTTTTAACTTTGTGTTCATGCGACACTAAGTTTATTTCG	172
Query	734	GTATTAGCATCTGTTTCCAAATGTTATCCCAGCCTTGAGGCAGGTTGTCCACGTGTTAC	793
Sbjct	171	GTATTAGCATCTGTTTCCAAATGTTATCCCAGCCTTGAGGCAGGTTGTCCACGTGTTAC	112
Query	794	TCACCCGTTTCGCCACTCACTTGAAAGGTGCAAGCACCTTTCGCTGTGCGTTTCGACTTGCA	853
Sbjct	111	TCACCCGTTTCGCCACTCACTTGAAAGGTGCAAGCACCTTTCGCTGTGCGTTTCGACTTGCA	52
Query	854	TGTATTAGGCACGACGCCAGCGTTTCATCCTGAGC	887
Sbjct	51	TGTATTAGGCACGCGCCAGCGTTTCATCCTGAGC	18

Figure 9. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 16/20 and the partial nucleotide sequence of the 16S rDNA of *Leuconostoc mesenteroides* ATCC 8293.

Query	7	GATGA-TGCTA-GTGTGGAGGGTTTCGCCCTTCAGTGCTGCAGCTAACAGCATTAAGC	64
Sbjct	815	GATGAATGCTAAGTGTGGAGGGTTTCGCCCTTCAGTGCTGCAGCTAAC-GCATTAAAGC	873
Query	65	ATTCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCGCA	124
Sbjct	874	ATTCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCGCA	933
Query	125	CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCCTTACCAGGTCTTGAC	184
Sbjct	934	CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCCTTACCAGGTCTTGAC	993
Query	185	ATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCA	244
Sbjct	994	ATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCA	1053
Query	245	TGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCT	304
Sbjct	1054	TGGTTGTCGTCAGCTCGTGTCTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCT	1113
Query	305	TATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGA	364
Sbjct	1114	TATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGA	1173
Query	365	GGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGGCTACACAGTGCTA	424
Sbjct	1174	GGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGGCTACACAGTGCTA	1233
Query	425	CAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATT	484
Sbjct	1234	CAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATT	1293
Query	485	TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGG	544
Sbjct	1294	TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGG	1353
Query	545	ATCAGCATGCCCGCGTGAATACGTTCCCGGGCCTTGTTACACACCGCCCGTCACACCATGA	604
Sbjct	1354	ATCAGCATGCCCGCGTGAATACGTTCCCGGGCCTTGTTACACACCGCCCGTCACACCATGA	1413
Query	605	GAGTTTGTAAACCCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGG	664
Sbjct	1414	GAGTTTGTAAACCCCAAAGTCGGTGGGGTAACCTTTTAGGAACCAGCCGCCTAAGGTGGG	1473
Query	665	ACAGATGATTAGGGTGAAG	683
Sbjct	1474	ACAGATGATTAGGGTGAAG	1492

Figure 10. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 19/20 and the partial nucleotide sequence of the 16S rDNA of *Lactiplantibacillus plantarum* NBRC 15891.

Query	11	CTCTCCCTGGGGA-CACCTTAATGCGTTAGCTTCGGCACTAAGAGGCGGAAACCTCTCAAC	69
Sbjct	894	CTCCCGACGGGGAACACTTAATGCGTTAGCTTCGGCACTAAGAGGCGGAAACCTCTCAAC	833
Query	70	ACCTAGTGTTCATCGTTTACGCGTGGACTACCAAGGTATCTAATCTGTTTGTCAACCA	120
Sbjct	834	ACCTAGTGTTCATCGTTTACGCGTGGACTACCAAGGTATCTAATCTGTTTGTCAACCA	775
Query	130	CACTTTCGAGCCTCAACGTCAGTTGCAGTCCAGTAAGCGCGCTTCGCCACTGGTGTCTTT	181
Sbjct	774	CACTTTCGAGCCTCAACGTCAGTTGCAGTCCAGTAAGCGCGCTTCGCCACTGGTGTCTTT	712
Query	190	CCATATATCTACGCGATCCACGCGTACACGTGAGTTCCACTTACTCTCTACTGCATCTTA	243
Sbjct	714	CCATATATCTACGCGATCCACGCGTACACGTGAGTTCCACTTACTCTCTACTGCATCTTA	650
Query	250	GTTAACCAAGTTTCCAATGCACCTCCGGAGTGAGCTCCGGGCTTTACCATCAGACTTAAT	303
Sbjct	654	GTTAACCAAGTTTCCAATGCACCTCCGGAGTGAGCTCCGGGCTTTACCATCAGACTTAAT	599
Query	310	AAACCGCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTCGGGACATACGTATT	365
Sbjct	594	AAACCGCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTCGGGACATACGTATT	538
Query	370	ACGCGCGCTCTGGCAGTATTTAGCCGTCCTTTCTGCGATGGTACGCTCAAACTAAA	422
Sbjct	534	ACGCGCGCTCTGGCAGTATTTAGCCGTCCTTTCTGCGATGGTACGCTCAAACTAAA	478
Query	430	TCATTTCCTATTCTAGCTGTTCTTCCCATACACAGTGCTTTACGACCCGGAAGCCTTCA	481
Sbjct	474	TCATTTCCTATTCTAGCTGTTCTTCCCATACACAGTGCTTTACGACCCGGAAGCCTTCA	411
Query	490	TCACACACGGCGGTGCTCCATCAGGCTTTGCGCCATTGTGGAGAGTTCCCTACTGCAG	541
Sbjct	414	TCACACACGGCGGTGCTCCATCAGGCTTTGCGCCATTGTGGAGAGTTCCCTACTGCAG	355
Query	550	CTCCCGTAGAGGTTTGGGCGGTGTCTCAGTCCCAATGTGGCGCATAGTCTCTCAACT	603
Sbjct	354	CTCCCGTAGAGGTTTGGGCGGTGTCTCAGTCCCAATGTGGCGCATAGTCTCTCAACT	299
Query	610	GGCTATGCATCATGTCTTGTAGGCCTTTACCCCAACCACTAACTATGCACGCGGAT	663
Sbjct	294	GGCTATGCATCATGTCTTGTAGGCCTTTACCCCAACCACTAACTATGCACGCGGAT	233
Query	670	CCATCTCTAGTGAGCGGAAGCGCTTTTAACTTTGTGTCATGCGACACTAAGTTTAT	722
Sbjct	234	CCATCTCTAGTGAGCGGAAGCGCTTTTAACTTTGTGTCATGCGACACTAAGTTTAT	175
Query	730	TCGGTATTAGCATCTGTTTCCAATGTTATCCCCAGCGCTTGAGGCAGGTGTGCCACGTG	781
Sbjct	174	TCGGTATTAGCATCTGTTTCCAATGTTATCCCCAGCGCTTGAGGCAGGTGTGCCACGTG	111
Query	790	TACTCACCGGTCGCGCACTACTTGAAGGTGCAAGCACTTTGCGTGTGCGTTCGACTT	841
Sbjct	114	TACTCACCGGTCGCGCACTACTTGAAGGTGCAAGCACTTTGCGTGTGCGTTCGACTT	55
Query	850	GCATGATTAGGCAAGCGCCAGCGCTTCATCTGAGC	896
Sbjct	54	GCATGATTAGGCAAGCGCCAGCGCTTCATCTGAGC	18

Figure 11. Comparison between the partial nucleotide sequence of the 16S rDNA of LAB 22/20 and the partial nucleotide sequence of the 16S rDNA of *Leuconostoc mesenteroides* ATCC 8293.

Lactobacillus helveticus 4/20, Lactobacillus helveticus 6/20, Lactobacillus helveticus 8/20 and Lactobacillus helveticus 9/20 exhibited the highest antimicrobial activity against Listeria monocytogenes and Enterococcus faecalis; Lactobacillus helveticus 4/20 and Lactobacillus helveticus 12/20 also manifested high antimicrobial activity against Staphylococcus aureus (Table 5.1). Lactiplantibacillus plantarum 5/20 and Lactiplantibacillus plantarum 13/20 showed the highest antimicrobial activity against Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis and Proteus vulgaris. Lactobacillus acidophilus 10/20 and Lactiplantibacillus plantarum 19/20 exhibited significantly lower antimicrobial activity than all newly isolated LAB strains (Table 5.2). Of the five Lactobacillus helveticus strains, Lactobacillus helveticus 12/20 demonstrated the highest inhibitory activity against Escherichia coli (Table 5.1). Among the representatives of the species Lactiplantibacillus plantarum, Lactiplantibacillus plantarum 5/20 manifested the highest antimicrobial activity against Escherichia coli (Table 5.2). Of the five Lactobacillus helveticus strains, Lactobacillus helveticus 4/20 exhibited the highest inhibitory activity against Staphylococcus aureus (Table 5.1). Among the representatives of the species Lactiplantibacillus plantarum, Lactiplantibacillus plantarum 5/20 and Lactiplantibacillus plantarum 13/20 showed the highest antimicrobial activity against Escherichia coli (Table 5.2). Of the five strains of Lactobacillus helveticus, Lactobacillus helveticus 4/20 and Lactobacillus helveticus 12/20 demonstrated the highest inhibitory activity against Salmonella abony (Table 5.1).

Table 5.1. Antimicrobial activity of *Lactobacillus helveticus* 4/20, *Lactobacillus helveticus* 6/20, *Lactobacillus helveticus* 8/20, *Lactobacillus helveticus* 9/20, *Lactobacillus helveticus* 12/20 against pathogenic bacteria. $d_{well}=7\text{mm}$. The LAB concentration was $10^{11} - 10^{12}$ CFU/cm³. "-" – no inhibition of the growth of pathogenic microorganisms by the LAB isolates was found.

LAB strain \ d_{iz} , [mm]		<i>Escherichia coli</i> ATCC 25922, 6×10^9 cfu/cm ³	<i>Staphylococcus aureus</i> ATCC 25093, $1,7 \times 10^{12}$ cfu/cm ³	<i>Salmonella abony</i> NTCC 6017, 2×10^{12} cfu/cm ³	<i>Proteus vulgaris</i> J, $1,5 \times 10^{12}$ cfu/cm ³	<i>Listeria monocytogenes</i> ATCC 19115, 4×10^{12} cfu/cm ³	<i>Enterococcus faecalis</i> ATCC 29212, $4,2 \times 10^{12}$ cfu/cm ³
4/20	CL	12,67±0,47	17,17±0,24	14,67±0,47	15,50±0,41	17,00±0,00	16,67±0,47
	BSS	11,17±0,24	20,17±0,24	9,67±0,47	12,33±0,47	14,17±0,24	13,33±0,47
	CFSN	10,50±0,41	10,17±0,24	10,17±0,24	12,00±0,00	15,17±0,24	11,33±0,47
	NCFSN	-	-	-	-	-	-
6/20	CL	12,17±0,24	15,00±0,00	11,33±0,47	14,33±0,47	18,33±0,47	16,50±0,41
	BSS	9,17±0,24	16,17±0,24	8,00±0,00	9,17±0,24	14,00±0,00	13,17±0,24
	CFSN	10,17±0,24	13,67±0,47	9,50±0,41	12,00±0,00	15,67±0,47	13,00±0,00
	NCFSN	-	-	-	-	-	-
8/20	CL	11,17±0,24	13,50±0,41	10,17±0,24	13,17±0,24	16,50±0,41	16,00±0,00
	BSS	8,00±0,00	12,17±0,24	8,33±0,47	8,17±0,24	13,17±0,24	12,17±0,24
	CFSN	10,50±0,41	10,50±0,41	8,00±0,00	10,00±0,00	14,67±0,47	13,17±0,24
	NCFSN	-	-	-	-	-	-
9/20	CL	11,50±0,41	16,00±0,00	11,67±0,47	12,50±0,41	18,67±0,47	18,67±0,47
	BSS	8,00±0,00	10,17±0,24	8,17±0,24	9,00±0,00	12,17±0,24	15,00±0,00
	CFSN	10,00±0,00	10,67±0,47	9,50±0,41	10,00±0,00	15,17±0,24	14,00±0,00
	NCFSN	-	-	-	-	-	-
12/20	CL	14,67±0,47	16,17±0,24	14,00±0,00	15,00±0,00	18,00±0,82	18,17±0,24
	BSS	9,17±0,24	12,33±0,47	10,00±0,00	10,00±0,00	15,17±0,24	13,17±0,24
	CFSN	12,00±0,00	13,17±0,24	10,17±0,24	13,50±0,41	15,67±0,47	13,00±0,00
	NCFSN	-	-	-	-	-	8,00±0,00

Table 5.2. Antimicrobial activity of *Lactiplantibacillus plantarum* 5/20, *Lactiplantibacillus plantarum* 13/20, *Lactiplantibacillus plantarum* 19/20, *Lactobacillus acidophilus* 10/20 against pathogenic bacteria. $d_{well}=7\text{mm}$. The LAB concentration was $10^{11} - 10^{12}$ CFU/cm³. "-" – no inhibition of the growth of pathogenic microorganisms by the LAB isolates was found.

LAB strain \ d_{iz} , [mm]		<i>Escherichia coli</i> ATCC 25922, 6×10^9 cfu/cm ³	<i>Staphylococcus aureus</i> ATCC 25093, $1,7 \times 10^{12}$ cfu/cm ³	<i>Salmonella abony</i> NTCC 6017, 2×10^{12} cfu/cm ³	<i>Proteus vulgaris</i> J, $1,5 \times 10^{12}$ cfu/cm ³	<i>Listeria monocytogenes</i> ATCC 19115, 4×10^{12} cfu/cm ³	<i>Enterococcus faecalis</i> ATCC 29212, $4,2 \times 10^{12}$ cfu/cm ³
5/20	CL	13,67±0,47	19,67±0,47	13,33±0,47	17,00±0,00	20,00±0,00	18,50±0,41
	BSS	9,17±0,24	17,33±0,47	9,17±0,24	10,00±0,00	16,17±0,24	14,67±0,47
	CFSN	11,17±0,24	14,67±0,47	11,33±0,47	13,17±0,24	17,67±0,47	13,50±0,41
	NCFSN	8,17±0,24	-	-	-	9,17±0,24	-
13/20	CL	11,17±0,24	19,50±0,41	10,17±0,24	14,50±0,41	17,17±0,24	17,67±0,47
	BSS	9,00±0,00	17,17±0,24	8,00±0,00	10,17±0,24	15,00±0,00	14,17±0,24
	CFSN	10,50±0,41	13,00±0,00	9,00±0,00	13,17±0,24	15,17±0,24	13,00±0,00
	NCFSN	-	-	-	-	8,00±0,00	-
19/20	CL	10,17±0,24	10,17±0,24	9,67±0,47	10,67±0,47	11,00±0,00	10,17±0,24
	BSS	8,00±0,00	9,17±0,24	8,00±0,00	8,17±0,24	8,00±0,00	8,67±0,47
	CFSN	8,67±0,47	8,00±0,00	8,50±0,41	9,00±0,00	9,00±0,00	9,17±0,24
	NCFSN	-	-	-	-	-	-
10/20	CL	10,17±0,24	8,00±0,00	9,00±0,00	10,17±0,24	9,67±0,47	8,17±0,24
	BSS	8,17±0,24	8,17±0,24	8,17±0,24	8,17±0,24	8,17±0,24	8,17±0,24
	CFSN	9,17±0,24	8,00±0,00	9,00±0,00	9,17±0,24	9,17±0,24	8,00±0,00
	NCFSN	-	-	-	-	-	-

Among the representatives of the species *Lactiplantibacillus plantarum*, *Lactiplantibacillus plantarum* 5/20 exhibited the highest antimicrobial activity against *Salmonella abony* (Table 5.2). Of the five strains *Lactobacillus helveticus*, *Lactobacillus helveticus* 4/20 and *Lactobacillus helveticus* 12/20 demonstrated the highest inhibitory activity against *Proteus vulgaris* (Table 5.1). Among the representatives of the species *Lactiplantibacillus plantarum*, *Lactiplantibacillus plantarum* 5/20 showed the highest antimicrobial activity against *Proteus vulgaris* (Table 5.2). Of the five *Lactobacillus helveticus* strains, *Lactobacillus helveticus* 6/20, *Lactobacillus helveticus* 9/20 and *Lactobacillus helveticus* 12/20 manifested the highest inhibitory activity against *Listeria monocytogenes* (Table 5.1). Among the representatives of the species *Lactiplantibacillus plantarum*, *Lactiplantibacillus plantarum* 5/20 exhibited the highest antimicrobial activity against *Listeria monocytogenes* (Table 5.2). Of the five *Lactobacillus helveticus* strains, *Lactobacillus helveticus* 9/20 and *Lactobacillus helveticus* 12/20 demonstrated the highest inhibitory activity against *Enterococcus faecalis* (Table 5.1). Among the representatives of the species *Lactiplantibacillus plantarum*, *Lactiplantibacillus plantarum* 5/20 and *Lactiplantibacillus plantarum* 13/20 exhibited the highest antimicrobial activity against *Enterococcus faecalis* (Table 5.2). The most pronounced antimicrobial activity against the test microorganisms included in the study was demonstrated by *Lactobacillus helveticus* 12/20 among the representatives of the species *Lactobacillus helveticus* and *Lactiplantibacillus plantarum* 5/20 among the representatives of the species *Lactiplantibacillus plantarum* (Table 5.1 and Table 5.2.).

When examining the antimicrobial activity of each newly isolated LAB strain against each pathogen included in the experiment, inhibition zones were observed when the culture liquid (CL), biomass in saline solution (BSS) and the cell-free supernatant (CFSN) were pipetted. For some strains, it was found that the CL and BSS had greater antimicrobial activity than the CFSN, which means that LAB suppress pathogenic microorganisms due to both competition for nutrients between them and inhibition by the lactic and other organic acids produced, as a result of which the pH decreases.

The present study highlights the significant antimicrobial potential of LAB strains isolated from the rose blossoms of *Rosa damascena* Mill., as evaluated through the well diffusion method. All tested LAB strains exhibited pronounced inhibitory activity against a spectrum of foodborne pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella abony*, *Proteus vulgaris*, *Listeria monocytogenes*, and *Enterococcus faecalis*. These findings reinforce the growing body of evidence supporting LAB as effective bio-preservatives and potential candidates for the development of functional foods and probiotics.

A key observation from this study is that the antimicrobial activity of the LAB strains was abolished upon neutralization of their organic acid content. This clearly suggests that the antimicrobial effects observed are primarily attributable to organic acid production—mainly lactic acid—and not due to bacteriocins, hydrogen peroxide, or other antimicrobial metabolites. This mode of inhibition, largely through acidification of the medium, aligns with previous findings reported for *Lactobacillus* and *Lactiplantibacillus* species, where the decrease in environmental pH creates unfavorable conditions for pathogen proliferation.

Among the tested strains, *Lactobacillus helveticus* 12/20 and *Lactiplantibacillus plantarum* 5/20 consistently demonstrated the most potent antimicrobial activity against nearly all tested pathogens. Notably, *L. helveticus* 12/20 exhibited strong inhibition against *E. coli*, *S. aureus*, *Salmonella abony*, *Proteus vulgaris*, *L. monocytogenes*, and *E. faecalis*, indicating its broad-spectrum efficacy. Likewise, *Lp. plantarum* 5/20 was particularly effective, showing significant inhibitory activity against *S. aureus*, *L. monocytogenes*, and *P. vulgaris*. These results underscore the strain-specific nature of antimicrobial efficacy, even within the same species, further emphasizing the necessity of individual assessment of LAB candidates for food and health applications.

The comparative analysis of different LAB species and strains revealed a pattern: *L. helveticus* strains, particularly *L. helveticus* 4/20, *L. helveticus* 6/20, *L. helveticus* 9/20, and *L. helveticus* 12/20, were more effective against Gram-positive bacteria such as *L. monocytogenes* and *E. faecalis*, while *Lp. plantarum* strains, especially *Lp. plantarum* 5/20 and *Lp. plantarum* 13/20, showed a broader spectrum, with potent activity against both Gram-positive and Gram-negative bacteria, including *E. coli* and *S.*

abony. This strain-level variability could be attributed to differences in organic acid profiles, acid tolerance, and metabolic capabilities.

The findings also underscore the potential of underexplored natural sources, such as the rose blossom of *Rosa damascena* Mill., as reservoirs of beneficial microorganisms. These results are especially relevant for the food industry, where there is a growing demand for natural preservatives and health-promoting microbial strains. The ability of certain strains to effectively inhibit foodborne pathogens suggests their promising application as components in biopreservatives, starter cultures, or next-generation probiotics.

The present study contributes valuable insights into the antimicrobial potential of novel LAB strains, reaffirming the critical importance of strain-specific evaluation. Future research should aim to characterize the organic acid profiles of these strains, explore their performance in food matrix systems, and evaluate their safety and efficacy *in vivo*.

3.2.2. Antibiotic Resistance of the Isolated LAB Strains

Knowledge of the antibiotic resistance of LAB with probiotic potential is essential. On the one hand, this can be considered as a significant criterion in the selection of probiotic cultures, due to the possibility of conducting combined therapy with an antibiotic and a probiotic, in order to restore the normal microflora of the gastrointestinal tract and/or urogenital tract [29]. On the other hand, a number of authors express the hypothesis that bacteria normally found in the body, including LAB, can serve as a source of antibiotic resistance genes, transferring them to various pathogenic microorganisms [30]. Therefore, one of the conditions for the selection of potentially probiotic strains is knowledge of their antibiotic sensitivity.

Thus, 20 antibiotics with different mechanisms of action from the main groups used in medical practice were selected and the sensitivity of the newly isolated LAB strains was tested. The results obtained from the studies conducted using the agar diffusion method of Bauer et al., 1966 [12,31] for 24 h are summarized in Table 6.1 and Table 6.2.

The antibiotic susceptibility patterns observed among the *Lactobacillus helveticus* and *Lactiplantibacillus plantarum* strains tested in this study revealed substantial variability, indicating the strain-specific nature of antibiotic resistance and susceptibility in this species. Given the increasing interest in LAB as probiotics and components of food starters, understanding their antibiotic resistance profiles is critical from both safety and therapeutic standpoints (Table 6.1 and Table 6.2).

When it comes to the *Lactobacillus helveticus* strains, among the antibiotics targeting cell wall synthesis, a heterogeneous resistance pattern was observed. *L. helveticus* 4/20 and *L. helveticus* 9/20 exhibited resistance to multiple β -lactam antibiotics including penicillin, ampicillin, and oxacillin, while strains such as *L. helveticus* 8/20 demonstrated broad sensitivity within this group (Table 6.1). Interestingly, vancomycin resistance was detected in *L. helveticus* 4/20, *L. helveticus* 9/20, and *L. helveticus* 12/20, which is noteworthy since LAB are often intrinsically resistant to glycopeptides due to the absence of the D-Ala-D-Lac target in their peptidoglycan precursors. The sensitivity of *L. helveticus* 6/20 to vancomycin may suggest a deviation from typical intrinsic resistance or experimental variability, warranting further genetic investigation. Regarding protein synthesis inhibitors, all *L. helveticus* strains showed resistance to lincomycin and most to amikacin, consistent with known intrinsic resistance mechanisms in LAB, including poor drug uptake or active efflux. However, variable responses to tetracyclines were observed: while *L. helveticus* 4/20 and *L. helveticus* 6/20 were resistant, *L. helveticus* 12/20 was sensitive.

Table 6.1. Antibiotic resistance of *Lactobacillus helveticus* 4/20, *Lactobacillus helveticus* 6/20, *Lactobacillus helveticus* 8/20, *Lactobacillus helveticus* 9/20, *Lactobacillus helveticus* 12/20.

#	Mechanism of action	Antibiotic		Concentration	4/20	6/20	8/20	9/20	12/20
1	Inhibitor of cell wall synthesis	Penicillin	P	10 E/disc	R	S	S	R	R
2		Bacitracin	Cm	0,07 E/disc	R	S	S	SR	SR
3		Piperacillin	P	100 µg/disc	S	R	S	S	SR
4		Ampicillin	A	10 µg/disc	R	R	S	R	R
5		Oxacillin	O	1 µg/disc	R	S	R	R	R
6		Amoxicillin	Ax	25 µg/disc	S	S	S	R	SR
7		Vancomycin	V	30 µg/disc	R	S	S	R	R
8	Inhibitor of protein synthesis	Tetracycline	T	30 µg/disc	R	R	S	R	S
9		Doxycycline	D	30 µg/disc	R	SR	SR	SR	S
10		Gentamicin	G	10 µg/disc	SR	R	S	SR	SR
11		Tobramycin	Tb	10 µg/disc	R	S	S	R	R
12		Amikacin	Am	30 µg/disc	R	R	SR	R	SR
13		Lincomycin	L	15 µg/disc	R	R	SR	R	SR
14		Chloramphenicol	C	30 µg/disc	S	SR	S	S	S
15		Erythromycin	E	15 µg/disc	SR	S	S	R	S
16	Inhibitor of the DNA synthesis and/or of cell division	Novobiocin	Nb	5 µg/disc	S	R	S	SR	R
17		Nalidixic acid	Nx	30 µg/disc	R	R	SR	R	R
18		Rifampin	R	5 µg/disc	SR	S	S	SR	S
19		Ciprofloxacin	Cp	5 µg/disc	R	R	SR	SR	SR

At $d_{iz} < 8$ mm – R, at $8 < d_{iz} < 16$ mm – SR, at $d_{iz} > 16$ mm – S.

Chloramphenicol sensitivity was broadly maintained across strains, aligning with its known efficacy against a wide range of Gram-positive bacteria. Erythromycin sensitivity in *L. helveticus* 6/20, *L. helveticus* 8/20, and *L. helveticus* 12/20 is particularly important, as macrolides are frequently used in clinical settings; resistance in *L. helveticus* 9/20 could reflect either acquired resistance or a variation in ribosomal binding sites. In the group of antibiotics that inhibit DNA synthesis or cell division, ciprofloxacin and nalidixic acid resistance was widespread, except in *L. helveticus* 8/20, which showed intermediate susceptibility. These findings may be due to mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase or topoisomerase IV, commonly implicated in fluoroquinolone resistance. Sensitivity to novobiocin and rifampin was more variable, with *L. helveticus* 6/20 being uniquely sensitive to rifampin, suggesting differential mechanisms of resistance, potentially at the level of RNA polymerase structure or function. The strain-dependent differences in antibiotic resistance profiles observed underscore the importance of individual strain screening, especially when considering LAB strains for probiotic or food industry applications. Moreover, the presence of resistance to clinically relevant antibiotics such as vancomycin and ciprofloxacin raises potential concerns regarding horizontal gene transfer, particularly in environments where LAB co-exist with pathogenic bacteria. Further genomic analysis is warranted to determine whether the resistance observed is due to intrinsic mechanisms or the acquisition of resistance genes. This is especially important given the safety implications of using *L. helveticus* strains in food or pharmaceutical products (Table 6.1).

Table 6.2. Antibiotic resistance of *Lactiplantibacillus plantarum* 5/20, *Lactiplantibacillus plantarum* 13/20, *Lactiplantibacillus plantarum* 19/20, *Lactobacillus acidophilus* 10/20.

#	Mechanism of action	Antibiotic		Concentration	5/20	13/20	19/20	10/20
1	Inhibitor of cell wall synthesis	Penicillin	P	10 E/disc	R	R	R	R
2		Bacitracin	Cm	0,07 E/disc	R	SR	SR	SR
3		Piperacillin	P	100 µg/disc	R	SR	S	S
4		Ampicillin	A	10 µg/disc	R	R	R	R
5		Oxacillin	O	1 µg/disc	R	R	R	R
6		Amoxicillin	Ax	25 µg/disc	S	SR	R	R
7		Vancomycin	V	30 µg/disc	R	R	R	R
8	Inhibitor of protein synthesis	Tetracycline	T	30 µg/disc	SR	S	R	R
9		Doxycycline	D	30 µg/disc	S	S	SR	SR
10		Gentamicin	G	10 µg/disc	SR	SR	SR	SR
11		Tobramycin	Tb	10 µg/disc	R	R	R	R
12		Amikacin	Am	30 µg/disc	R	SR	R	R
13		Lincomycin	L	15 µg/disc	R	SR	R	R
14		Chloramphenicol	C	30 µg/disc	S	S	S	S
15		Erythromycin	E	15 µg/disc	R	S	R	R
16	Inhibitor of the DNA synthesis and/or of cell division	Novobiocin	Nb	5 µg/disc	SR	R	SR	SR
17		Nalidixic acid	Nx	30 µg/disc	R	R	R	R
18		Rifampin	R	5 µg/disc	SR	S	SR	SR
19		Ciprofloxacin	Cp	5 µg/disc	R	SR	SR	SR

At $d_{iz} < 8$ mm – R, at $8 < d_{iz} < 16$ mm – SR, at $d_{iz} > 16$ mm – S.

When it comes to the *Lactiplantibacillus plantarum* strains, among the cell wall synthesis inhibitors, resistance to β -lactam antibiotics was consistently observed across all *Lactiplantibacillus plantarum* strains. All three strains showed resistance to penicillin (Table 6.2), ampicillin, oxacillin, and vancomycin – an expected finding considering the intrinsic resistance of many LAB to β -lactams and glycopeptides due to variations in their cell wall structure and low-affinity penicillin-binding proteins. However, strain-specific differences were noted in response to bacitracin, piperacillin, and amoxicillin. For instance, *Lactiplantibacillus plantarum* 5/20 was sensitive only to amoxicillin, whereas *Lactiplantibacillus plantarum* 13/20 and *Lactiplantibacillus plantarum* 19/20 demonstrated intermediate susceptibility to amoxicillin and bacitracin, respectively. These subtle differences could reflect either adaptive phenotypic variation or distinct underlying resistance mechanisms, such as efflux pump activity or cell envelope permeability. In terms of protein synthesis inhibitors, tobramycin and amikacin resistance was universal across all *Lactiplantibacillus plantarum* strains, aligning with known intrinsic resistance in LAB to aminoglycosides due to their inability to penetrate the thick peptidoglycan layer in the absence of oxygen. Lincomycin resistance was also observed in all three *Lactiplantibacillus plantarum* strains, suggesting possible intrinsic mechanisms or conserved ribosomal protection elements. However, the response to other antibiotics such as tetracycline, doxycycline, erythromycin, and gentamicin varied between *Lactiplantibacillus plantarum* strains. *Lactiplantibacillus plantarum* 13/20 was sensitive to tetracycline and erythromycin, while *Lactiplantibacillus plantarum* 5/20 and *Lactiplantibacillus plantarum* 19/20 were resistant. Notably, chloramphenicol sensitivity was retained in all strains, a favorable observation given its historical effectiveness against Gram-positive bacteria and its use as a marker in probiotic screening. The intermediate responses to gentamicin and doxycycline across multiple strains suggest either partial resistance mechanisms or borderline

susceptibility thresholds that warrant further MIC-based analysis. For DNA synthesis and cell division inhibitors, resistance to nalidixic acid was observed in all *Lactiplantibacillus plantarum* strains, which is consistent with previously reported resistance in LAB due to mutations in DNA gyrase or topoisomerase IV. Ciprofloxacin resistance was also prevalent but exhibited intermediate susceptibility in strains *Lactiplantibacillus plantarum* 13/20 and *Lactiplantibacillus plantarum* 19/20, suggesting potential variability in fluoroquinolone target affinity or efflux mechanisms. Rifampin sensitivity in *Lactiplantibacillus plantarum* 13/20 and intermediate susceptibility in others points toward differing *rpoB* gene sequences or promoter activity. Resistance to novobiocin in *Lactiplantibacillus plantarum* 13/20 and intermediate responses in the other two strains align with the strain-dependent nature of resistance against this DNA gyrase inhibitor. The data reveal that while *Lp. plantarum* strains share common resistance traits—particularly against aminoglycosides, β -lactams, and quinolones—there is clear inter-strain heterogeneity that could impact their use in functional foods or clinical applications. Once again, the occurrence of resistance to clinically important antibiotics such as erythromycin and ciprofloxacin in some strains is a potential concern, especially in the context of horizontal gene transfer within the gut microbiota. These findings underscore the necessity of thorough safety assessments for each *L. plantarum* strain before inclusion in probiotic formulations, particularly when these strains may encounter antibiotic-selective environments in the host. Whole-genome sequencing, plasmid analysis, and conjugation assays would provide deeper insight into the genetic basis of resistance and the potential risk of horizontal gene transfer (Table 6.2).

Resistance to β -lactam antibiotics, including penicillin, ampicillin, oxacillin, and amoxicillin, was evident in *L. acidophilus* 10/20, which aligns with previously reported intrinsic resistance mechanisms in lactobacilli (Table 6.2). These mechanisms are thought to involve low-affinity penicillin-binding proteins (PBPs) and limited cell wall permeability to β -lactams. Interestingly, this strain showed sensitivity to piperacillin and intermediate sensitivity to bacitracin, suggesting partial or incomplete resistance mechanisms to certain β -lactams and polypeptide antibiotics.

This variability may reflect differences in the structural targets of these drugs or differential expression of resistance genes. In the category of protein synthesis inhibitors, *L. acidophilus* 10/20 demonstrated resistance to several commonly used antibiotics, including tetracycline, tobramycin, amikacin, lincomycin, and erythromycin. Resistance to aminoglycosides such as tobramycin and amikacin is well-documented in *Lactobacillus* species and is generally considered intrinsic due to their anaerobic or microaerophilic growth, which limits aminoglycoside uptake. Resistance to macrolides (erythromycin) and lincosamides (lincomycin), however, may be mediated by acquired genes such as *erm(B)* or *lnu(A)* and warrants further investigation to determine the risk of horizontal gene transfer. The strain retained sensitivity to chloramphenicol, a broad-spectrum antibiotic, and showed intermediate susceptibility to doxycycline and gentamicin, suggesting that some protein synthesis pathways remain vulnerable to inhibition. The intermediate susceptibility may indicate reduced expression of resistance mechanisms, low-level efflux activity, or partial permeability to these compounds. With respect to DNA synthesis and cell division inhibitors, *L. acidophilus* 10/20 was resistant to nalidixic acid and demonstrated intermediate sensitivity to ciprofloxacin, rifampin, and novobiocin. Resistance to quinolones such as nalidixic acid is common in lactobacilli and may arise from mutations in DNA gyrase or topoisomerase IV. The partial susceptibility to ciprofloxacin and rifampin may suggest incomplete resistance or adaptive tolerance, but further genomic analysis would be required to confirm whether these resistance traits are chromosomal or potentially mobile. The antibiotic profile of *L. acidophilus* 10/20 indicates a predominance of resistance to antibiotics across all three functional groups—cell wall synthesis, protein synthesis, and DNA replication inhibitors—with a few key exceptions. The retention of sensitivity to chloramphenicol and piperacillin, and intermediate sensitivity to several others, offers a promising basis for safe probiotic application, provided these traits are confirmed to be non-transferable (Table 6.2)

3.3. Batch Cultivation of *Lactiplantibacillus plantarum* 5/20 in a Laboratory Bioreactor with Continuous Stirring in MRS-Broth Medium. Determination of the Process Kinetics and Identification of Model Parameters

The possibility of obtaining a probiotic concentrate of active cells of *Lactiplantibacillus plantarum* 5/20 in MRS-broth medium was investigated. For this purpose, batch cultivation of the studied strain was carried out with correction and maintenance of pH=5.7 in a laboratory bioreactor with mechanical stirring. The dynamics of biomass accumulation during the process was monitored (Figure 12). The lag phase of the strain was extremely short and difficult to determine. Therefore, according to equation 2, the induction period (the time from the lag phase during which the cells begin to synthesize the necessary cellular structures and enzymes and to transition from a non-adapted to an adapted state to the composition of the medium and the cultivation conditions) and the adaptation rate constant k_0 were determined. According to the model, for this strain the induction period (τ_0) was 0.835 h, which was about 50 minutes. The adaptation rate constant (k_0) for this strain was relatively high – 0.500 h⁻¹. The short induction period and the high adaptation rate constant show that the strain quickly and easily adapted to the medium and cultivation conditions. From the 2nd to the 8th hour, intensive growth of the culture in the exponential phase was observed. From the 8th to the 12th hour, the strain entered the phase of slower growth, and at the 12th hour it entered the stationary phase, when a high concentration of viable cells of 7.1.10¹² cfu/cm³ was achieved. From the 12th to the 24th hour the concentration of viable cells increased slightly and was about 2.4.10¹³ cfu/cm³.

Based on these experimental data, mathematical modeling of the growth kinetics was performed and the maximum growth rate μ_m and the coefficient of intrapopulation competition β , which are important bioprocess parameters of the culture, were determined. A comparison was made between the experimental data and those obtained from the models. The results of these studies are presented in Figure 12, and the values of the kinetic parameters are shown in Table 7.

Table 7. Kinetic parameters of the process of batch cultivation of *Lactiplantibacillus plantarum* 5/20 in a bioreactor with mechanical stirring at 37 °C.

Batch cultivation of <i>Lactiplantibacillus plantarum</i> 5/20				
μ, h^{-1}	$\beta, cm^3/(CFU.h)$	$X_{kp}, LogN$	R^2	Error
0,130	0,0095	13,63	0,9995	0,778

From the data presented in Figure 12 it is clear that the models were very well in agreement with the experimental results. This was also confirmed by the high correlation coefficient and the low value of the identification error R^2 (Table 7). In addition, the model gave a theoretical value of the maximum biomass concentration of 13.63 LogN, which was very close to the experimental one - 13.36 LogN. This allows the derived mathematical model and the identified kinetic parameters in it to be used for forecasting and prediction of the fermentation process in the studied time interval, as well as for its scaling up and control. The data presented in Table 8 indicate that *Lactiplantibacillus plantarum* 5/20 was characterized by a relatively high maximum specific growth rate - 0.130 h⁻¹ and a low coefficient of intrapopulation competition - 0.0095 cm³/(CFU.h). The relatively high maximum specific growth rate and low coefficient of intrapopulation competition indicate that optimal conditions had been created for the growth of the studied strain.

Figure 13 presents the change in pH and oxidation-reduction potential. The data presented in Figure 9 show that the pH value rapidly decreased from 6.19 at the beginning of the process to 5.7 at the 4th hour, which once again testified to the rapid growth of the strain. After that, the pH value was maintained constant until the end of the fermentation process. The oxidation-reduction potential was relatively constant until the 4th hour and ranged between 114 – 118 mV, after which it began to decrease, and this trend was maintained until the end of the fermentation process, when its value was -89 mv.

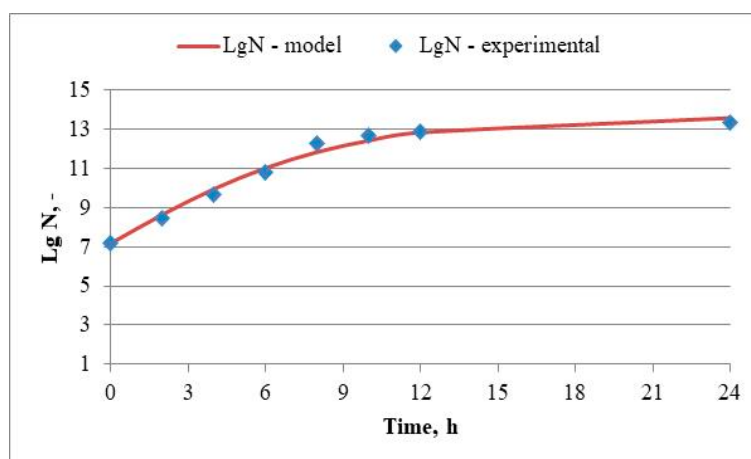


Figure 12. Comparison of experimental data with the logistic curve model for biomass accumulation during batch cultivation of *Lactiplantibacillus plantarum* 5/20 in a bioreactor with mechanical stirring at 37 °C.

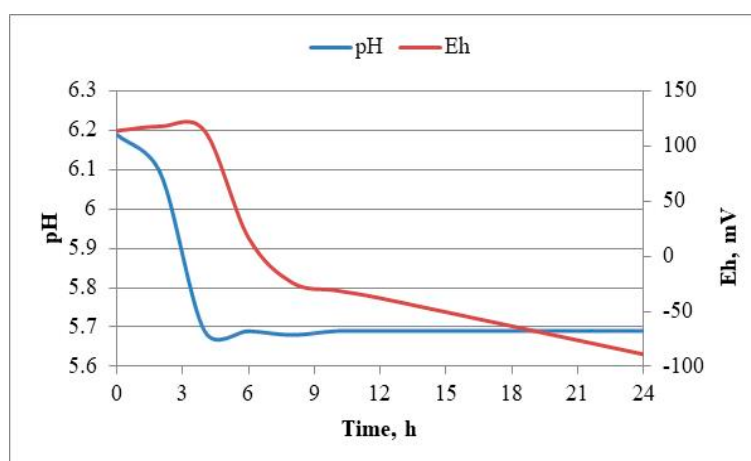


Figure 13. Change in the oxidation-reduction potential and the pH in the batch cultivation of *Lactiplantibacillus plantarum* 5/20 in a bioreactor with mechanical stirring at 37 °C.

3.4. Freeze-Drying

The freeze-drying method is appropriate when the moisture content of the raw materials is over 30%. The most common food products contain significant amounts of free water (from 70 to 90%) for different types of products. Like all preservation methods, freeze-drying (lyophilization) requires the products to be of high quality. Pre-treatment of production-valuable strains or mixed cultures is especially necessary. Pre-treatment aims to create conditions that guarantee maximum survival of bacterial cultures during freeze-drying. The vitality of bacterial cultures in the freeze-dried preparation is determined by the ability of bacterial cells to restore their initial biochemical properties upon rehydration. For lactic acid bacteria, these are fermentative, proteolytic, aroma-forming, antimicrobial and other activities. When microorganisms are stored in a dry state, they are in anabiosis. In this state, it is assumed that the metabolic processes are greatly slowed down, close to the cessation of all life processes - nutrition, respiration and reproduction. Since the cells are deprived of water and oxygen, metabolic processes are also terminated. The survival of different types of microorganisms subjected to freeze-drying is different. The LAB viability and in particular the microflora of Bulgarian yogurt, show that the survival of the studied and selected LAB strains during freeze-drying was also influenced by the growth stage at the time of drying [32]. The survival of bacterial cells also depends on the environment in which they are in the drying process. A number of substances contained in the nutrient media for cultivating microorganisms have a protective effect and preserve bacterial cells during drying. The concentration of bacterial cells also affects their

survival during freeze-drying. At a concentration of cultures of lactic acid bacteria of 10^{10} CFU/cm³ and higher, greater survival is observed [33]. Of essential importance for the survival of microorganisms is the method of supplying heat to freeze-dried cultures; with conductive heating the survival of lactic acid streptococci is high, and of *L. bulgaricus*, which is very sensitive to freeze-drying, it is low [34]. After completion of the fermentation process, the biomass was immobilized in a hydrocolloid matrix. After freezing to a temperature of -40°C , it was subjected to sublimation. The microbiological status of the obtained lyophilisate was determined (Table 8 and Figure 14).

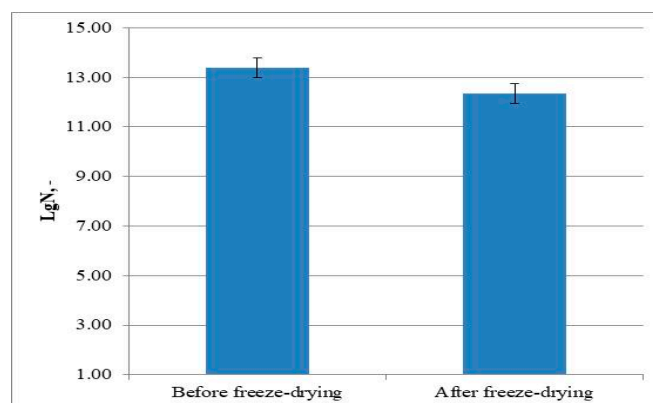


Figure 14. Survival of *Lactiplantibacillus plantarum* 5/20 cells during the freeze-drying process.

Table 8. Microbiological status of the lyophilized *Lactiplantibacillus plantarum* 5/20.

Type of pathogenic microorganisms tested	According to BDS	Analysis result
Total number of mesophilic aerobic and facultative anaerobic bacteria	No more than 800	180
<i>Escherichia coli</i> in 0.1 g of the product	Not to be detected any	Not detected
Sulphite-reducing clostridia in 0.1 g of the product	Not to be detected any	Not detected
<i>Salmonella</i> sp. in 25.0 g of the product	Not to be detected any	Not detected
Coagulase-positive staphylococci in 1.0 g of the product	Not to be detected any	Not detected
Spores of microscopic molds, CFU/g	No more than 100	Not detected
Yeasts, CFU/g	No more than 100	Not detected

The titer of active cells in the dry product after freeze-drying was determined and the concentration of active cells was found to be 2.3×10^{12} cfu/g, with a survival rate of 92.52% (Figure 14 and Table 8). The high survival rate indicates that the strain was xerotolerant and was little affected by the freeze-drying conditions. The resistance of the strain to damaging factors during freeze-drying makes it valuable for production purposes, since not all lactobacilli show such high survival and resistance to production processes.

4. Conclusions

11 strains were isolated from rose blossom of *Rosa damascena* Mill. They were identified to species level by applying cultural, microbiological, physiological-biochemical (API 50 CHL) and molecular-genetic (16S rRNA gene sequencing) identification methods. Among the isolates, there were 5 strains of the species *Lactobacillus helveticus* (4/20; 6/20; 8/20; 9/20 and 12/20), 3 strains of the species *Lactiplantibacillus plantarum* (5/20; 13/20 and 19/20), 1 strain of *Lactobacillus acidophilus* (10/20) and 2 strains of *Leuconostoc mesenteroides* (16/20 and 22/20). All newly isolated LAB strains exhibited high antimicrobial activity against the pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella abony*, *Proteus vulgaris*, *Listeria monocytogenes*, *Enterococcus faecalis*. The strains *Lactiplantibacillus*

plantarum 5/20, *Lactobacillus helveticus* 6/20 and *Lactobacillus acidophilus* 10/20 were resistant to most of the antibiotics used in clinical practice, which in turn opens up the possibility of their joint inclusion in the complex therapy in the composition of probiotics that would be administered together with the corresponding antibiotic. A batch fermentation process was carried out with *Lactiplantibacillus plantarum* 5/20 and the kinetic parameters of the fermentation process to obtain a concentrate with a high content of viable cells (10^{13} CFU/cm³) were determined. A freeze-dried preparation with a high content of viable cells (over 10^{12} CFU/g) was obtained. After additional studies of the probiotic properties of the newly isolated LAB strains, they can be included in the development of probiotic preparations and starter cultures for various types of functional foods and beverages.

Author Contributions: Conceptualization, Z. D. and R.D-K.; methodology, R.D-K. and B. G. .; software, R. D-K., G. K. and B. G.; validation, Z. D., R.D-K. and G. K.; formal analysis, P. Z., Y. G., B. G. and V. S; resources, Z. D.; data curation, R. D.-K and G. K.; writing—original draft preparation, R. D.-K., V. S., P. Z., B. G., Y. G.; writing—review and editing, R. D.-K., Z. D. and G. K; supervision, Z. D.; funding acquisition, G. K and P. Z.

Funding: The research in this article was funded by the project "Investigation of the synergistic action of plant extracts and lactic acid bacteria and ensuring sustainability in the production of fermented foods", National Program "Young scientists and postdoctoral students - 2", Ministry of Education and Science, Bulgaria

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Yu, A.O.; Leveau, J.H.; Marco, M.L. Abundance, diversity and plant-specific adaptations of plant-associated lactic acid bacteria. *Environ. Microbiol. Rep.* **2020**, *12*, 16–29.
2. Ibrahim, S.A.; Ayivi, R.D.; Zimmerman, T.; Siddiqui, S.A.; Altemimi, A.B.; Fidan, H.; Esatbeyoglu, T.; Bakhshayesh, R.V. Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods* **2021**, *10*, 3131.
3. Lingga, R.; Astuti, R.; Bahtera, N.; Adibrata, S.; Nurjannah, N.; Ahsaniyah, S.; Irawati, I. Effect of additional probiotic in cattle feed on cattle's consumption and growth rate. *Int. J. Sci. Technol. Manag.* **2022**, *3*, 1418–1425.
4. Shivsharan, U.; Bhitre, M. Antimicrobial activity of lactic acid bacterial isolates. *Int. Res. J. Pharm.* **2013**, *4*, 197–201.
5. Nadirova, S.; Sinyavskiy, Y. Biotechnological approaches to the creation of new fermented dairy products. *Eurasian J. Appl. Biotechnol.* **2023**, *4*, 128–136. <https://doi.org/10.11134/btp.4.2022.16>
6. Bayu, H.; Irwanto, R.; Dalimunthe, N.; Lingga, R. Isolation and identification of lactic acid bacteria from *Channa* sp. as potential probiotic. *J. Pembelajaran Biol. Nukleus* **2023**, *9*, 75–84. <https://doi.org/10.36987/jpbn.v9i1.3551>
7. Ar, I.; Laksmi, B.; Astawan, M.; Fujiyama, K.; Arief, I. Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian local beef. *Asian J. Anim. Sci.* **2014**, *9*, 25–36. <https://doi.org/10.3923/ajas.2015.25.36>
8. Pamungkaningtyas, F.; Mariyatun, M.; Kamil, R.; Setyawan, R.; Hasan, P.; Wiryohanjoyo, D.; Rahayu, E. Sensory evaluation of yogurt-like set and yogurt-like drink produced by indigenous probiotic strains for market test. *Indones. Food Nutr. Prog.* **2018**, *15*, 1. <https://doi.org/10.22146/ifnp.31010>
9. Pundir, R.K.; Rana, S.; Kashyap, N.; Kaur, A. Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study. *J. Appl. Pharm. Sci.* **2013**, *3*, 85–93. <https://doi.org/10.7324/JAPS.2013.30317>
10. Wang, J.; Lu, C.; Xu, Q.; Li, Z.; Song, Y.; Zhou, S.; Luo, X. Bacterial diversity and lactic acid bacteria with high alcohol tolerance in the fermented grains of soy sauce aroma type baijiu in North China. *Foods* **2022**, *11*, 1794. <https://doi.org/10.3390/foods11121794>
11. Aritionang, S.; Roza, E.; Rossi, E.; Purwati, E.; Husmaini, H. Isolation and identification of lactic acid bacteria from okara and evaluation of their potential as candidate probiotics. *Pak. J. Nutr.* **2017**, *16*, 618–628. <https://doi.org/10.3923/pjn.2017.618.628>

12. Hudzicki, J. Kirby-Bauer disk diffusion susceptibility test protocol. *Am. Soc. Microbiol.* **2009**, *15*, 1–23. Available online: <https://asm.org/protocols/kirby-bauer-disk-diffusion-susceptibility-test-pro> (accessed on 27 April 2025).
13. Sanger, F.; Nicklen, S.; Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463–5467. <https://doi.org/10.1073/pnas.74.12.5463>
14. Kostov, G.; Denkova, R.; Ivanova, P.; Denkova, I. Kinetics of batch fermentation in the cultivation of a probiotic strain of lactic acid bacteria. *Acta Univ. Cibiniensis Ser. E Food Technol.* **2015**, *19*, 61–72. Available online: [https://saiapm.ulbsibiu.ro/cercetare/ACTA_E/AUCFT2015_19\(1\)_61-72.pdf](https://saiapm.ulbsibiu.ro/cercetare/ACTA_E/AUCFT2015_19(1)_61-72.pdf) (accessed on 27 April 2025).
15. Kostov, G.; Denkova, R.; Ivanova, P.; Denkova, I. Microbial growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in a complex nutrient medium (MRS-broth). *ECMS Proc.* **2022**, 135–142. Available online: https://www.scs-europe.net/dlib/2022/ecms2022acceptedpapers/0135_simo_ecms2022_0028.pdf (accessed on 27 April 2025).
16. Kostov, G.; Denkova-Kostova, R.; Ivanova, P.; Denkova, I. New approach to modelling the kinetics of the fermentation process in cultivation of lactic acid bacteria. *Proc. Eur. Conf. Model. Simul.* **2018**, 212–218. Available online: <https://www.scs-europe.net/dlib/2018/2018-0212.htm> (accessed on 27 April 2025).
17. Denkova, R.; Denkova, Z.; Yanakieva, V.; Georgieva, L. Highly active freeze-dried probiotic concentrates with long shelf life. *Food Environ. Saf.* **2013**, *12*, 225–232. Available online: <https://fens.usv.ro/index.php/FENS/article/download/169/167> (accessed on 27 April 2025).
18. Bulgarian State Standard (BDS) EN ISO 4833:2004. Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of microorganisms—Colony-count technique at 30 °C. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2004.
19. Bulgarian State Standard (BDS) ISO 15214:2002. Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of mesophilic lactic acid bacteria—Colony-count technique at 30 degrees C. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2002.
20. Bulgarian State Standard (BDS) EN ISO 4833-1:2013/A1:2022. Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 degrees C by the pour plate technique; Amendment 1. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2022.
21. Bulgarian State Standard (BDS) ISO 16649-2:2014. Microbiology of the food chain—Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli*—Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β -D-glucuronide. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2014.
22. Bulgarian State Standard (BDS) EN ISO 6579-1:2017/A1:2020. Microbiology of the food chain—Horizontal method for the detection, enumeration and serotyping of *Salmonella*—Part 1: Detection of *Salmonella* spp.; Amendment 1: Broader range of incubation temperatures and clarification of testing of samples with high background flora. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2020.
23. Bulgarian State Standard (BDS) EN ISO 6888-1:2021/A1:2023. Microbiology of the food chain—Horizontal method for the enumeration of *Staphylococcus aureus*—Part 1: Technique using Baird-Parker agar medium; Amendment 1. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2023.
24. Bulgarian State Standard (BDS) ISO 15213:2023. Microbiology of the food chain—Horizontal method for the detection and enumeration of *Clostridium* spp.—Colony-count technique. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2023.
25. Bulgarian State Standard (BDS) ISO 6611:2006. Milk and milk products—Enumeration of colony-forming units of yeasts and/or moulds—Colony-count technique at 25 degrees C. Bulgarian Institute for Standardization: Sofia, Bulgaria, 2006.
26. Majhenič, A.Č.; Lorbeg, P.M.; Treven, P. Enumeration and identification of mixed probiotic and lactic acid bacteria starter cultures. In *Probiotic Dairy Products*; CRC Press: Boca Raton, FL, USA, 2017; pp. 207–251.
27. Singh, S.; Goswami, P.; Singh, R.; Heller, K.J. Application of molecular methods for identification of lactic acid bacteria. *LWT—Food Sci. Technol.* **2009**, *42*, 448–457.
28. Song, Y.L.; Kato, N.; Matsumiya, Y.; Liu, C.X.; Kato, H.; Watanabe, K. Identification of *Lactobacillus* species of human origin by a commercial kit, API50CHL. *Rinsho Biseibutsu Jinsoku Shindan Kenkyukai Shi* **1999**, *10*, 77–82.

29. Charteris, W.P.; Kelly, P.M.; Morelli, L.; Collins, J.K. 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. *J. Appl. Microbiol.* **1998**, *84*, 759–768.
30. Mathur, S.; Singh, R. Antibiotic resistance in food lactic acid bacteria—A review. *Int. J. Food Microbiol.* **2005**, *105*, 281–295.
31. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496.
32. Fonseca, F.; Cenard, S.; Passot, S. Freeze-drying of lactic acid bacteria. In *Methods in Molecular Biology*; Humana Press: New York, NY, USA, 2015; Volume 1257, pp. 477–488. https://doi.org/10.1007/978-1-4939-2193-5_24.
33. Tsvetkov, T.; Brankova, R. Viability of micrococci and lactobacilli upon freezing and freeze-drying in the presence of different cryoprotectants. *Cryobiology* **1983**, *20*, 318–323. [https://doi.org/10.1016/0011-2240\(83\)90020-2](https://doi.org/10.1016/0011-2240(83)90020-2).
34. Montel Mendoza, G.; Pasteris, S.E.; Otero, M.C.; Nader-Macías, M.E.F. Survival and beneficial properties of lactic acid bacteria from ranculture subjected to freeze-drying and storage. *Benef. Microbes* **2014**, *5*, 381–390. <https://doi.org/10.3920/BM2013.0068>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.