








## Article

# Hypoglycemic, Antioxidant Activities, and Probiotic Characteristics of *Lacticaseibacillus rhamnosus* LBUX2302 Isolated from Stool Samples of Neonates

Pedro A. Reyes-Castillo <sup>1,†</sup>, Ana Laura Esquivel-Campos <sup>2,†</sup> , Edgar Torres-Maravilla <sup>3</sup> , Eduardo Zúñiga-León <sup>2</sup> , Felipe Mendoza-Pérez <sup>2</sup> , Rosa González-Vázquez <sup>4,5</sup>, María Guadalupe Córdova-Espinoza <sup>4,5,6</sup> , María Angélica Gutiérrez-Nava <sup>7</sup> , Raquel González-Vázquez <sup>8,\*</sup>  and Lino Mayorga-Reyes <sup>2,\*</sup>

- <sup>1</sup> Doctorado en Ciencias Biológicas y de la Salud, Universidad Autonoma Metropolitana, Mexico City 04960, Mexico; 2202802526@alumnos.xoc.uam.mx
  - <sup>2</sup> Laboratorio de Biotecnología, Departamento de Sistemas Biológicos, Universidad Autonoma Metropolitana Unidad Xochimilco, Mexico City 04960, Mexico; aesquivel@correo.xoc.uam.mx (A.L.E.-C.); jezuniga@correo.xoc.uam.mx (E.Z.-L.); fmendoza@correo.xoc.uam.mx (F.M.-P.)
  - <sup>3</sup> Facultad de Medicina Mexicali, Universidad Autonoma de Baja California, Mexicali 21000, Mexico; edgar.torres.maravilla@uabc.edu.mx
  - <sup>4</sup> Laboratorio de Bacteriología Médica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (IPN), Mexico City 11350, Mexico; rosagonzalez@yahoo.com.mx (R.G.-V.); mixtlipp@yahoo.com.mx (M.G.C.-E.)
  - <sup>5</sup> Unidad Médica de Alta Especialidad, Hospital de Especialidades, “Dr. Antonio Fraga Mouret”, Centro Médico Nacional La Raza, Instituto Mexicano del Seguro Social (IMSS), Mexico City 02990, Mexico
  - <sup>6</sup> Laboratorio de Inmunología, Escuela Militar de Graduados de Sanidad, Mexico City 11200, Mexico
  - <sup>7</sup> Laboratorio de Ecología Microbiana, Departamento de Sistemas Biológicos, Universidad Autonoma Metropolitana Unidad Xochimilco, Ciudad de Mexico 04960, Mexico; agutz@correo.xoc.uam.mx
  - <sup>8</sup> Laboratorio de Biotecnología, Departamento de Sistemas Biológicos, Secihti-Universidad Autonoma Metropolitana Unidad Xochimilco, Mexico City 04960, Mexico
- \* Correspondence: rgonzalezv@correo.xoc.uam.mx (R.G.-V.); lmayorga@correo.xoc.uam.mx (L.M.-R.)
- † These authors contributed equally to this work.



Academic Editor: Pabulo Rampelotto

Received: 22 March 2025

Revised: 2 May 2025

Accepted: 13 May 2025

Published: 18 May 2025

**Citation:** Reyes-Castillo, P.A.; Esquivel-Campos, A.L.; Torres-Maravilla, E.; Zúñiga-León, E.; Mendoza-Pérez, F.; González-Vázquez, R.; Córdova-Espinoza, M.G.; Gutiérrez-Nava, M.A.; González-Vázquez, R.; Mayorga-Reyes, L. Hypoglycemic, Antioxidant Activities, and Probiotic Characteristics of *Lacticaseibacillus rhamnosus* LBUX2302 Isolated from Stool Samples of Neonates. *Life* **2025**, *15*, 804. <https://doi.org/10.3390/life15050804>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** *Lacticaseibacillus* species have shown potential in managing hyperglycemia, hypercholesterolemia, and oxidative stress, depending on the strain and species. This study aimed to isolate a novel *Lacticaseibacillus rhamnosus* strain from healthy newborns and assess its hypoglycemic and antioxidative activity, along with other probiotic properties. A non-hemolytic *L. rhamnosus* LBUX2302 was isolated, and it exhibited survival rates of 2.7%, 22%, and 27.5% at pH 2, 3, and 5 for 120 min. It metabolized various carbon sources and showed resistance to gentamicin, dicloxacillin, and penicillin; coaggregated with *Salmonella typhi* ATCC14028, *Staphylococcus aureus* STCC6538, and *Escherichia coli* O157:H7. *L. rhamnosus* LBUX2302 showed hydrophobicity, autoaggregation, and adhesion to HaCat, HeLa, MCF-7, SK-LU-1, and SW620 cell lines. It also exhibited extracellular activity of bile salt hydrolase. Enzymatic inhibition assays revealed 66% and 24% inhibitions of  $\alpha$ -amylase and  $\alpha$ -glucosidase, respectively. Its cell-free supernatant inhibited DPPH (89%), hydroxyl (81%), and superoxide anion radicals (61%). Also, antioxidant activity was observed in whole cells and cell fragments. Finally, the presence of ferulic acid activity was detected. The results highlight *L. rhamnosus* LBUX2302 as a promising probiotic with hypoglycemic and antioxidant effects, warranting further in vivo evaluation for its possible inclusion in functional food and health formulations.

**Keywords:** *Lacticaseibacillus rhamnosus*; probiotics; bile salt hydrolase activity; antioxidant effect; adhesion

## 1. Introduction

Lactic acid bacteria (LAB) comprise the *Lactacaseibacillus* genus, in which the *rhamnosus* species is included [1]. LAB have been isolated from various sources, such as fermented dairy and non-dairy products, the gastrointestinal tract of humans, animals, and insects, as well as human breast milk [2,3]. FAO/WHO suggested that potential probiotics should be capable of surviving passage through the digestive tract. This means they must be resistant to gastric juices and be able to grow in the presence of bile under conditions found in the intestines. As with any bacteria, antibiotic resistance exists among some LAB, and this resistance may be related to chromosomal- or plasmid-located genes [4].

Subsequently, their functional properties should be assessed, including adhesion to the epithelial surface, antimicrobial and antioxidant activity, hydrophobicity, and self-aggregation [5,6]. LAB can attach to the intestinal epithelium via various mechanisms, including passive forces, electrostatic and hydrophobic interactions, steric forces, lipoteichoic acids, and distinct surface structures. This ability to adhere may inhibit pathogenic bacteria from binding to intestinal cells [7].

LAB can reduce cholesterol through the enzymatic activity of bile salt hydrolase (BSH) [8]. Certain *Lactacaseibacillus* strains have shown hypoglycemic potential, attributed to their ability to inhibit the enzymatic activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase. This inhibition slows glucose uptake, promoting a more stable glycemic profile, and suggests a potential therapeutic application for the management of type 2 diabetes mellitus (T2DM) [9,10]. T2DM and oxidative stress are closely linked due to hyperglycemia-induced overproduction of reactive oxygen species (ROS), such as superoxide anion ( $O_2^{\cdot -}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $OH^{\cdot -}$ ). Some *Lactacaseibacillus* strains have shown antioxidant activity by increasing different free radical scavenging activities and superoxide dismutase enzyme activity, which could help to reduce oxidative stress, thus impacting glucose homeostasis [11].

*L. rhamnosus* isolated from cheese showed antibacterial effects against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [12]. *Lactacaseibacillus rhamnosus* LR22 has been reported as beneficial against constipation [13]. Due to the above, this work focused on isolating a new strain of *L. rhamnosus* from healthy newborns and evaluating its hypoglycemic and antioxidative activity, along with other probiotic properties.

## 2. Materials and Methods

### 2.1. Strain Isolation

LAB isolation was performed using stool samples from healthy newborns from an obstetric clinic of the public health service in Mexico. The research protocol was approved by the Ethics Committee of the clinic under registration number 36,068. Details about isolation are found in Reyes-Castillo et al. (2023) [10]. We did not handle the stool samples directly as we received the purified strains, which were subsequently examined for their morphology and Gram staining characteristics.

### 2.2. Molecular Identification

A Wizard<sup>®</sup> Genomic DNA Purification Kit (Promega, Tokyo, Japan) was used to perform gDNA extraction, following the manufacturer's protocol. The primers used to amplify a segment of the genomic DNA of our isolated strain were 27F (AGAGTTTGATCMG-GCTCAG) and 1491R (TACGGYTACCTTGTTAGGATT). The conditions for the PCR reaction were according to Galkiewicz and Kellogg, 2008 [14]. The amplified product was sequenced by the Integrated Microbiome Resource (Halifax, NS, Canada). Sequence alignment and comparisons were conducted using MEGA5 and NCBI's Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (accessed on 18 May 2024).

### 2.3. Phylogeny Analysis

The phylogenetic tree was constructed using MEGA software (version 11.0.13) [15] by the Maximum Likelihood method with the Kimura 2-parameter model [16]. The 16S rRNA gene sequences were obtained from seven *Lactobacillus* species deposited in the NCBI database. Bootstrap values of the tree were computed by resampling 1000 replications.

### 2.4. Catalase Test

A 12 h culture plate of pure bacterial cells was used to evaluate catalase activity by placing a drop of hydrogen peroxide on the center of a clean slide. Then, using a sterile bacteriological loop or stick, a small amount of the bacterial colony was transferred and mixed with the H<sub>2</sub>O<sub>2</sub> drop. The reaction was observed immediately. The positive control was *Staphylococcus aureus* ATCC6538 [17], while *Lactocaseibacillus casei* isolated from a commercial product was the negative control. This strain was used as the control in the next experiments. Catalase-positive bacteria produced bubbles due to the release of oxygen, whereas catalase-negative bacteria did not generate bubbles. Triplicates of the test were performed [18].

### 2.5. Hemolysis Test

A bacterial suspension containing  $1 \times 10^9$  CFU/mL was seeded onto blood agar and incubated at 37 °C for 48 h. *S. aureus* ATCC6538 [17] and *L. casei* [18] were used as controls. Hemolysis was considered positive when complete red blood cell lysis was observed, whereas the absence of hemolysis was classified as a negative result. Triplicates of the test were performed [10].

### 2.6. Growth Kinetics on Different Carbon Sources

Bacterial growth was monitored over 10 h at 2 h intervals using glucose, sucrose, fructooligosaccharides (FOS), lactose, lactulose, and raffinose at 0.01% (*w/v*) concentrations in a minimal medium (pH 7) containing 0.02 g/L NaCl, 1 g/L (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 0.02 g/L, CaCl<sub>2</sub> ·H<sub>2</sub>O, 0.4 g/L MgSO<sub>4</sub> ·7-H<sub>2</sub>O, 0.72 g/L, K<sub>2</sub>H PO<sub>4</sub>, 0.72 g/L, KH<sub>2</sub> PO<sub>4</sub>, and 0.01% (*w/v*) yeast extract. Bacterial growth was measured using a microplate reader and analyzed with Gen5<sup>®</sup> software (version 2x). Triplicates of the test were performed [10].

### 2.7. Antimicrobial Activity

This activity was evaluated against *E. coli* ATCC25922 [19], *E. coli* O157:H7 [20], *Salmonella typhi* ATCC14028 [21], and *S. aureus* ATCC6538 [17] by diffusion plate assay, as reported by Reyes-Castillo et al. (2023) [10]. Absence of inhibition was considered as negative (−), weak inhibition as (+), and strong inhibition as (++). Antimicrobial resistance testing was conducted in triplicate. *L. casei* was used as a control [18]. Triplicates of the test were performed [9].

### 2.8. Antibiotic Resistance

This ability was assessed by the diffusion test, using Multibac discs for Gram-positive bacteria of Investigación Diagnostica, Ciudad de Mexico, Mexico, according to Reyes-Castillo et al. (2023) [10]. The discs contained 1 µg of dicloxacillin, 5 µg of ciprofloxacin, 10 µg of ampicillin and gentamicin, 15 µg of erythromycin, 25 µg of trimethoprim-sulfamethoxazole, 30 µg of cefotaxime, cephalothin, clindamycin, vancomycin, and tetracycline, and 10 U of penicillin. Triplicates of the test were performed. *L. casei* was used as a control.

### 2.9. pH Resistance

Prior to the experiment, a standard growth curve of *L. rhamnosus* and *L. casei* (control) was determined to establish the amount of CFU needed to inoculate 10 mL of MRS broth to obtain, after 8 h of incubation at 37 °C,  $1 \times 10^9$  CFU/mL. To prepare the inoculum of the desired concentration, 10 mL of MRS was inoculated and incubated at 37 °C for 24 h. Then, each culture was centrifuged for 5 min at  $10,000 \times g$ , the pellet was washed twice with PBS (0.1 M, pH 7) and suspended in PBS adjusted to different pH values (1.5, 2, 3, and 5) using 1N HCl and incubated at 37 °C for 120 min. The viability was determined by the plate count method [18] at 0, 15, 30, 60, and 120 min. Using the following expression, the percentage of viability was determined under the test conditions. Triplicates of the test were performed [22].

$$\% \text{ Viability} = \left( \frac{\text{UFC}/\text{mL}_{\text{time min}}}{\text{UFC}/\text{mL}_{\text{time0 min}}} \right) * 100$$

### 2.10. Hydrophobicity

Hydrophobicity was evaluated according to Vinderola et al. 2003 [23] to assess non-specific adhesion (the ability to adhere to hydrocarbons). Previously, a suspension of *L. rhamnosus* with an optical density (OD) of 1.0 at 600 nm ( $A_0$ ) was prepared. This suspension was vigorously mixed for 1 min with xylene in a 1:3 ratio and incubated at 37 °C for one hour. The aqueous component was separated, and its  $\text{OD}_{600 \text{ nm}}$  was measured ( $A_1$ ). *L. casei* was the control strain. Triplicates of the test were performed. Hydrophobicity was defined as follows:

$$\% \text{ Hidrophobicity} = \left( 1 - \left( \frac{A_1}{A_0} \right) \right) * 100$$

### 2.11. Autoaggregation

Before the experiment, a bacterial culture was prepared as in the hydrophobicity test and was considered  $A_0$ . The suspension was incubated at 37 °C for 2 h. Then, 0.1 mL was transferred and mixed with 1.9 mL of PBS.  $\text{OD}_{600 \text{ nm}}$  was determined ( $A_1$ ). *L. casei* was the control strain. Triplicates of the test were performed [24]. Autoaggregation was defined as follows:

$$\% \text{ Autoaggregation} = \left( 1 - \left( \frac{A_1}{A_0} \right) \right) * 100$$

### 2.12. Coaggregation

This assay involved preparing a cell suspension as described in the hydrophobicity tests and mixing 1 mL of the suspension with an equal volume of each pathogen (*E. coli*, *S. typhi*, and *S. aureus*). The  $\text{OD}_{600 \text{ nm}}$  was measured and designated as  $A_0$ . The mix was then incubated for 2 h at 37 °C, after which the  $\text{OD}_{600 \text{ nm}}$  was measured and considered as  $A_t$ . *L. casei* was used as the control strain. Triplicates of the test were performed. The coaggregation percentage was defined as in Zuo et al. (2023) [24].

$$\% \text{ Coaggregation} = (A_0 - A_T / A_0) * 100$$

### 2.13. Qualitative Assay of Bile Salt Tolerance

For bile salt tolerance assessment, the method of Gao et al. (2021) [9] was used. In brief, 10 µL of a fresh culture of the isolated strain was inoculated onto plates containing MRS agar containing 0.1%, 0.3%, and 0.5% *w/v* of glycocholic acid (GcCA), glycodeoxycholic acid (GDxCA), taurocholic acid (TcCA), taurodeoxycholic acid (TDxCA), and oxgall (OXGL) (Sigma Aldrich, St. Louis, MO, USA). Each assay was incubated aerobically for 48 h at

37 °C. The appearance of a halo surrounding the colony after incubation was considered an indicator of tolerance to bile salt (BS). Triplicates of the test were performed.

#### 2.14. Bile Salt Hydrolase (BSH) Activity

This activity was assessed using whole cells (WC) according to González-Vázquez et al. (2015) [18], using an inoculum of  $1 \times 10^9$  CFU/mL in PBS containing 0.5% *w/v* of GcCA, GDxCA, TcCA, TDxCA, and OXGL and incubated for 48 h. *L. casei* was used as a control. A standard curve of glycine was established. Triplicates of the test were performed. BSH activity was calculated as follows:

$$\% \text{ BSH activity} = \frac{\text{Glycin concentration}_{\text{sample}}}{\text{Glycin concentration}_{\text{control}}} * 100$$

#### 2.15. 2,2-Diphenyl-1 Picrylhydrazyl (DPPH) Radical Inhibitory Activity

To analyze the inhibition of DPPH radical activity by *L. rhamnosus* LBUX02, a solution containing 1 mL of 0.2 mmol/L of DPPH solubilized in CH<sub>3</sub>OH was mixed with 1 mL of WC or cell-free supernatant (CFs), or cell fragments Cfg, and maintained in darkness for 30 min. OD<sub>517 nm</sub> was measured. *L. casei* was the control strain. Triplicates of the test were performed. DPPH inhibitory activity was calculated as described by Gao et al. (2021) [9]:

$$\% \text{ DPPH inhibitory activity} = 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} * 100$$

#### 2.16. Hydroxyl Radical Inhibitory Activity

In this assay, 1 mL of WC, CFs, or Cfg was mixed with 1.0 mL of phenanthroline (2.5 mM), 1 mL of FeSO<sub>4</sub> (2.5 mM), and 1 mL of PBS (pH 7.4), then the mixture was incubated at 37 °C for 90 min. To inhibit the reaction, 1 mL of 20 mM H<sub>2</sub>O<sub>2</sub> was added, and the OD<sub>517 nm</sub> was measured. *L. casei* was the control strain. Triplicates of the test were performed. Inhibitory ability was determined as described by Yan et al. (2019) [25]:

$$\% \text{ Hydroxyl radical inhibitory activity} = 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} * 100$$

#### 2.17. Superoxide Anion Radical Inhibitory Activity

The inhibitory activity was evaluated using 1 mL per sample of WC, CFs, or Cfg mixed with 3 mL of Tris-HCl solution (pH 8.2) and incubated at 25 °C for 20 min. Afterwards, 0.4 mL of pyrogallol (25 mM) was added, and the reaction was maintained at room temperature for 4 min. Then, 0.5 mL of HCl was added to inhibit the reaction, and OD<sub>325 nm</sub> was measured. *L. casei* was the control strain. Triplicates of the test were performed. Inhibitory activity was determined as follows [25]:

$$\% \text{ Superoxide anion radical inhibitory activity} = \left( 1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right) * 100$$

#### 2.18. Qualitative Ferulic Acid Activity (EFA)

EFA activity on the plate was tested in agreement with the methodology reported by Tomaro-Duchesneau et al. 2012 [26]. Before the assay, LAB strains were inoculated in MRS broth supplemented with 1% ethyl 4-hydroxy-3-methoxycinnamate (EFA), 1.33 mM, and then they were incubated anaerobically under standard conditions. Meanwhile, MRS EFA agar plates were prepared using MRS agar (pH = 6.5 and 1.5% *w/v*). Once tempered, 0.3 mL of EFA (prepared at 10% *w/v* in N, N-dimethylformamide) was added per 20 mL of agar. Sterile Whatman #3 paper discs, impregnated with LAB strains (MRS-EFA culture), were



placed onto the dried MRS EFA plates, and incubated at 37 °C for 48 h. No activity was considered as a negative (−) test, a moderate test was considered as positive (+), and total activity was considered as double positive (++). *L. casei* was the control strain. Triplicates of the test were performed.

#### 2.19. Inhibition of $\alpha$ -Amylase

This experiment was carried out according to Won et al. (2021) [27]. In brief, a previous culture of *L. rhamnosus* was prepared. The experiment was carried out by plating the bacteria at a concentration of  $1 \times 10^9$  CFU/mL onto MRS broth and incubating at 37 °C for 24 h. Afterwards, the culture was centrifuged for 15 min at  $8000 \times g$  at 4 °C. Then, 250  $\mu$ L of  $\alpha$ -amylase (pre-incubated at 25 °C for 10 min, 0.5 mg/mL) was mixed with 250  $\mu$ L of CFs. The mix was then incubated at 25 °C for 10 min with 250  $\mu$ L of 1% (*w/v*) starch solution in 0.02 M PBS. To finish the reaction, 500  $\mu$ L of DNS dye reagent was added, and the test was boiled for 5 min, then cooled to room temperature and diluted fourfold with distilled water. OD<sub>540 nm</sub> was determined using 1 mL of the diluted solution. *L. casei* was the control strain. Triplicates of the test were performed. The percentage of  $\alpha$ -amylase inhibition was defined as follows:

$$\% \alpha - \text{amylase inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) * 100$$

#### 2.20. Inhibition of $\alpha$ -Glucosidase

The inhibition of this enzyme was determined according to Won et al. (2021) [27]. *L. rhamnosus* strain ( $1 \times 10^9$  CFU/mL) was grown on MRS broth under standard conditions. Then the culture was centrifuged at  $8000 \times g$  for 15 min at 4 °C. A volume of 25  $\mu$ L of supernatant was used to mix it with 150  $\mu$ L of 0.01 M PBS (pH 7.0) and 75  $\mu$ L of *p*-nitrophenyl glucopyranoside (PNPG) solution (0.2 M). This mixture was incubated at 37 °C for 10 min. To start the reaction, 50  $\mu$ L of  $\alpha$ -glucosidase (0.17 U/mL) was added. The reaction was incubated at 37 °C for 10 min, and it was finished by adding 1 mL of 0.1 M Na<sub>2</sub>CO<sub>3</sub>. OD<sub>405 nm</sub> was quantified to determine the *p*-nitrophenol released. The experiment was performed in triplicate, with *L. casei* as the control strain. The percentage of  $\alpha$ -glucosidase inhibition was defined as follows:

$$\% \alpha - \text{glucosidase inhibition} = \left( 1 - \frac{(C - D)}{(A - B)} \right) * 100$$

#### 2.21. Adhesion to Cancer Cell Lines

Cell adhesion assays were performed using different cancer cell lines, including SW-620 (colon cancer), Hella (cervical cancer), MCF-7 (breast cancer), HaCaT (aneuploid immortal keratinocyte cell), and SK-LU-1 (lung adenocarcinoma). Dulbecco's Modified Eagle's medium (Lonza, Basel, Suiza), with 5% fetal bovine serum (Gibco), 1% L-glutamine (Lonza, Basel, Suiza), and 1% antibiotics (penicillin/streptomycin, Gibco), was used for all cell line proliferations. Then,  $4 \times 10^5$  cells per well were seeded and incubated under standard conditions with 5% CO<sub>2</sub>. Triplicates of the test were performed [28].

Before the adhesion assay, a fresh culture of *L. rhamnosus* at  $1 \times 10^8$  CFU/mL was prepared. The bacteria were then incubated with the cancer cell lines at 37 °C with 5% CO<sub>2</sub> for 1 h. The culture was washed with PBS and 300  $\mu$ L of trypsin-EDTA (Gibco, Waltham, MA, USA). Adherent *Lactobacillus* cells were quantified by plate counting and expressed as CFU/mL. Adhesion was reported as the percentage of adhered bacterial cells with respect to the *Lactobacillus* initially added.

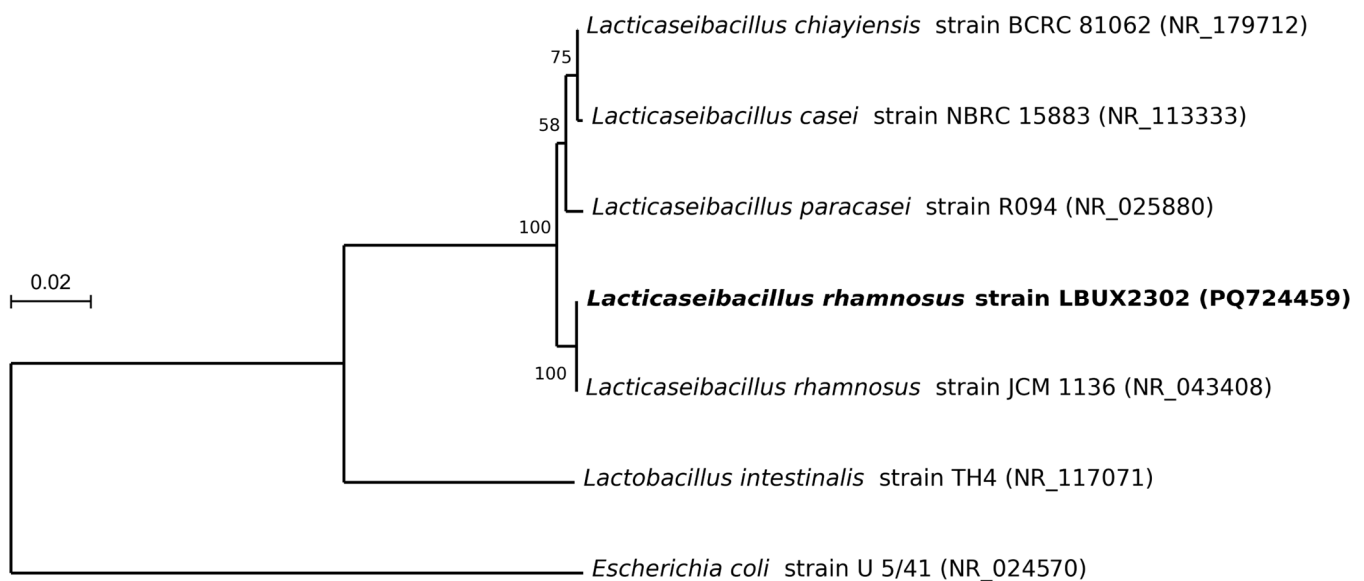
### 2.22. Statistical Analysis

Significant differences in all experiments were determined using the Kruskal–Wallis test, followed by Dunn’s post hoc test with a 95% confidence interval, performed using GraphPad Prism 5.01 software.

## 3. Results

### 3.1. Identification and Phylogeny

The isolate obtained from stool samples exhibited a rod-like morphology and was Gram-positive. The DNA amplified via PCR was sequenced, and BLASTN (version 2.16.1+) analysis revealed 99.6% identity with *Lactacaseibacillus rhamnosus* (Figure 1). The strain was designated as *L. rhamnosus* LBUX2302 (GenBank accession: PQ724459.1).

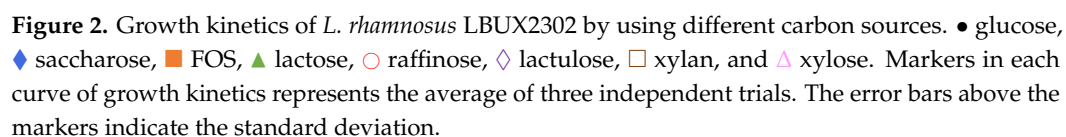


**Figure 1.** Phylogeny analysis based on 16S rRNA gene sequences from 7 bacteria including species of *Lactacaseibacillus*, *Lactobacillus* and *Escherichia*. The percent numbers at the nodes indicate the levels of bootstrap support based on Maximum Likelihood analyses of 1000 replicates.

### 3.2. Growth Kinetics on Different Carbon Sources

*L. rhamnosus* LBUX2302 exhibited optimum growth at pH 7.0. and was able to metabolize all tested carbohydrates (Figure 2). The highest growth rate was observed in glucose (control). The exponential phase for glucose and saccharose lasted between 3 h, whereas for FOS, it was 1 h; however, no significant differences were detected. Differences ( $p \leq 0.05$ ) between glucose and lactose, raffinose, lactulose, xylan, and xylose were found. Sucrose showed significant differences compared to lactulose and xylose (Figure 1). Additionally, FOS exhibited significant differences compared to lactulose, xylan, and xylose. For raffinose, lactulose, xylan, and xylose, an initial phase was observed during the first hour, followed by an exponential phase between 1 and 3 h. After 4 h, the strain maintained stable metabolic activity over time (Figure 2).

Significant differences ( $p \leq 0.05$ ) in the growth of *L. rhamnosus* LBUX2302 when utilizing glucose vs. lactose, raffinose, lactulose, xylan, and xylose; sucrose vs. lactulose, and xylose; and FOS vs. lactulose, xylan, and xylose were found.



Neither *L. rhamnosus* LBUX2302 nor *L. casei* exhibited catalase or hemolytic activity. Conversely, *S. aureus* (negative control) displayed both activities, suggesting that the isolates are safe for use, which is an essential criterion for probiotic microorganisms (Table 1A).

**Table 1.** Biochemical and functional characterization of *L. rhamnosus* LBUX2302.

[illegible]



Table 1. Cont.

	(E) % Hydrophobicity	%Autoaggregation	
<i>L. casei</i>	0	94	
<i>L. rhamnosus</i> LBUX2302	61	54	
	(F) % Coaggregation		
	<i>E. coli</i> ATCC25922	<i>S. aureus</i> ATCC6538	<i>S. typhi</i> ATCC14028
<i>L. casei</i>	7.3	25	10
<i>L. rhamnosus</i> LBUX2302	74	77	69
	(G) Ferulic Acid Activity		
<i>L. casei</i>	+		
<i>L. rhamnosus</i> LBUX2302	+		

(−) indicates absence of inhibition, (+) indicates weak inhibition, and (++) indicates strong pathogen and no pathogen inhibition. Va: vancomycin; Am: ampicillin; Dc: dicloxacillin; Cf: cephalothin; Pe: penicillin; Cfx: cefotaxime; Ge: gentamicin; Clm: clindamycin; E: erythromycin; Te: tetracycline; Cpf: ciprofloxacin, and Stx: trimethoprim-sulfamethoxazole. Sensitive (s); resistant (r) to antibiotics and bile salts. Glycocholic acid (GcCA), taurocholic acid (TcCA), glycodeoxycholic acid (GDxCA), taurodeoxycholic acid (TDxCA), oxgall (OXGL). Ferulic acid activity (+) indicates presence of activity or (−) indicates absence of activity.

### 3.4. Antimicrobial Activity

In the case of antimicrobial properties, *L. rhamnosus* LBUX2302 and *L. casei* strains inhibited the growth of pathogenic or non-pathogenic bacteria, either completely or moderately. The *L. rhamnosus* LBUX2302 strain totally inhibited *S. typhi* ATCC14028, a bacterium in which virulent genes such as *fmA* = fimbria production factor, *invA* = invasion factor, *SpvR* and *SpvC* = systemic infection (inhibition activation of macrophage), and *Stn* = enterotoxigenic substances production have been reported [21]. Also, it inhibited the *E. coli* ATCC25922 strain, a non-pathogenic strain commonly used as a control strain for antibiotic susceptibility testing [19]. In the case of *E. coli* 0157:H7, it was partially inhibited; this bacterium has shown pathogenicity that primarily affects children, the elderly, and immunosuppressed individuals, causing mild cases of diarrhea to hemolytic uremic syndrome [20]. *L. rhamnosus* LBUX2302 inhibited the *S. aureus* ATCC6538 strain, which has been used as a control for antibiotic susceptibility testing [17] (Table 1B).

### 3.5. Antibiotic Resistance

Probiotics must be evaluated for their antibiotic resistance and pathogenicity [17]. *L. rhamnosus* LBUX2302 and *L. casei* strains were sensitive to several antibiotics, exhibiting resistance to  $\beta$ -lactam and aminoglycoside antibiotics (Table 1C). *L. rhamnosus* LBUX2302 showed resistance to gentamicin (aminoglycoside), dicloxacillin, and penicillin ( $\beta$ -lactam antibiotics). *L. casei* specifically showed resistance to  $\beta$ -lactam antibiotics, such as vancomycin, dicloxacillin, and penicillin, and to protein synthesis inhibitors such as gentamicin.

### 3.6. Bile Salt Tolerance

Bile salt tolerance is an essential characteristic for evaluating the viability of LAB in the intestine. *L. rhamnosus* LBUX2302 and *L. casei* showed tolerance to primary and secondary bile salts at concentrations of 0.1%, 0.3%, and 0.5% (Table 1D).

### 3.7. Hydrophobicity and Autoaggregation

The cell surface hydrophobicity of *L. rhamnosus* LBUX2302 was 61%. The control strain did not exhibit hydrophobicity. A significant difference was observed between the strains. Autoaggregation in probiotics plays a fundamental role in their adhesion to the intestinal

epithelium. *L. rhamnosus* LBUX2302 exhibited an autoaggregation rate of (53.5%), which was lower than that of the control strain (Table 1E).

### 3.8. Coaggregation

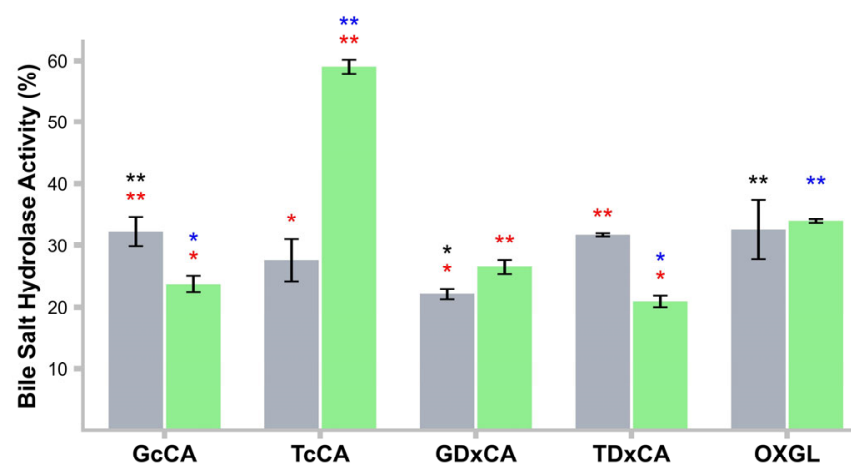
*L. rhamnosus* LBUX2302 showed a high average percentage of 70% of coaggregation with other microorganisms, specifically *E. coli* ATCC25922, *S. aureus* ATCC6538, and *S. typhi* ATCC14028, compared to the coaggregation ability of *L. casei* (Table 1F).

### 3.9. Ferulic Acid Activity

Both *L. rhamnosus* LBUX2302 and the control strain exhibited ferulic acid activity (Table 1G).

### 3.10. BSH Activity in CFs

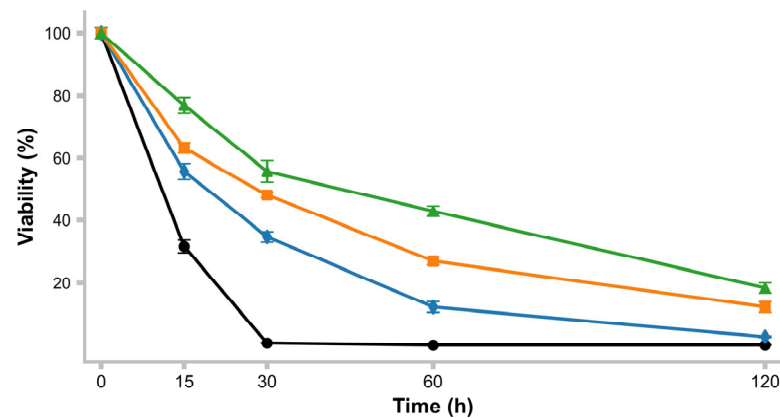
Both *L. rhamnosus* LBUX2302 (32.2%) and *L. casei* (24%) exhibited BSH activity in GcCA, with no significant differences. Regarding TcCA, the control strain (58%) showed greater BSH activity than the *L. rhamnosus* LBUX2302 strain (30%). However, *L. rhamnosus* LBUX2302 (33%) displayed higher BSH activity in the presence of TDxCA versus the control strain (20%) (Figure 3). Significant differences in BSH activity were found in *L. rhamnosus* LBUX2302 between GcCA vs. GDxCA and GDxCA vs. OXGL. For *L. casei*, significant differences were found in GcCA vs. TcCA, TcCA vs. TDxCA, and GcCA and TDxCA with OXGL. When comparing both strains, significant differences were present in all bile salts except OXGL.



**Figure 3.** BSH activity of ■ *L. rhamnosus* LBUX2302 and ■ *L. casei*. \* in black indicates significant differences  $p \leq 0.05$  between bile salts to *L. rhamnosus* LBUX2302, \* in blue to *L. casei* and in red between both strains in the same bile salt. Each bar represents the average of three independent trials. Two bars that share the same number of asterisks of the same color indicate that there is no statistically significant differences between them. In contrast, bars with a different number of asterisks of the same color indicate a statistically significant difference. Error bars indicate the standard deviation.

### 3.11. pH Survival

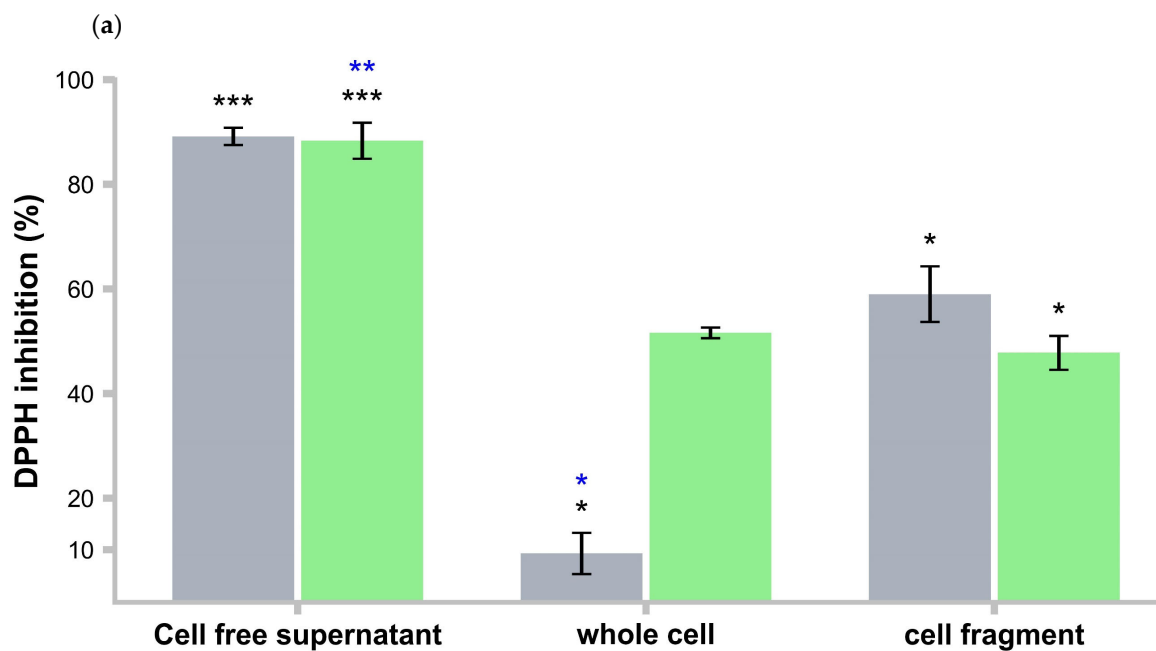
The survival rate at different pH levels is shown in Figure 4. *L. rhamnosus* LBUX2302 was unable to survive at pH 1.5 for 120 min. However, at pH 2, 3, and 5, survival rates were 2.7%, 22%, and 27.5%, respectively, after 120 min, with no significant differences detected.



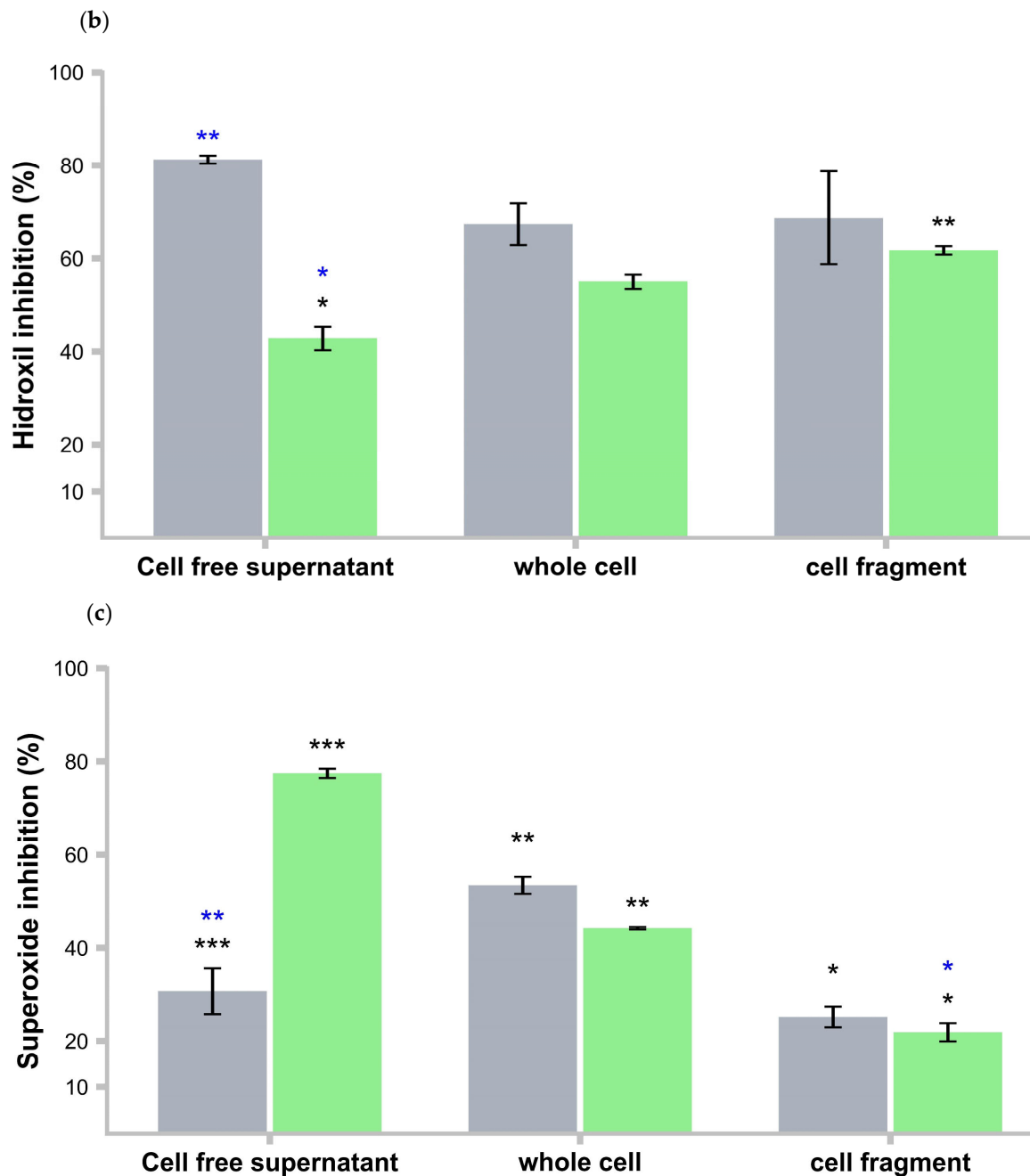
**Figure 4.** Percentage of survival under different pH 1.5 (●), 2 (◆), 3 (■), 5 (▲) of *L. rhamnosus* LBUX2302 and *L. casei*. Markers in each curve of viability curve represents the average of three independent trials. Error bars above the markers indicate the standard deviation.

### 3.12. Antioxidant Activity

The CFs of *L. rhamnosus* LBUX2302 and *L. casei* strains exhibited high antioxidant inhibition of DPPH. In contrast, the WC of *L. casei* demonstrated an antioxidant capacity 15 times greater than that of the *L. rhamnosus* LBUX2302 strain. For *L. rhamnosus* LBUX2302, a significant difference ( $p \leq 0.05$ ) was observed among the three treatments. Regarding *L. casei*, significant differences ( $p \leq 0.05$ ) were found between CFs and Cfg. (Figure 5a). The CFs of *L. rhamnosus* LBUX2302 were able to eliminate 80% of hydroxyl radicals, whereas the CFs of *L. casei* exhibited a 43% inhibition rate ( $p \leq 0.05$ ). No significant differences were observed among the different cell fractions of *L. rhamnosus* LBUX2302. However, for *L. casei*, significant differences ( $p \leq 0.05$ ) were found between CFs and Cfg. Additionally, significant differences ( $p \leq 0.05$ ) were observed between both strains in the CFs fractions (Figure 5b).



**Figure 5.** Cont.

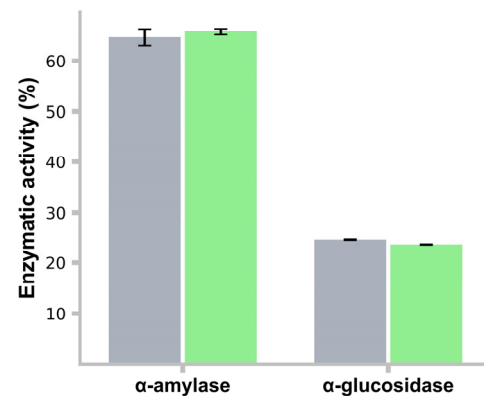


**Figure 5.** Antioxidant activity of ■ *L. rhamnosus* LBUX2302 and ■ *L. casei* (a) % of DPPH inhibition, (b) Hydroxyl anion inhibition, (c) Superoxide anion inhibition. Each bar represents the average of three independent trials. Error bars indicate the standard deviation. \* in black means differences between different evaluations in the same strain. \* in blue means differences among the strains between the treatments ( $p \leq 0.05$ ). Two bars that share the same number of asterisks of the same color indicate that there is no statistically significant differences between them. In contrast, bars with a different number of asterisks of the same color indicate a statistically significant difference.

Regarding superoxide anion radical inhibition, *L. rhamnosus* LBUX2302 and the control strain exhibited significant differences ( $p \leq 0.05$ ) among their respective cell fractions. When comparing superoxide inhibition across the different cell fractions between the two strains, significant differences ( $p \leq 0.05$ ) were observed in both the CFs and the Cfg (Figure 5c).

### 3.13. $\alpha$ -Amylase and $\alpha$ -Glucosidase Activity Inhibition

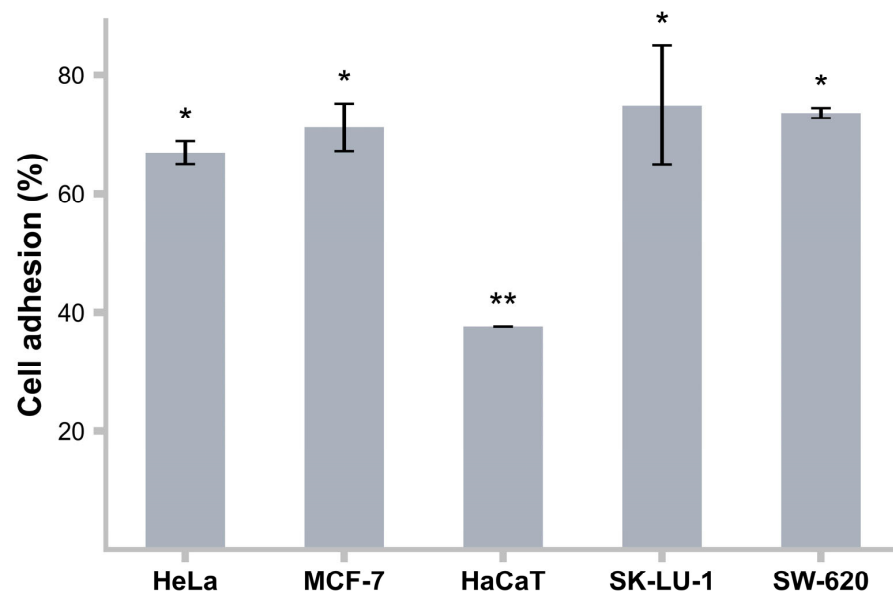
The *L. rhamnosus* LBUX2302 strain exhibited 64.5% inhibition of  $\alpha$ -amylase activity, showing no significant differences compared to the control strain (64%). Regarding  $\alpha$ -glucosidase inhibition, *L. rhamnosus* LBUX2302 (23%) and the control strain (22.5%) demonstrated lower inhibition compared to  $\alpha$ -amylase activity, with no significant differences (Figure 6). Between the two strains for either enzymatic activity, no significant differences were found.



**Figure 6.**  $\alpha$ -amylase and  $\alpha$ -glucosidase activity inhibition of  $\blacksquare$  *L. rhamnosus* LBUX2302 and  $\blacksquare$  *L. casei*. Each bar represents the average of three independent trials. Error bars indicate the standard deviation. No significant differences were found.

### 3.14. Adhesion to Cancer Cell Lines

*L. rhamnosus* LBUX2302 exhibited a higher adhesion capacity to MCF-7, SK-LU-1, and SW-620 cell lines (71%, 75%, and 74%) with respect to HaCaT (38%). These results suggest that adhesion depends on the origin of the cell line and its interaction with the strain species (Figure 7).



**Figure 7.** Cell adhesion ability of *L. rhamnosus* LBUX2302 to different cellular lines. \* Indicates significant differences ( $p \leq 0.05$ ). Each bar represents the average of three independent experiments. Error bars indicate the standard deviation. Two bars that share the same number of asterisks indicate that there is no statistically significant differences between them. In contrast, bars with a different number of asterisks indicate a statistically significant difference.

#### 4. Discussion

The phenotypic characterization of probiotic microorganisms provides valuable information about their potential applications in the food, animal, and human health industries. The isolation of novel microorganisms with hypoglycemic and antioxidant properties requires systematic investigation. T2DM is a chronic disease characterized by hyperglycemia and insulin resistance. Genetic factors, dietary habits, obesity, sedentary lifestyle, and lack of exercise contribute to its development [29,30]. Postprandial glucose is considered the most important parameter in controlling the risk of diabetes. One of the strategies to keep postprandial glucose at adequate levels is the inhibition of the  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes, which catalyze the digestive process of carbohydrates. Conventional treatment involves pharmacological intervention, which may have adverse effects. An alternative approach is the use of probiotic bacteria to manage the disease [27,31,32]. The inhibitory activity shown by *L. rhamnosus* LBUX2302 (60% to 25%) over  $\alpha$ -amylase and  $\alpha$ -glucosidase is consistent with previously reported findings [31,33]. This inhibition may contribute to reducing blood glucose levels and slowing glucose absorption. Our results serve as an indicator for scaling up to in vivo evaluations to confirm the potential of *L. rhamnosus* LBUX2302 as a therapeutic agent for hyperglycemia. *Lactiplantibacillus plantarum* CCFM0236 has demonstrated the ability to inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase, thereby reducing postprandial hyperglycemia. Huligere et al. (2023) [34] have demonstrated that different *Lactocaseibacillus* strains present in the human gut possess  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity, reducing blood glucose responses in vitro.

*L. rhamnosus* LBUX2302 also exhibited strong antioxidant activity, particularly in the CFs, with high DPPH, hydroxyl, and superoxide anion inhibitory capacities. These antioxidative properties of *L. rhamnosus* LBUX2302 can be applied in fermented beverages and animal feed consumption since it is possible to reduce oxidative damage caused by stress and limit the use of synthetic antioxidants. This reduction in oxidative damage is probably due to the action of enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, and vitamins C and E, or to the action of bioactive peptides, which are polysaccharides such as the ones produced by the intestinal microbiota, as suggested by Lepecka et al. (2023) [35]. Our results agree with those reported by Won et al. (2021) [27] and Wanget al. (2022) [36], who described *L. plantarum* and *L. paracasei* strains that showed high hypoglycemic, antioxidant, and probiotic properties. Oxidative stress, which implies a disequilibrium between the production of ROS and the defense system against oxidation, contributes to DM complications, the damage of genetic material, oxidation of proteins, and peroxidation of lipids [37]. Kaprasob et al. (2019) [38] have suggested that bioprocessing of cashews and apple juice through fermentation with *L. plantarum* and *L. casei* was effective for obtaining antioxidant nutraceuticals, which were relevant to DM2. Antioxidant activity has been reported for various LAB, including *L. rhamnosus*, *L. lactis*, and *L. plantarum* [39]. This activity has been associated with probiotic-induced glycemic improvement, suggesting that probiotics may modulate oxidative stress and contribute to glucose regulation [34,40].

Bile salt tolerance is essential for some probiotics to maintain viability in the intestinal environment. *L. rhamnosus* LBUX2302 and *L. casei* exhibited tolerance to various bile salt concentrations, which was mediated by the activation of surface proteins [41]. BSH activity plays a crucial role in the host metabolism, affecting energy regulation, lipid absorption, and cholesterol metabolism [42]. In this study, *L. rhamnosus* LBUX2302 and *L. casei* exhibited BSH activity in primary and secondary bile salts, as well as OXGL, which may contribute to cholesterol and triglycerides reduction [43]. Dong and Lee (2018) [44] have reported that BSH activity in *L. rhamnosus* E9 showed a preference for glycocholic bile salts. This activity could be attributed to the genes encoding this enzyme in *L. rhamnosus* [45].



On the other hand, *L. casei* exhibited a higher affinity for TcCA compared to other bile salts. Notably, in human bile salts, the proportion of glycine to taurine conjugates is 3:1 [46]. Similar BSH activity results have been reported for *L. casei* by various authors. González-Vázquez (2015) [18] identified BSH activity in *L. casei* Shirota using TcCA and TDxCA as substrates, with higher activity observed in TcCA. Lee et al. (2023) [47] have reported that supplementation with *L. reuteri* NCIMB 30242 could alleviate serum cholesterol levels by inhibiting intracellular cholesterol synthesis and promoting intestinal cholesterol excretion.

Autoaggregation, coaggregation, and hydrophobicity are key factors influencing bacterial adhesion [48]. *L. rhamnosus* LBUX2302 exhibited 54% of autoaggregation (Table 1E), indicating possible potential biofilm formation and prolonged colonization in the digestive tract [49,50]. A hydrophobicity assay revealed a 61% affinity to hydrocarbons (Table 1E), aligning with previously reported values for *L. rhamnosus* strains [51].

The autoaggregation of probiotic bacteria is an essential process for adhesion and is considered the first step in bacterial colonization [52]. This process leads to the formation of structured bacterial communities that facilitate cell-to-cell interaction and communication, the exchange of genetic material, adherence, and colonization in different environments [53]. Our results were superior to those reported by Grigoryan et al. (2018) [54] and Zawistowska-Rojek et al. (2022) [55], who have found autoaggregation percentages of 44.3% to *L. rhamnosus* INA-5.1%, 39.4% to *L. rhamnosus* R-2002, 10.1% to *L. rhamnosus* LrA, 14.6% to *L. rhamnosus* LrB, 15% to *L. rhamnosus* LrC, and 12.5% to *L. rhamnosus* LrD. In contrast, Sophatha et al. (2020) [56] have reported high autoaggregation levels in *L. rhamnosus* SD4 and SD11 (55–60%), which are similar to the results obtained in our study. The literature indicates that autoaggregation percentages can vary within the same species, likely due to differences in the probiotic strain's origin and incubation time [57]. *L. rhamnosus* LBUX2302 showed a higher adhesion capacity in several cell lines, such as human colon cancer, cervical cancer, human breast cancer, and lung adenocarcinoma, which indicates that this microorganism could have new functional properties not yet explored. Thananimit, et al. (2022) [58] demonstrated that *L. rhamnosus* SD1, SD4, and SD11 exhibit high adhesion capacity in Caco-2 and HIEC-6 cells, with adhesion percentages ranging from 39% to 80%. However, no previous studies have reported the adhesion of *Lactocaseibacillus* in cell lines such as Hela, MCF-7, HaCaT, SK-LU-1, and SW-260. Evaluating adhesion in these cell lines could reveal new probiotic functions.

The coaggregation assay demonstrated strong adhesion of *L. rhamnosus* LBUX2302 to non-pathogenic and pathogenic bacteria, particularly *E. coli* ATCC25922, *S. aureus* ATCC6538, and *S. typhi* ATCC14028 (Table 1F), suggesting its potential to prevent pathogen colonization [59]. Our results were consistent with those reported by Cozzolino et al. (2020) [51], who found that *L. rhamnosus* GG exhibited high coaggregation with pathogenic bacteria, including *P. mirabilis* and *E. coli*. In contrast, Zawistowska-Rojek et al. (2022) [55] have reported lower coaggregation values for *L. rhamnosus* GG with *E. coli*, *S. typhimurium*, and *E. faecalis*. High coaggregation values indicate a strong capacity for barrier formation, which can prevent pathogenic colonization [59].

*L. rhamnosus* LBUX2302 showed weak antimicrobial inhibition against *E. coli* O157:H and *S. aureus* ATCC6538. Inturri et al. (2019) [60] have demonstrated that *L. rhamnosus* HN001 inhibited *E. coli* ATCC25922, *E. coli* ATCC35218, and *S. typhi* STN12. Davoodabadi et al. (2015) [61] have found that *L. rhamnosus* S19 inhibited various *E. coli* strains, including those with enteroaggregative, antihemorrhagic, antiinvasive, enteropathogenic, and enterotoxigenic activities. Furthermore, Johnson-Henry et al. (2008) [62] have reported that *L. rhamnosus* GG protects epithelial monolayers against the influence of *E. coli* O157:H7 on tight junctions claudin-1 and ZO-1.

The potential mechanism by which *L. rhamnosus* SCB0119 induces *S. aureus* cell death may involve genomic instability due to interference with DNA repair pathways through the expression of the genes that encode ATP synthase, responsible for ATP hydrolysis [63]. Finally, probiotic antimicrobial activity can be mediated through multiple mechanisms, including organic acid production leading to acidification, bacteriocin production, competition for nutrients and adhesion sites in the host mucosa, and the induction of proinflammatory responses that facilitate pathogen elimination, both in vitro and in vivo [64–66]. Nonetheless, additional studies are required to elucidate the specific mechanism underlying the antimicrobial activity of *L. rhamnosus* LBUX2302.

The isolation of microorganisms with probiotic characteristics must include an evaluation of their abilities to tolerate relevant conditions, such as acidity and bile acids. The acidity that probiotics face in the gastrointestinal tract is a factor that influences their viability [67]. In this study, *L. rhamnosus* LBUX2302 exhibited a survival range between 22 and 27.5% at pH 2 and 3 over 120 min. Probiotics are intrinsically acid-resistant; they must withstand the harsh conditions of the stomach to reach the intestine alive, where they can colonize and exert beneficial effects [68]. The resistance of microorganisms to acidic pH may be attributed to a univariant gradient between cytoplasmic and extracellular pH, as well as proton extrusion via the F<sub>0</sub>F<sub>1</sub>-ATPase mechanism, which facilitates the survival in the gastrointestinal tract [69].

Safety is a crucial criterion for bacterial strains used for the food industry [70]. According to the European Food Safety Authority (EFSA), bacterial identification and hemolytic activity are among the primary criteria for assessing probiotic safety. In this study, we found no hemolytic activity in *L. rhamnosus* LBUX2302 or the control strain (Table 1A). Wang et al. (2021) [71] similarly reported an absence of hemolytic activity in LAB isolated from infant feces. Other LAB, such as *L. rhamnosus* CA15, *L. rhamnosus* CWKu-12, and *L. rhamnosus* SS73, have also been found to lack hemolytic activity [72,73] to produce hemolysin proteins, unlike the *S. aureus* species.

Antibiotics are one of the alternatives for treating infectious diseases caused by Gram-positive and Gram-negative bacteria in humans and animals. Their overuse has led to the emergence of antibiotic-resistant bacteria, which has caused an international public health problem [74]. Additionally, we assessed the antibiotic profile of *L. rhamnosus* LBUX2302, which exhibited resistance to gentamicin, dicloxacillin, and penicillin. LAB have been previously reported to exhibit intrinsic resistance to aminoglycosides (gentamicin) [75], which indicates that there are no genes that encode transferable resistance determinants and that it may be due to chromosomal zones of the bacteria [10]. Therefore, according to the qualified presumption of safety criteria, they should be considered safe. Furthermore, another author has suggested that the antibiotic resistance detected in *L. rhamnosus* GG is natural [76]. It is important to note that antibiotic-resistant LAB do not necessarily represent a health risk; nevertheless, additional research is required to confirm whether antibiotic-resistant genes in LAB are capable of being transferred [36]. In 2023, Shahali et al. [77] reported a high percentage of resistance to gentamicin, streptomycin, and ciprofloxacin in many LAB strains. The potential mechanism of gentamicin resistance in LAB strains is the absence of an antibiotic transporter, as gentamicin susceptibility is associated with the ability of the antibiotic to cross the bacterial membrane [78]. Previous studies have suggested that *Lactobacillus* spp. is susceptible to penicillin and  $\beta$ -lactams. Our findings indicate that *L. rhamnosus* LBUX2302 exhibited resistance to dicloxacillin and penicillin. These results are consistent with those reported by Hasan et al. (2020) [79], who found that *L. rhamnosus* MT539286 was resistant to the  $\beta$ -lactam antibiotics amoxicillin and oxacillin. Although *L. rhamnosus* is recognized as safe (GRAS) and has been granted a Qualified Presumption of Safety (QPS) status [80], it can still present antibiotic resistance and potentially transfer

resistance genes to other bacteria via mobile genetic elements such as plasmids. Due to the need to perform a genomic characterization of *L. rhamnosus* LBUX2302, we are currently analyzing its genome to determine whether resistance to  $\beta$ -lactams and aminoglycosides is transferable or encoded at the chromosomal level. Specifically, we aim to identify intrinsic resistance genes related to the antibiotics gentamicin, penicillin, and dicloxacillin.

The *Lactobacillus* genus can efficiently grow on mono-, di-, tri-, and oligosaccharides [81]. Carbohydrates serve as an essential energy source for both the host and gut microbiota. In general, *Lactobacillus* species can metabolize these carbohydrates, producing compounds of interest for health and food industries [82,83]. In this study, we observed that *L. rhamnosus* LBUX2302 was able to metabolize monosaccharides and oligosaccharides (Figure 1), likely due to the expression of genes encoding glycosyl hydrolase enzymes. Our results are consistent with those reported by Ceapa et al. (2015) [84], who found that *L. rhamnosus* GG could utilize a wide range of mono-, di-, and polysaccharides, and polyols. In addition, Li et al. (2024) [85] have identified several genes encoding enzymes associated with carbohydrate metabolism in *L. rhamnosus* LR-ZB1107-01, including glycoside hydrolases, glycosyltransferases, esterases, and carbohydrate-binding modules. Furthermore, *L. rhamnosus* LR-ZB1107-01 possesses a complex phosphoenolpyruvate (PEP)–phosphotransferase system (PTS), which is responsible for the phosphorylation and transportation of sugars such as saccharose, lactose, maltose/glucose, mannitol, cellobiose, mannose, and fructose into the cell [85,86]. These sugar metabolism and transport systems are likely involved in the environmental adaptation capacity of *L. rhamnosus*. Moreover, we found that *L. rhamnosus* LBUX2302 can utilize FOS for growth, a result similar to the one reported by Kaewarsar et al. (2023) [87]. Additionally, Niu et al. (2023) [88] suggested that *L. rhamnosus* AS1.2466T can grow in the presence of FOS and, when administered simultaneously in mice, extends its colonization time in the intestine and ileum. The proposed mechanism for FOS degradation involves the fructanhydrolase enzyme, which has been identified in *L. paracasei* 1195 and functions in conjunction with the mannose PTS transporter complex [89]. However, this mechanism has not yet been identified in *L. rhamnosus* species. Therefore, a key perspective for future research that we suggest is to sequence the complete genome of *L. rhamnosus* LBUX2302 and annotate the genes involved in all the activities determined in this study, and to test the effects in vivo models to demonstrate safety and corroborate the activities found.

## 5. Conclusions

This study indicates that *L. rhamnosus* LBUX2302 is a safe strain with promising probiotic properties. It demonstrates tolerance to bile salts, high hydrophobicity, and the ability to autoaggregate and congregate, along with strong adhesion capacity to MCF-7, SK-LU-1, and SW-620 cell lines. Furthermore, it exhibits significant hypoglycemic and antioxidant potential. However, further genomic characterization is necessary to confirm its safety and functionality at the genetic level. Once this characterization is completed, *L. rhamnosus* LBUX2302 could be considered for in vivo studies and potential applications in the pharmaceutical and food industries.

**Author Contributions:** Conceptualization, L.M.-R., R.G.-V. (Raquel González-Vázquez), M.A.G.-N., E.T.-M.; Methodology, P.A.R.-C., E.Z.-L., R.G.-V. (Raquel González-Vázquez), A.L.E.-C.; Investigación, L.M.-R., R.G.-V. (Raquel González-Vázquez), M.A.G.-N., E.T.-M., P.A.R.-C., E.Z.-L., F.M.-P., R.G.-V. (Rosa González-Vázquez), M.G.C.-E.; Resources, L.M.-R., R.G.-V. (Raquel González-Vázquez), M.A.G.-N.; Data curation, P.A.R.-C., E.Z.-L., R.G.-V. (Raquel González-Vázquez), L.M.-R.; Writing—original draft, review and editing, L.M.-R., R.G.-V. (Raquel González-Vázquez), M.A.G.-N., E.T.-M., P.A.R.-C., E.Z.-L., A.L.E.-C., F.M.-P., R.G.-V. (Rosa González-Vázquez), M.G.C.-E.; Supervision, L.M.-

R., R.G.-V. (Raquel González-Vázquez), M.A.G.-N., E.T.-M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by (i) General Rectory of Universidad Autonoma Metropolitana, through 2024 call for research project proposals addressing current challenges; and (ii) Rectory of the Universidad Autonoma Metropolitana, campus Xochimilco, through the support program for final projects 2024.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Instituto Mexicano del Seguro Social (protocol code 36068 accepted on 23 August 2023). Nevertheless, we did not handle the stools samples directly as we received the purified strains.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The genomic sequencing data of *L. rhamnosus* LBUX2302 have been registered in Gen Bank under the accession code PQ724459.1.

**Acknowledgments:** Pedro Reyes-Castillo acknowledges Secihti for the scholarship number 2020-000026-02NACF-13604.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## Abbreviations

Am: ampicillin; BS: bile salt; BSH: Bile salt hydrolase; Cf: cephalothin; Cfg: cell fragments; CFs: cell-free supernatant; CFU: colony forming unit; Cfx: cefotaxime; Clm: clindamycin; Cpf: ciprofloxacin; Dc: dicloxacillin; DM2: type 2 diabetes; DNS: 3,5-Dinitrosalicylic acid; DM: Diabetes mellitus; DPPH: 2,2-diphenyl-1-picrylhydrazyl; E: erythromycin; EFA: ferulic acid activity; EFSA: European Food Safety Authority; FAO/WHO: Food and Agriculture Organization/codex alimentarius; FOS: fructooligosaccharides; GcCA: glycocholic acid; GDxCA: glycodeoxycholic acid; Ge: gentamicin; GRAS: generally recognized as safe; HaCat: spontaneously immortalized human keratinocyte cell line; HeLa: Henrietta Lacks cell line; H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide; LAB: Lactic acid bacteria; NCBI: National Center of Biotechnology Information; MCF-7: breast cancer cell line; MRS: de Man Rogosa medium; OD: optical density; OH<sup>-</sup>: hydroxyl radicals; OXGL: oxgall; O<sup>-2</sup>: superoxide anion; PBS: Phosphate buffer solution; Pe: penicillin; PEP: phosphoenolpyruvate; PNPG: *p*-nitrophenyl glucopyranoside; QPS: Qualified Presumption of Safety; ROS: reactive oxygen species; SK-LU-1: human lung adenocarcinoma cell line; Stx: trimethoprim-sulfamethoxazole; SW620: adherent cell line isolated from the large intestine of a patient with Dukes-C colorectal cancer; TcCA: taurocholic acid; TDxCA: taurodeoxycholic acid; Te: tetracycline; Va: vancomycin; WC: whole cells.

## References

1. Zheng, J.; Wittouck, S.; Salvetti, E.; Franz, C.; Harris, H.M.B.; Mattarelli, P.; O'Toole, P.W.; Pot, B.; Vandamme, P.; Walter, J.; et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 2782–2858. [[CrossRef](#)] [[PubMed](#)]
2. Mora-Villalobos, J.A.; Montero-Zamora, J.; Barboza, N.; Rojas-Garbanzo, C.; Usaga, J.; Redondo-Solano, M.; Schroedter, L.; Olszewska-Widdrat, A.; López-Gómez, J.P. Multi-product lactic acid bacteria fermentations: A review. *Fermentation* **2020**, *6*, 23. [[CrossRef](#)]
3. Rossi, F. Special Issue “Functional Characterization of Lactic Acid Bacteria”: Editorial. *Microorganisms* **2023**, *11*, 1190. [[CrossRef](#)]
4. Hotel, A.C.P.; Cordoba, A. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Prevention* **2001**, *5*, 1–10.
5. Latif, A.; Shehzad, A.; Niazi, S.; Zahid, A.; Ashraf, W.; Iqbal, M.W.; Rehman, A.; Riaz, T.; Aadil, R.M.; Khan, I.M.; et al. Probiotics: Mechanism of action, health benefits and their application in food industries. *Front. Microbiol.* **2023**, *14*, 1216674. [[CrossRef](#)]



6. Saarela, M.; Mogensen, G.; Fonden, R.; Matto, J.; Mattila-Sandholm, T. Probiotic bacteria: Safety, functional and technological properties. *J. Biotechnol.* **2000**, *84*, 197–215. [\[CrossRef\]](#)
7. Poimenidou, S.V.; Skarveli, A.; Saxami, G.; Mitsou, E.K.; Kotsou, M.; Kyriacou, A. Inhibition of *Listeria monocytogenes* Growth, Adherence and Invasion in Caco-2 Cells by Potential Probiotic Lactic Acid Bacteria Isolated from Fecal Samples of Healthy Neonates. *Microorganisms* **2023**, *11*, 363. [\[CrossRef\]](#)
8. de Vos, W.M.; Tilg, H.; Van Hul, M.; Cani, P.D. Gut microbiome and health: Mechanistic insights. *Gut* **2022**, *71*, 1020–1032. [\[CrossRef\]](#)
9. Gao, J.; Li, X.; Zhang, G.; Sadiq, F.A.; Simal-Gandara, J.; Xiao, J.; Sang, Y. Probiotics in the dairy industry—Advances and opportunities. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 3937–3982. [\[CrossRef\]](#)
10. Reyes-Castillo, P.A.; González-Vázquez, R.; Torres-Maravilla, E.; Bautista-Hernández, J.I.; Zúñiga-León, E.; Leyte-Lugo, M.; Mateos-Sánchez, L.; Mendoza-Pérez, F.; Gutiérrez-Nava, M.A.; Reyes-Pavón, D.; et al. Bifidobacterium longum LBUX23 Isolated from Feces of a Newborn; Potential Probiotic Properties and Genomic Characterization. *Microorganisms* **2023**, *11*, 1648. [\[CrossRef\]](#)
11. Wang, H.; Li, L. Comprehensive Evaluation of Probiotic Property, Hypoglycemic Ability and Antioxidant Activity of Lactic Acid Bacteria. *Foods* **2022**, *11*, 1363. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Afshari, A.; Hashemi, M.; Tavassoli, M.; Eraghi, V.; Noori, S.M.A. Probiotic bacteria from 10 different traditional Iranian cheeses: Isolation, characterization, and investigation of probiotic potential. *Food Sci. Nutr.* **2022**, *10*, 2009–2020. [\[CrossRef\]](#) [\[PubMed\]](#)
13. He, Y.; Na, R.; Niu, X.; Xiao, B.; Yang, H. *Lactobacillus rhamnosus* and *Lactobacillus casei* affect various stages of Gardnerella species biofilm formation. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 568178. [\[CrossRef\]](#)
14. Galkiewicz, J.P.; Kellogg, C.A. Cross-kingdom amplification using bacteria-specific primers: Complications for studies of coral microbial ecology. *Appl. Environ. Microbiol.* **2008**, *74*, 7828–7831. [\[CrossRef\]](#)
15. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [\[CrossRef\]](#)
16. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [\[CrossRef\]](#)
17. Makarova, O.; Johnston, P.; Walther, B.; Rolff, J.; Roesler, U. Complete Genome Sequence of the Disinfectant Susceptibility Testing Reference Strain *Staphylococcus aureus* subsp. *aureus* ATCC 6538. *Genome Announc.* **2017**, *5*, e00293-17. [\[CrossRef\]](#)
18. Gonzalez-Vazquez, R.; Azaola-Espinosa, A.; Mayorga-Reyes, L.; Reyes-Nava, L.A.; Shah, N.P.; Rivera-Espinoza, Y. Isolation, Identification and Partial Characterization of a *Lactobacillus casei* Strain with Bile Salt Hydrolase Activity from Pulque. *Probiotics Antimicrob. Proteins* **2015**, *7*, 242–248. [\[CrossRef\]](#)
19. Minogue, T.D.; Daligault, H.A.; Davenport, K.W.; Bishop-Lilly, K.A.; Broomall, S.M.; Bruce, D.C.; Chain, P.S.; Chertkov, O.; Coyne, S.R.; Freitas, T. Complete genome assembly of *Escherichia coli* ATCC 25922, a serotype O6 reference strain. *Genome Announc.* **2014**, *2*, 10-1128. [\[CrossRef\]](#)
20. Hilborn, E.D.; Mshar, P.A.; Fiorentino, T.R.; Dembek, Z.F.; Barrett, T.J.; Howard, R.T.; Cartter, M.L. An outbreak of *Escherichia coli* O157 [ratio] H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. *Epidemiol. Infect.* **2000**, *124*, 31–36. [\[CrossRef\]](#)
21. Agbankpe, A.J.; Dougnon, T.V.; Balarabe, R.; Deguenon, E.; Baba-Moussa, L. In vitro assessment of antibacterial activity from *Lactobacillus* spp. strains against virulent *Salmonella* species isolated from slaughter animals in Benin. *Vet. World* **2019**, *12*, 1951. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Su, J.; Wang, T.; Li, Y.-Y.; Li, J.; Zhang, Y.; Wang, Y.; Wang, H.; Li, H. Antioxidant properties of wine lactic acid bacteria: *Oenococcus oeni*. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 5189–5202. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Vinderola, C.G.; Reinheimer, J.A. Lactic acid starter and probiotic bacteria: A comparative “in vitro” study of probiotic characteristics and biological barrier resistance. *Food Res. Int.* **2003**, *36*, 895–904. [\[CrossRef\]](#)
24. Zuo, F.; Yu, R.; Feng, X.; Chen, L.; Zeng, Z.; Khaskheli, G.B.; Ma, H.; Chen, S. Characterization and in vitro properties of potential probiotic *Bifidobacterium* strains isolated from breast-fed infant feces. *Ann. Microbiol.* **2016**, *66*, 1027–1037. [\[CrossRef\]](#)
25. Yan, F.; Li, N.; Yue, Y.; Wang, C.; Zhao, L.; Evvie, S.E.; Li, B.; Huo, G. Screening for Potential Novel Probiotics With Dipeptidyl Peptidase IV-Inhibiting Activity for Type 2 Diabetes Attenuation in vitro and in vivo. *Front. Microbiol.* **2020**, *10*, 2855. [\[CrossRef\]](#)
26. Tomaro-Duchesneau, C.; Saha, S.; Malhotra, M.; Coussa-Charley, M.; Al-Salami, H.; Jones, M.; Labbé, A.; Prakash, S. *Lactobacillus fermentum* NCIMB 5221 has a greater ferulic acid production compared to other ferulic acid esterase producing *Lactobacilli*. *Int. J. Probiotics Prebiotics* **2012**, *7*, 23–32.
27. Won, G.; Choi, S.I.; Park, N.; Kim, J.E.; Kang, C.H.; Kim, G.H. In Vitro Antidiabetic, Antioxidant Activity, and Probiotic Activities of *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* Strains. *Curr. Microbiol.* **2021**, *78*, 3181–3191. [\[CrossRef\]](#)
28. González-Vázquez, R.; Zúñiga-León, E.; Torres-Maravilla, E.; Leyte-Lugo, M.; Mendoza-Pérez, F.; Hernández-Delgado, N.C.; Pérez-Pastén-Borja, R.; Azaola-Espinosa, A.; Mayorga-Reyes, L. Genomic and biochemical characterization of *bifidobacterium pseudocatenulatum* JCLA3 isolated from human intestine. *Microorganisms* **2022**, *10*, 2100. [\[CrossRef\]](#)

29. Punthakee, Z.; Goldenberg, R.; Katz, P. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Can. J. Diabetes* **2018**, *42* (Suppl. S1), S10–S15. [\[CrossRef\]](#)
30. Salis, S.; Virmani, A.; Priyambada, L.; Mohan, M.; Hansda, K.; Beaufort, C. ‘Old Is Gold’: How Traditional Indian Dietary Practices Can Support Pediatric Diabetes Management. *Nutrients* **2021**, *13*, 4427. [\[CrossRef\]](#)
31. Kumari, V.B.C.; Huligere, S.S.; Alotaibi, G.; Al Mouslem, A.K.; Bahaiddin, A.A.; Shivanandappa, T.B.; Ramu, R. Antidiabetic Activity of Potential Probiotics *Limosilactobacillus* spp., *Levilactobacillus* spp., and *Lacticaseibacillus* spp. Isolated from Fermented Sugarcane Juice: A Comprehensive In Vitro and In Silico Study. *Nutrients* **2023**, *15*, 1882. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Li, X.; Wang, N.; Yin, B.; Fang, D.; Jiang, T.; Fang, S.; Zhao, J.; Zhang, H.; Wang, G.; Chen, W. Effects of *Lactobacillus plantarum* CCFM0236 on hyperglycaemia and insulin resistance in high-fat and streptozotocin-induced type 2 diabetic mice. *J. Appl. Microbiol.* **2016**, *121*, 1727–1736. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Muganga, L.; Liu, X.; Tian, F.; Zhao, J.; Zhang, H.; Chen, W. Screening for lactic acid bacteria based on antihyperglycaemic and probiotic potential and application in synbiotic set yoghurt. *J. Funct. Foods* **2015**, *16*, 125–136. [\[CrossRef\]](#)
34. Huligere, S.S.; Chandana Kumari, V.B.; Alqadi, T.; Kumar, S.; Cull, C.A.; Amachawadi, R.G.; Ramu, R. Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitions of fermented batters. *Front. Microbiol.* **2023**, *13*, 1042263. [\[CrossRef\]](#)
35. Łepecka, A.; Szymański, P.; Okoń, A.; Zielińska, D. Antioxidant activity of environmental lactic acid bacteria strains isolated from organic raw fermented meat products. *Lwt* **2023**, *174*, 114440. [\[CrossRef\]](#)
36. Wang, G.; Chen, Y.; Xia, Y.; Song, X.; Ai, L. Characteristics of probiotic preparations and their applications. *Foods* **2022**, *11*, 2472. [\[CrossRef\]](#)
37. Ardeshtarijani, E.; Tabatabaei-Malazy, O.; Mohseni, S.; Qorbani, M.; Larijani, B.; Baradar Jalili, R. Effect of probiotics supplementation on glucose and oxidative stress in type 2 diabetes mellitus: A meta-analysis of randomized trials. *DARU J. Pharm. Sci.* **2019**, *27*, 827–837. [\[CrossRef\]](#)
38. Kaprasob, R.; Sarkar, D.; Kerdchoechuen, O.; Laohakunjit, N.; Khanongnuch, C.; Shetty, K. Beneficial lactic acid bacteria based bioprocessing of cashew apple juice for targeting antioxidant nutraceutical inhibitors as relevant antidotes to type 2 diabetes. *Process Biochem.* **2019**, *82*, 40–50. [\[CrossRef\]](#)
39. Kim, S.; Lee, J.Y.; Jeong, Y.; Kang, C.-H. Antioxidant Activity and Probiotic Properties of Lactic Acid Bacteria. *Fermentation* **2022**, *8*, 29. [\[CrossRef\]](#)
40. Sathiyaseelan, A.; Saravanakumar, K.; Han, K.; Naveen, K.V.; Wang, M.-H. Antioxidant and Antibacterial Effects of Potential Probiotics Isolated from Korean Fermented Foods. *Int. J. Mol. Sci.* **2022**, *23*, 10062. [\[CrossRef\]](#)
41. Kebouchi, M.; Galia, W.; Genay, M.; Soligot, C.; Lecomte, X.; Awussi, A.A.; Perrin, C.; Roux, E.; Dary-Mourot, A.; Le Roux, Y. Implication of sortase-dependent proteins of *Streptococcus thermophilus* in adhesion to human intestinal epithelial cell lines and bile salt tolerance. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 3667–3679. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Bustos, A.Y.; Font de Valdez, G.; Fadda, S.; Taranto, M.P. New insights into bacterial bile resistance mechanisms: The role of bile salt hydrolase and its impact on human health. *Food Res. Int.* **2018**, *112*, 250–262. [\[CrossRef\]](#)
43. Hernández-Gómez, J.G.; López-Bonilla, A.; Trejo-Tapia, G.; Ávila-Reyes, S.V.; Jiménez-Aparicio, A.R.; Hernández-Sánchez, H. In vitro bile salt hydrolase (BSH) activity screening of different probiotic microorganisms. *Foods* **2021**, *10*, 674. [\[CrossRef\]](#)
44. Dong, Z.; Lee, B.H. Bile salt hydrolases: Structure and function, substrate preference, and inhibitor development. *Protein Sci.* **2018**, *27*, 1742–1754. [\[CrossRef\]](#)
45. Kaya, Y.; Kök, M.Ş.; Öztürk, M. Molecular cloning, expression and characterization of bile salt hydrolase from *Lactobacillus rhamnosus* E9 strain. *Food Biotechnol.* **2017**, *31*, 128–140. [\[CrossRef\]](#)
46. Miyazaki, T.; Ueda, H.; Ikegami, T.; Honda, A. Upregulation of Taurine Biosynthesis and Bile Acid Conjugation with Taurine through FXR in a Mouse Model with Human-like Bile Acid Composition. *Metabolites* **2023**, *13*, 824. [\[CrossRef\]](#)
47. Lee, M.; Park, J.; Kim, O.K.; Kim, D.; Han, M.J.; Kim, S.H.; Kim, T.H.; Lee, J. *Lactobacillus reuteri* NCIMB 30242 (LRC) Inhibits Cholesterol Synthesis and Stimulates Cholesterol Excretion in Animal and Cell Models. *J. Med. Food* **2023**, *26*, 529–539. [\[CrossRef\]](#)
48. Guan, C.; Chen, X.; Jiang, X.; Zhao, R.; Yuan, Y.; Chen, D.; Zhang, C.; Lu, M.; Lu, Z.; Gu, R. In vitro studies of adhesion properties of six lactic acid bacteria isolated from the longevous population of China. *RSC Adv.* **2020**, *10*, 24234–24240. [\[CrossRef\]](#)
49. Rajab, S.; Tabandeh, F.; Shahraky, M.K.; Alahyaribeik, S. The effect of *Lactobacillus* cell size on its probiotic characteristics. *Anaerobe* **2020**, *62*, 102103. [\[CrossRef\]](#)
50. Wang, S.; Li, L.; Yu, L.; Tian, F.; Zhao, J.; Zhai, Q.; Chen, W. Natural aggregation of *Lactobacillus*: Mechanisms and influencing factors. *Food Biosci.* **2024**, *62*, 105007. [\[CrossRef\]](#)
51. Cozzolino, A.; Vergalito, F.; Tremonte, P.; Iorizzo, M.; Lombardi, S.J.; Sorrentino, E.; Luongo, D.; Coppola, R.; Di Marco, R.; Succi, M. Preliminary Evaluation of the Safety and Probiotic Potential of *Akkermansia muciniphila* DSM 22959 in Comparison with *Lactobacillus rhamnosus* GG. *Microorganisms* **2020**, *8*, 189. [\[CrossRef\]](#) [\[PubMed\]](#)



52. Monteagudo-Mera, A.; Rastall, R.A.; Gibson, G.R.; Charalampopoulos, D.; Chatzifragkou, A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 6463–6472. [[CrossRef](#)] [[PubMed](#)]
53. Isenring, J.; Geirnaert, A.; Lacroix, C.; Stevens, M.J.A. Bistable auto-aggregation phenotype in *Lactiplantibacillus plantarum* emerges after cultivation in in vitro colonic microbiota. *BMC Microbiol.* **2021**, *21*, 268. [[CrossRef](#)]
54. Grigoryan, S.; Bazukyan, I.; Trchounian, A. Aggregation and Adhesion Activity of Lactobacilli Isolated from Fermented Products In Vitro and In Vivo: A Potential Probiotic Strain. *Probiotics Antimicrob. Proteins* **2018**, *10*, 269–276. [[CrossRef](#)]
55. Zawistowska-Rojek, A.; Kośmider, A.; Stepień, K.; Tyski, S. Adhesion and aggregation properties of Lactobacillaceae strains as protection ways against enteropathogenic bacteria. *Arch. Microbiol.* **2022**, *204*, 285. [[CrossRef](#)]
56. Sophatha, B.; Piwat, S.; Teanpaisan, R. Adhesion, anti-adhesion and aggregation properties relating to surface charges of selected Lactobacillus strains: Study in Caco-2 and H357 cells. *Arch. Microbiol.* **2020**, *202*, 1349–1357. [[CrossRef](#)]
57. Krausova, G.; Hyrslova, I.; Hynstova, I. In Vitro Evaluation of Adhesion Capacity, Hydrophobicity, and Auto-Aggregation of Newly Isolated Potential Probiotic Strains. *Fermentation* **2019**, *5*, 100. [[CrossRef](#)]
58. Thananimit, S.; Pahumunto, N.; Teanpaisan, R. Characterization of short chain fatty acids produced by selected potential probiotic lactobacillus strains. *Biomolecules* **2022**, *12*, 1829. [[CrossRef](#)]
59. Tuo, Y.; Yu, H.; Ai, L.; Wu, Z.; Guo, B.; Chen, W. Aggregation and adhesion properties of 22 Lactobacillus strains. *J. Dairy. Sci.* **2013**, *96*, 4252–4257. [[CrossRef](#)]
60. Inturri, R.; Trovato, L.; Volti, G.L.; Oliveri, S.; Blandino, G. In vitro inhibitory activity of Bifidobacterium longum BB536 and Lactobacillus rhamnosus HN001 alone or in combination against bacterial and Candida reference strains and clinical isolates. *Heliyon* **2019**, *5*, e02891. [[CrossRef](#)]
61. Davoodabadi, A.; Soltan Dallal, M.M.; Lashani, E.; Tajabadi Ebrahimi, M. Antimicrobial Activity of Lactobacillus spp. Isolated From Fecal Flora of Healthy Breast-Fed Infants Against Diarrheagenic Escherichia coli. *Jundishapur J. Microbiol.* **2015**, *8*, e27852. [[CrossRef](#)] [[PubMed](#)]
62. Johnson-Henry, K.C.; Donato, K.A.; Shen-Tu, G.; Gordanpour, M.; Sherman, P.M. Lactobacillus rhamnosus strain GG prevents enterohemorrhagic Escherichia coli O157:H7-induced changes in epithelial barrier function. *Infect. Immun.* **2008**, *76*, 1340–1348. [[CrossRef](#)]
63. Peng, H.; Zhou, G.; Yang, X.M.; Chen, G.J.; Chen, H.B.; Liao, Z.L.; Zhong, Q.P.; Wang, L.; Fang, X.; Wang, J. Transcriptomic Analysis Revealed Antimicrobial Mechanisms of Lactobacillus rhamnosus SCB0119 against Escherichia coli and Staphylococcus aureus. *Int. J. Mol. Sci.* **2022**, *23*, 15159. [[CrossRef](#)]
64. Yasmin, I.; Saeed, M.; Khan, W.A.; Khaliq, A.; Chughtai, M.F.J.; Iqbal, R.; Tehseen, S.; Naz, S.; Liaqat, A.; Mehmood, T.; et al. In vitro Probiotic Potential and Safety Evaluation (Hemolytic, Cytotoxic Activity) of Bifidobacterium Strains Isolated from Raw Camel Milk. *Microorganisms* **2020**, *8*, 354. [[CrossRef](#)]
65. Kim, M.J.; Ku, S.; Kim, S.Y.; Lee, H.H.; Jin, H.; Kang, S.; Li, R.; Johnston, T.V.; Park, M.S.; Ji, G.E. Safety Evaluations of Bifidobacterium bifidum BGN4 and Bifidobacterium longum BORI. *Int. J. Mol. Sci.* **2018**, *19*, 1422. [[CrossRef](#)]
66. Žuntar, I.; Petric, Z.; Bursać Kovačević, D.; Putnik, P. Safety of Probiotics: Functional Fruit Beverages and Nutraceuticals. *Foods* **2020**, *9*, 947. [[CrossRef](#)]
67. Gunzburg, W.H.; Aung, M.M.; Toa, P.; Ng, S.; Read, E.; Tan, W.J.; Brandtner, E.M.; Dangerfield, J.; Salmons, B. Efficient protection of microorganisms for delivery to the intestinal tract by cellulose sulphate encapsulation. *Microb. Cell Fact.* **2020**, *19*, 216. [[CrossRef](#)]
68. Ayyash, M.M.; Abdalla, A.K.; AlKalbani, N.S.; Baig, M.A.; Turner, M.S.; Liu, S.-Q.; Shah, N.P. Invited review: Characterization of new probiotics from dairy and nondairy products—Insights into acid tolerance, bile metabolism and tolerance, and adhesion capability. *J. Dairy. Sci.* **2021**, *104*, 8363–8379. [[CrossRef](#)]
69. Lund, P.A.; De Biase, D.; Liran, O.; Scheler, O.; Mira, N.P.; Cetecioglu, Z.; Fernández, E.N.; Bover-Cid, S.; Hall, R.; Sauer, M.; et al. Understanding How Microorganisms Respond to Acid pH Is Central to Their Control and Successful Exploitation. *Front. Microbiol.* **2020**, *11*, 556140. [[CrossRef](#)]
70. Laulund, S.; Wind, A.; Derkx, P.M.F.; Zuliani, V. Regulatory and Safety Requirements for Food Cultures. *Microorganisms* **2017**, *5*, 28. [[CrossRef](#)]
71. Wang, X.; Wang, W.; Lv, H.; Zhang, H.; Liu, Y.; Zhang, M.; Wang, Y.; Tan, Z. Probiotic Potential and Wide-spectrum Antimicrobial Activity of Lactic Acid Bacteria Isolated from Infant Feces. *Probiotics Antimicrob. Proteins* **2021**, *13*, 90–101. [[CrossRef](#)] [[PubMed](#)]
72. Pino, A.; Vaccalluzzo, A.; Caggia, C.; Balzaretto, S.; Vanella, L.; Sorrenti, V.; Ronkainen, A.; Satokari, R.; Randazzo, C.L. Lactocaseibacillus rhamnosus CA15 (DSM 33960) as a Candidate Probiotic Strain for Human Health. *Nutrients* **2022**, *14*, 4902. [[CrossRef](#)] [[PubMed](#)]
73. Rodríguez Díaz, J.A.; Hernández García, J.E.; Sebastián Frizzo, L.; Fernández León, K.J.; Sánchez, L.; Solenzal Valdivia, Y. Caracterización in vitro de propiedades probióticas de Lactobacillus ssp. aislados del tracto digestivo de abejas. *Rev. Salud Anim.* **2021**, *43*.

74. Serwecińska, L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water* **2020**, *12*, 3313. [[CrossRef](#)]
75. Li, Y.; Li, L.; Kromann, S.; Chen, M.; Shi, L.; Meng, H. Antibiotic resistance of *Lactobacillus* spp. and *Streptococcus thermophilus* isolated from Chinese fermented milk products. *Foodborne Pathog. Dis.* **2019**, *16*, 221–228. [[CrossRef](#)]
76. Capurso, L. Thirty Years of *Lactobacillus rhamnosus* GG: A Review. *J. Clin. Gastroenterol.* **2019**, *53*, S1–S41. [[CrossRef](#)]
77. Shahali, A.; Soltani, R.; Akbari, V. Probiotic *Lactobacillus* and the potential risk of spreading antibiotic resistance: A systematic review. *Res. Pharm. Sci.* **2023**, *18*, 468–477. [[CrossRef](#)]
78. Campedelli, I.; Mathur, H.; Salvetti, E.; Clarke, S.; Rea, M.C.; Torriani, S.; Ross, R.P.; Hill, C.; O'Toole, P.W. Genus-Wide Assessment of Antibiotic Resistance in *Lactobacillus* spp. *Appl. Environ. Microbiol.* **2019**, *85*, e01738–18. [[CrossRef](#)]
79. Hasan, M.; Arif, A.; Hasnain, A.; Abbas, T. Antibiotic susceptibility and antibacterial activity of neutralized cell-free supernatant of *Lactobacillus rhamnosus* MT539286 against Foodborne and Clinical pathogens. *Int. J. Endorsing Health Sci. Res. (IJEHSR)* **2020**, *9*, 4–9. [[CrossRef](#)]
80. Anisimova, E.; Gorokhova, I.; Karimullina, G.; Yarullina, D. Alarming Antibiotic Resistance of *Lactobacilli* Isolated from Probiotic Preparations and Dietary Supplements. *Antibiotics* **2022**, *11*, 1557. [[CrossRef](#)]
81. Chamberlain, M.; O'Flaherty, S.; Cobián, N.; Barrangou, R. Metabolomic Analysis of *Lactobacillus acidophilus*, *L. gasseri*, *L. crispatus*, and *Lactocaseibacillus rhamnosus* Strains in the Presence of Pomegranate Extract. *Front. Microbiol.* **2022**, *13*, 863228. [[CrossRef](#)] [[PubMed](#)]
82. Indira, M.; Venkateswarulu, T.C.; Abraham Peele, K.; Nazneen Bobby, M.; Krupanidhi, S. Bioactive molecules of probiotic bacteria and their mechanism of action: A review. *3 Biotech.* **2019**, *9*, 306. [[CrossRef](#)] [[PubMed](#)]
83. Mora-Flores, L.P.; Moreno-Terrazas Casildo, R.; Fuentes-Cabrera, J.; Pérez-Vicente, H.A.; de Anda-Jáuregui, G.; Neri-Torres, E.E. The Role of Carbohydrate Intake on the Gut Microbiome: A Weight of Evidence Systematic Review. *Microorganisms* **2023**, *11*, 1728. [[CrossRef](#)]
84. Ceapa, C.; Lambert, J.; van Limpt, K.; Wels, M.; Smokvina, T.; Knol, J.; Kleerebezem, M. Correlation of *Lactobacillus rhamnosus* Genotypes and Carbohydrate Utilization Signatures Determined by Phenotype Profiling. *Appl. Environ. Microbiol.* **2015**, *81*, 5458–5470. [[CrossRef](#)]
85. Li, Q.-Q.; Zeng, S.-P.; Liang, M.-H.; Yousaf, M.; Wu, Y.-P.; Tang, J.; Xiong, J.; Liu, D.-M. Safety and metabolism characteristics of *Lactocaseibacillus rhamnosus* LR-ZB1107-01 based on complete genome and corresponding phenotype. *LWT* **2024**, *204*, 116443. [[CrossRef](#)]
86. Jeckelmann, J.-M.; Erni, B. Carbohydrate transport by group translocation: The bacterial phosphoenolpyruvate: Sugar phosphotransferase system. *Bact. Cell Walls Membr.* **2019**, *92*, 223–274.
87. Kaewarsar, E.; Chaiyasut, C.; Lailerd, N.; Makhmruang, N.; Peerajan, S.; Sirilun, S. Optimization of mixed inulin, fructooligosaccharides, and galactooligosaccharides as prebiotics for stimulation of probiotics growth and function. *Foods* **2023**, *12*, 1591. [[CrossRef](#)]
88. Niu, Z.; Zou, M.; Bei, T.; Zhang, N.; Li, D.; Wang, M.; Li, C.; Tian, H. Effect of fructooligosaccharides on the colonization of *Lactobacillus rhamnosus* AS 1.2466T in the gut of mice. *Food Sci. Human. Wellness* **2023**, *12*, 607–613. [[CrossRef](#)]
89. Zunga, M.; Yebra, M.J.; Monedero, V. Complex Oligosaccharide Utilization Pathways in *Lactobacillus*. *Curr. Issues Mol. Biol.* **2021**, *40*, 49–80. [[CrossRef](#)]

**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.