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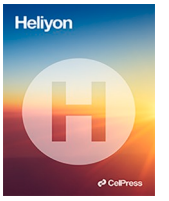

Research article

Probiotic capacity of commensal lactic acid bacteria from the intestine of Guinea pigs (*Cavia porcellus*)

Yamid A. Pinchao [a,*], Liliana Serna-Cock [a], Oswaldo Osorio Mora [b]

[a] *Faculty of Engineering and Administration, Universidad Nacional de Colombia, Palmira, Colombia*
[b] *Faculty of Agroindustrial Engineering, Universidad de Nariño, San Juan de Pasto, Colombia*

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ABSTRACT

The intricate balance of intestinal microbiota is significantly influenced by the pivotal role of indigenous lactic acid bacteria (LAB). These LAB not only contribute to antimicrobial activity and enhance animal health and productivity but also serve as defense against intestinal infections. In the present study, the probiotic potential of LAB strains isolated from various intestinal sections of adult and young guinea pigs (*Cavia porcellus*) was comprehensively assessed. Strains belonging to the genera *Ligilactobacillus*, *Weissella*, *Enterococcus*, and *Limosilactobacillus* were also identified. The antibacterial activities of the LAB strains against *Salmonella typhimurium, Escherichia coli,* and *Staphylococcus aureus* were quantified. Exopolysaccharide production, adherence capacity, antibiotic resistance, and bile salt tolerance (0.15 %, 0.30 %, and 0.45 %) of LAB were quantified. Further analyses focused on the effects of pH (2.9, 5.0, 6.4, and 7.4), temperature (40, 50, and 60 °C) and NaCl concentrations (3.5 % and 6.5 % w/v) on LAB growth. Strains GCI9 and GDE10 (*Ligilactobacillus salivarius*), isolated from the cecum and intestine of guinea pigs, exhibited significant antimicrobial activity against *S. typhimurium, E. coli* and *S. aureus.* Remarkable adherence capacity to porcine gastric mucin was demonstrated by *L. salivarius* strains, specifically ACI1, GCI9, and GDE10, with the highest exopolysaccharide levels produced by ACI1 and GCI9 (1.71 and 1.76 mg/mL, respectively). The probiotic potential was further underscored by remarkable bile salt tolerance, especially in strain GDE10, and substantial exopolysaccharide production. These strains displayed notable adaptability to varying environmental conditions, including NaCl concentrations at 3.5 % and 6.5 %, temperatures ranging from 40 to 60 °C, and pH levels of 2.9, 5.0, 6.4, and 7.4. This comprehensive assessment of the probiotic properties of *L. salivarius* strains, particularly ACI1, GCI9, and GDE10, shows promise for the development of probiotic formulations aimed at enhancing the intestinal health of guinea pigs.

1. Introduction

Peru holds the distinction of being the world leader in guinea pig production, comprising a significant 77.6 %) of the global market, followed by Ecuador, Bolivia, and Colombia [1]. The continuous growth of guinea pig breeding in these countries is attributed to its integral role in traditional and cultural gastronomy, coupled with a commendable 40 % profit margin for commercialization [1].

In Colombia, the department of Nariño is the epicenter of guinea pig production is, contributing to 95 % of the national production,

* Corresponding author.
E-mail address: yapinchaop@unal.edu.co (Y.A. Pinchao).

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benefiting approximately 30.000 producer families with an annual output of 2.6 million guinea pigs [2]. However, the expansion of commercial-scale production has ushered in challenges such as elevated morbidity and mortality rates, along with the overreliance on antibiotics to combat infectious diseases caused by *Salmonella typhimurium, Escherichia coli, Staphylococcus aureus*, and other parasites [3,4]. An intriguing avenue for mitigating these challenges lies in the utilization of probiotic bacteria isolated from guinea pig intestines. Studies conducted by Killer et al. [5] and Sechovcová et al. [6] showcase the isolation of *Lactobacillus caviae* and *Alloscardovia venturai,* both belonging to the lactobacillus and bifidobacterium species respectively. Furthermore, Palakawong Na Ayudthaya [7] identified a *Streptococcus* species from guinea pig fecal samples. The exploration of probiotic bacteria derived from the host itself has gained momentum in recent decades as a natural resource to optimize animal production and prevent infections [8,9]. These live organisms contribute to the improvement of intestinal microbial balance and exhibit efficacy against various disorders, including inflammatory diseases and bacterial infections [10,11]. Notably, studies indicate that strains display enhanced probiotic capacity when applied to the same source from which they are isolated [7,8,12–14]. Therefore, leveraging strains with probiotic potential from guinea pig intestines for inclusion in their diets becomes a promising strategy.

Several factors determine the probiotic capacity of a strain, including its adherence capacity, antimicrobial activity, tolerance to diverse temperatures, pH fluctuations, NaCl concentrations, production of exopolysaccharides (EPS), and antibiotic resistance [9,10,15,16].

The importance of molecularly identifying strains with probiotic capacity is underscored, and molecular techniques, particularly polymerase chain reaction (PCR)-based methods, play a pivotal role in their characterization and specific detection. Notably, 16S rDNA sequencing, involving single- or double-gene amplification from an appropriate DNA sample, facilitates the determination and comparison of nucleotide sequences with those of other bacteria, thereby elucidating their phylogeny [5,13]. Thus, the objective of this study was to identify lactic acid bacteria (LAB) isolated from guinea pig intestines and assess their probiotic capacity.

2. Materials and methods

2.1. Reagents

Nutrient agar, de Mann–Rogosa and Sharpe (MRS) broth, peptone water, and glucose were obtained from PANREAC (Castellar del Vallès, Barcelona). Ethanol, xylene, and phosphate buffered saline were obtained from Merck (Darmstadt, Germany). MacConkey agar, pepsin, pancreatin, bile salts, and aniline blue were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Isolation of LAB

This study was approved by the Research Ethics Committee of the Universidad Nacional de Colombia, Palmira Campus and the Ministry of Environment, Republic of Colombia (Contract No. 121 of 2016). Four 4-month-old adult guinea pigs and four 15-day-old healthy young guinea pigs were obtained from Botana farm, Universidad de Nariño, Colombia. The guinea pigs were sacrificed using a captive bolt gun by holding the animals on a metal table. With the head firmly supported on the table, the captive bolt gun was placed at the midline between the eyes and ears so that the bolt could penetrate the thalamus/midbrain regions, and the carotid arteries and jugular veins were separated bilaterally until total exsanguination [17]. Intestines were aseptically extracted for LAB isolation.

A total of 24 LAB samples were collected from the intestinal mucosa of the cecum, colon, and small intestine. Each swab was immersed in a tube containing sterile peptone. From the resulting dispersion of each intestinal section, 1 mL was serially diluted to 10^{-4} in sterile water. Bacterial colonies were seeded on MRS agar with the addition of 0.3 % aniline blue [18]. Bacterial colonies were seeded by depletion for 48 h at 37 °C. The seeding procedure was repeated until pure cultures were obtained and the strains were coded using letters and numbers. The first letter A or G were used to indicate whether the strain was obtained from an adult or young guinea pig, and the second and third letters CI, CO, or DE were used to indicate the intestinal section from which it was obtained, the cecum, colon, or intestine, respectively. The code was used to indicate the number of consecutively isolated colonies. Pure colonies were subjected to Gram staining and catalase assays. Finally, pure gram-positive, non-spore-forming, and catalase-negative colonies were seeded in MRS broth and cryopreserved in glycerol at −40 °C for molecular identification.

2.3. Molecular identification of LAB

The cryopreserved strains were reactivated by placing them in MRS broth for 24 h at 37 °C and were subsequently replicated on MRS agar for 48 h at 37 °C. DNA was extracted from the reactivated strains using a DNA extraction kit (GenElute Promega Wizard Genomic DNA Isolation, Sigma-Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions. The extracted DNA was subjected to molecular size comparison using a GeneRuler 1 kb Plus DNA Ladder (Thermo Scientific™, V. A. Graiciuno 8, Vilnius, LT-02241 Lithuania). After size confirmation, the DNA samples were subjected to PCR. For amplification of the 16S ribosomal gene, PCR was performed using the GoTaq G2 Flexi DNA Polymerase Amplification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The samples were then placed in a thermal cycler (PTC 100, Bio-Rad, Edison, NJ, USA). The initial denaturation was performed at 95 °C for 5 min for 30 cycles and the second denaturation at 95 °C for 30 s for 30 cycles. Posteriorly, the hybridization (amplification) was performed at 50 °C for 60 s and 30 cycles and the final extension at 72 °C for 40 s and 30 cycles. The melting temperature of the primers was 50 °C. 16S rDNA was amplified from the isolated genomic DNA [18].

PCR was performed using primer pairs 27F (5′-AGA-GTTTGATCCTGGCTCAG-3′) and 1495R (5′-CTACGGCTACCTTGTTACGA-3′). The size and amount of PCR products were verified on a 1 % agarose gel-TBE 1X with 1 h run conditions at 100 V using the Thermo

Scientific™ GeneRuler 100 pb Plus DNA Ladder 100 pb Plus DNA Ladder. The PCR products were subjected to Sanger sequencing at the Corporation Research Center (contract number 105S-2021-SEC; Bogotá, Colombia).

To identify each LAB, regions with low sequence quality were removed using BioEdit software version 7.7.1. Edited sequences were compared with sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool. The sequences were registered in the NCBI to obtain their respective access codes.

To construct a phylogenetic tree, the 16S rRNA gene sequences of different isolates were aligned using BioEdit version 7.7.1. Phylogenetic analysis was performed using the neighbor-joining method with the following settings in the PAUP software: phylogeny test: bootstrap method, 1000 reps; bootstraps under 50 at branch node were removed; substitution model - Kimura 2-parameter model; rates and patterns: uniform rates. The nucleotide substitution model was determined using JModelTest V.2.1.10, by employing the Bayesian Information Criterion for model selection. Reference sequences from GenBank were used to establish internal and external roots in the analysis.

2.4. Measuring the antimicrobial activity of LAB

The antimicrobial activity of molecularly identified LAB was measured against the pathogens *S. typhimurium* (ATCC® 25241™), *E. coli* (ATCC® 25922™), and *S. aureus* (ATCC® 25923™), following the methodology outlined in a study by Serna Cock et al. [19].

Each LAB strain was cultured in 25 mL of MRS broth, and each pathogenic strain was cultured in 5 mL of Mueller–Hinton broth. From each LAB, 15 mL was centrifuged at 4000 rpm for 30 min, and the resulting pellet was adjusted to a cell concentration of 1.8×10^9 cells/mL with peptonized water, with an optical density (OD) of 0.6 at 600 nm. To determine whether the antimicrobial activity was attributed to lactic acid or other metabolites produced by the strains, the supernatant was adjusted to pH 7.0 with NaOH (1 M) (antagonism test) [16]. For pathogenic strains, the inoculum was adjusted to an OD of 0.1 at 600 nm, equivalent to 0.5 on the McFarland scale.

Each pathogenic strain was inoculated onto Mueller–Hinton agar. A Sensi-Disc™ ampicillin/sulbactam served as a positive control on each agar plate. Three holes were made in the Mueller–Hinton agar using a sterile punch, and 15 μL of LAB suspension was inoculated at the base of each hole. A cylinder of sterile MRS agar (diameter 7 mm) was placed over the holes, and an additional 15 μL of LAB suspension was inoculated on the surface of the cylinder. The plates were incubated at 37 °C for 48 h, and the resulting inhibition halo was measured using the ImageJ software (Imagen j® 1.40 g, Wayne Rasband, National Institutes of Health, USA). Inhibition tests against each pathogen were conducted in triplicate for each strain.

For the antagonism test, each pathogenic strain was inoculated onto Mueller–Hinton agar by massive seeding with swabs of pathogenic bacteria. Three holes were made in the Mueller–Hinton agar using a sterile punch, and each hole was filled with 100 μL of neutralized supernatant. Amoxicillin (10 mg/mL) was used as a positive control, and 100 μL of Mueller–Hinton medium served as the negative control. The plates were incubated at 30 °C for 24 h, and the resulting inhibition halos were measured using the ImageJ software.

2.5. Measurement of adhesion capacity

The assessment of LAB adhesion capacity followed the methodology outlined in a study by Garcia-Gonzalez et al. [20] with slight modifications. Porcine gastric mucin type III was dissolved in phosphate-buffered saline (PBS) at a final concentration of 10 mg/mL. Subsequently, 100 μL of the resulting solution was added to each well of the plate for immobilization at 4 °C for 48 h (96 wells were used for each LAB). Following this, two washes were performed with PBS, and a solution of fetal bovine serum (20 mg/mL) was added to each well for a saturation stage for 24 h at 4 °C. The LAB were pre-inoculated in MRS broth at 37 °C for 48 h. Twenty milliliters of each culture was centrifuged at 4000 rpm for 30 min, and the supernatant was discarded. The respective biomasses were adjusted to a cell concentration of 2.8×10^9 cell/mL (OD of 0.60 at 600 nm using PBS buffer). A total of 50 μL of each LAB suspension were added to the respective wells and incubated for 2 h at 37 °C. Subsequently, the supernatants from each well were removed, and two washes with sterile PBS were performed to remove unattached bacteria. Finally, adherent bacteria were resuspended in 0.25 % trypsin/ethylenediamine tetraacetic acid solution for 2 min. Ten microliters of the contents of each well was used to count the adherent bacterial cells for each strain, and the cell concentration was determined using Neubauer chamber counting by adding trypan blue (1 % w/v) to each sample in a 1:1 ratio. The results are expressed as a percentage of adherence, as detailed in the equation.

$$\%A = (\#\ adherent\ cells\ /\ \#\ initial\ cells) \times 100$$

where %A is the percentage of adhesion, #adherent cells are the cells of each strain that adhered to the wells, and #initial cells are the cells initially added to each well.

2.6. Antibiotic resistance of selected LAB

Each selected strain was inoculated into 25 mL of MRS broth, and after 48 h, 15 mL was centrifuged at 4000 rpm for 30 min. The precipitates were adjusted with peptone water to a cell concentration of 1.8×10^9 cells/mL and an OD of 0.6 at 600 nm. For antibiotic resistance assays, each LAB was evaluated on MRS agar plates, compared to microbial sensitivity discs of eight antibiotics (lincomycin (2 μg), clindamycin (2 μg), penicillin G (10 u), ampicillin/sulbactam (20 μg), ampicillin (10 μg), ciprofloxacin (5 μg), and enrofloxacin (5 μg)). The inhibition halo of each antibiotic was measured using the ImageJ software [21]. The assays were performed in triplicate

for each LAB strain.

2.7. Production of EPS

LAB were cultured in 20 mL of MRS broth supplemented with 2 % (w/v) glucose at 37 °C for 72 h. Bacterial cells were removed by centrifugation at 7800 rpm for 20 min. Seven milliliters of supernatant was recovered from each culture, and 95 % (v/v) cold ethanol (4 °C) was added at a 1:1 ratio to precipitate EPS. The resulting solution was centrifuged at 7800 rpm for 20 min to facilitate precipitation. The supernatant was discarded, and the pellet was dried at 37 °C for 24 h. EPS was calculated using the following equation:

$$EPS_{(mg/mL)} = Tube_{final} - Tube_{initial}$$

where $EPS_{(g)}$ is the amount of EPS produced, Tub_{final} is the weight of the tube at the end of the extraction procedure, and $Tube_{initial}$ is the weight of the empty tube.

2.8. Effect of NaCl on the growth of LAB

Survival of LAB at different NaCl concentrations was evaluated as previously described [22], with slight modifications. Each of the LAB was reactivated in tubes with MRS medium at 37 °C for 24 h. Six tubes were prepared using 9 mL of MRS broth. An additional three tubes were adjusted to 3.5 % (w/v) NaCl concentration, and three tubes were adjusted to 6.5 % (w/v) NaCl concentration. To each tube, 1 mL of each reactivated LAB was added and immediately incubated at 37 °C for 5 h. Each tube was measured for absorbance (600 nm) before and after incubation in a Genesys 180 UV–Visible Spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). The experiments were performed in triplicate, and the results were expressed as percent growth (%C).

$$\%C = ((Abs_2 - Abs_1) / Abs_2) \times 100$$

where Abs_2 is the absorbance after incubation and Abs_1 is the absorbance before incubation.

2.9. Effect of temperature on LAB growth

The thermal resistance of LAB was evaluated at 40, 50, and 60 °C following the methodology provided by Teles Santos et al. [23] with some modifications. The strains were grown for 24 h in MRS broth. Cultures of each strain were replicated to tubes with MRS broth and kept in a water bath at 40, 50, and 60 °C, for 5 min, and immediately cooled in an ice bath. The tubes were incubated at 37 °C for 2 h. Each tube was measured for Absorbance (600 nm) before and after incubation. The experiments were performed in triplicate, and the results were expressed as percentage growth using the equation (%C).

2.10. Effect of pH on LAB growth

LAB were grown at different pH values (2.9, 5.0, 6.4, and 7.4) following the protocol given by Abushelaibi et al. [15], with some modifications. The strains were grown for 24 h in MRS broth. Each strain was replicated in tubes containing MRS broth with pH adjusted to 2.9, 5.0, 6.4, and 7.4 (pH-meter Orion Star A211, equipped with pH/ATC Ultra Triode probe, Thermo Scientific). The replicated tubes were incubated for 2 h at 37 °C. The absorbance (600 nm) of each tube was measured before and after the incubation. Replicates were performed in triplicates, and the results were expressed as percent growth using the equation (%C).

2.11. Bile salt tolerance

LAB were grown in different concentrations of bile salts (Sigma–Aldrich), following a previously described protocol [24]. The strains were grown overnight in MRS broth. A volume of 100 μL of each strain was then replicated in tubes containing 900 μL of MRS broth with bile salt concentrations of 0.15 %, 0.30 %, and 0.45 %. The tubes were incubated for 6 h at 37 °C. The growth of each strain was assessed by measuring its absorbance at 600 nm at 0 and 6 h using a previously described spectrophotometer. All tests were conducted in triplicate and the results were expressed as a percentage of growth, calculated using the equation (%C).

2.12. Statistical analysis

All data were expressed as the mean ± standard deviation. One-way analysis of variance and Fisher's least significant difference test were used to detect significant differences between groups using the statistical software STATGRAPHICS Centurion XVI, version 16.1.03. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Isolation and molecular identification of LAB

A total of 29 presumptive LAB strains were isolated from 24 samples collected from the intestines, cecum, and colons of adult and

young guinea pigs. All strains were non-spore-forming, gram-positive, and catalase-negative bacilli. Table 1 presents the results of molecular identification of LAB. All strains presented an E value of 0.0.

The phylogenetic relationship between the 16S rRNA gene sequences used in the identification of the strains is shown in Fig. 1, which illustrates the phylogenetic analysis of the BAL sequences from the conserved 16S gene used for identification. This tree shows four groups or clades that reflect the taxonomic diversity of the isolated strains. The 4 clades include strains closely related with the genera *Ligilactobacillus, Enterococcus, Weissella,* and *Limosilactobacillus*.

Based on the genera and species shown in Fig. 1, the strains ACI1, GCI9, GDE10, GCI19, ACI10, ACO3, ACO10, GDE2, and ADE13 were selected for probiotic capacity testing.

3.2. Antimicrobial activity

Table 2 shows the antimicrobial activities of the 10 strains selected against the three pathogens. *Ligilactobacillus salivarius* (GCI9) and *Enterococcus hirae* (ACO10) inhibited all three pathogens. These strains were obtained from the cecum of a young guinea pig and colon of an adult guinea pig. The antimicrobial activity of ACO10 was higher against *E. coli* and *S. typhimurium,* showing statistically significant differences with the other strains studied. *Lactobacillus salivarius* (ACI1 and GDE10), *Weissella viridescens* (GDE2), and *E. hirae* (GCI19) inhibited at least one pathogen (these strains were from the cecum and small intestine of young and adult guinea pigs). The strains *Limosilactobacillus alvi* (ACI10), *Weissella paramesenteroides* (ACO3), *Weissella cibaria* (ADE13), and *Weissella confusa* (ACI2) did not inhibit any of the pathogens (these strains were isolated from the adult guinea pig cecum, colon, and small intestine). The results of the antagonism test using the neutralized supernatant did not show inhibitory halos, indicating that the antimicrobial activity of the strains was due to lactic acid.

3.3. Adhesion capacity

Table 3 shows the average values of the percentage of adhesion of LAB isolated from guinea pig intestines to type III porcine gastric mucin.

According to the results obtained, the strains with the highest adherence capacity were *Lactobacillus salivarius, Ligilactobacillus salivarius* and *W. cibaria* (strains ACI1, GCI9 and ADE13, respectively).

3.4. Antibiotic resistance

Table 4 shows the values of the antibiotic inhibition halos for each LAB strain isolated from guinea pig intestines.

Table 1
Molecular identification of lactic acid bacteria.

Strain code	Sequence size (bp)	Consulting cover	% Identifier	Molecular identification	Accession
ACI1	1213	94 %	98.53 %	*Ligilactobacillus salivarius*	OQ092276.1
ACI10	1263	96 %	94,82 %	*Limosilactobacillus alvi*	OQ092277.1
GCO2	1189	100 %	98,91 %	*Ligilactobacillus salivarius*	OQ092278.1
ADE3	1253	99 %	98,17 %	*Ligilactobacillus salivarius*	OQ092279.1
ACO3	1189	96 %	97,76 %	*Weissella paramesenteroides*	OQ092280.1
GCI4	1322	96 %	97,52 %	*Ligilactobacillus salivarius*	OQ092281.1
ACI5	1249	94 %	97,99 %	*Ligilactobacillus salivarius*	OQ092282.1
GCI6	1316	94 %	97,14 %	*Ligilactobacillus salivarius*	OQ092283.1
ADE7	1317	91 %	98,77 %	*Ligilactobacillus salivarius*	OQ092284.1
GCO8	1305	97 %	97,29 %	*Ligilactobacillus salivarius*	OQ092285.1
GCI9	1250	99 %	98,18 %	*Ligilactobacillus salivarius*	OQ092286.1
GDE10	1267	99 %	96,08 %	*Ligilactobacillus salivarius*	OQ092287.1
ACI11	1275	97 %	96,27 %	*Ligilactobacillus salivarius*	OQ092288.1
ACO12	1282	97 %	96,43 %	*Ligilactobacillus salivarius*	OQ092289.1
GDE13	1302	97 %	96,65 %	*Ligilactobacillus salivarius*	OQ092290.1
GCO14	1285	99 %	96,07 %	*Ligilactobacillus salivarius*	OQ092291.1
ADE15	1283	98 %	96,51 %	*Ligilactobacillus salivarius*	OQ092292.1
ACI2	1170	89 %	98,03 %	*Weissella confusa*	OQ092293.1
ACI8	1156	91 %	97,18 %	*Weissella viridescens*	OQ092294.1
GCI19	1223	100 %	97,66 %	*Enterococcus hirae*	OQ092295.1
GCI10	1320	99 %	97,74 %	*Enterococcus hirae*	OQ092296.1
ACI13	943	89 %	85,46 %	*Limosilactobacillus reuteri*	OQ092297.1
GCO6	1216	93 %	97,82 %	*Enterococcus faecium*	OQ092298.1
ACO7	1125	97 %	98,03 %	*Enterococcus hirae*	OQ092299.1
ACO10	1227	93 %	97,76 %	*Enterococcus hirae*	OQ092300.1
GDE2	1130	88 %	97,73 %	*Weissella viridescens*	OQ092301.1
ADE13	1115	91 %	97,12 %	*Weissella cibaria*	OQ092302.1
ADE5	1184	92 %	97,91 %	*Enterococcus hirae*	OQ092303.1
ADE6	1187	95 %	97,01 %	*Enterococcus hirae*	OQ092304.1

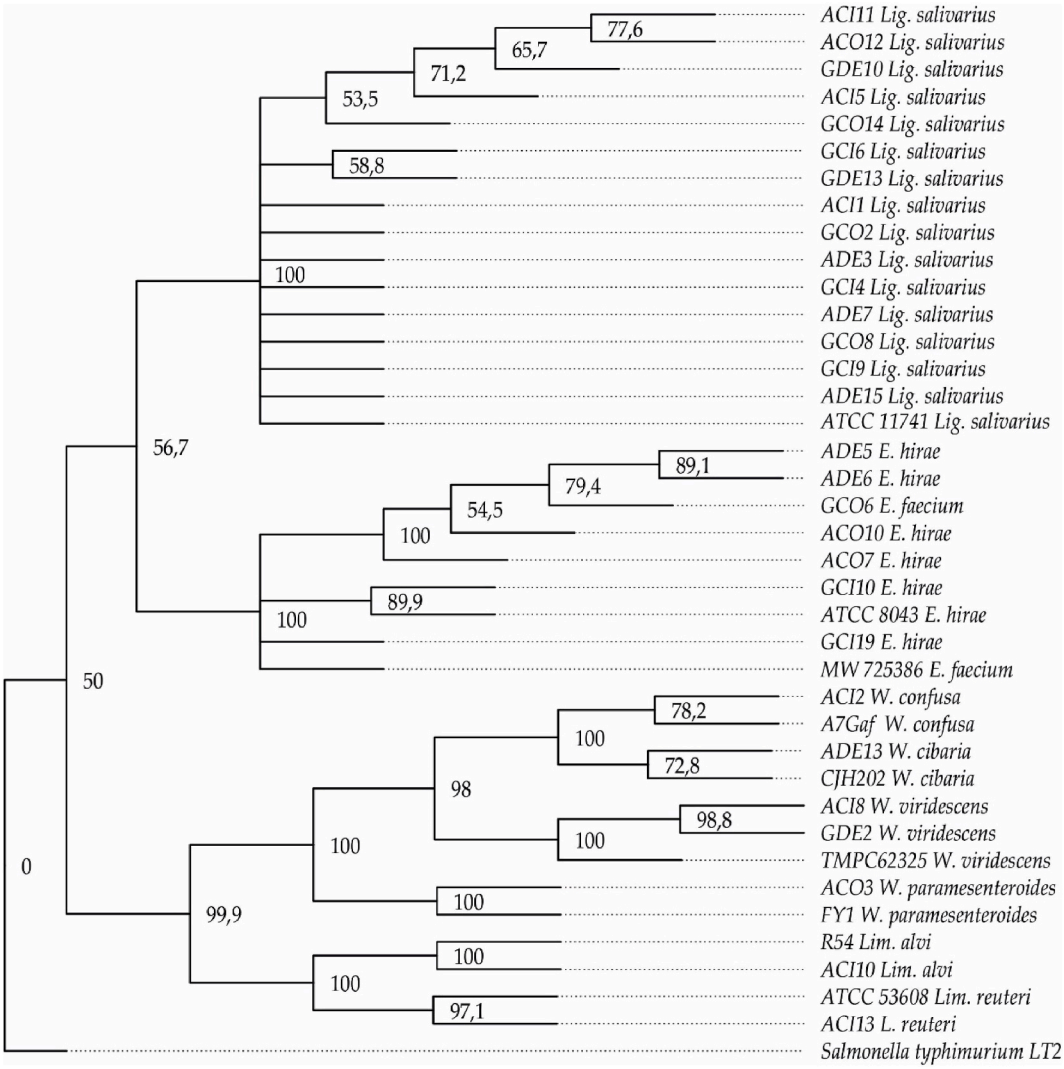

Fig. 1. Phylogenetic tree obtained from the sequences of bacteria isolated from the intestinal mucosa of guinea pigs and constructed using the neighbor-joining method.

Table 2
Antimicrobial activity of strains isolated from guinea pig intestines against pathogenic bacteria.

Strain	Escherichia coli	Staphylococcus aureus	Salmonella typhimurium
ACI1	–	$13{,}32 \pm 0{,}19^{A}$	–
GCI9	$12{,}0 \pm 0{,}42^{C}$	$13{,}65 \pm 0{,}40^{A}$	$10{,}67 \pm 0{,}43^{A}$
GDE10	–	$13{,}33 \pm 0{,}18^{A}$	–
ACI10	–	–	–
ACO3	–	–	–
ACO10	$12{,}67 \pm 0{,}33^{B}$	$11{,}67 \pm 0{,}33^{B}$	$13{,}0 \pm 0{,}58^{B}$
GDE2	$11{,}33 \pm 0{,}33^{A}$	$13{,}0 \pm 0{,}58^{A}$	–
ADE13	–	–	–
GCI19	–	–	$12{,}0 \pm 0^{C}$
ACI2	–	–	–

All data were represented as mean ± standard deviation (n = 3). Superscripts with different letters in the same column indicate significant differences ($p < 0.05$) as analyzed using Fisher's least significant difference test.

Table 3
Percentage of adherence to type III porcine gastric mucin of strains isolated from guinea pig intestines.

Strain	% A
ACI1	$11,33 \pm 0,87^{H}$
GCI9	$10,72 \pm 0,94^{G}$
GDE10	$3,37 \pm 0,51^{A}$
ACI10	$8,29 \pm 0,95^{E}$
ACO3	$7,8133 \pm 0,28^{CD}$
ACO10	$9,81 \pm 0,45^{F}$
GDE2	$5,78 \pm 0,84^{B}$
ADE13	$11,1 \pm 0,39^{GH}$
GCI19	$7,41 \pm 0,27^{C}$
ACI2	$8,02 \pm 0,98^{DE}$

Table 4
Antibiotic sensitivity of strains isolated from guinea pig intestines (R = resistant, I = intermediate, S = sensitive).

Strain	Lincomycin	Clindamycin	Penicillin G	Ampicillin/sulbactam	Ampicillin	Ciprofloxacin	Enrofloxacin	Erythromycin
ACI1	R	S	S	S	S	R	R	R
GCI9	R	I	S	S	S	R	R	I
GDE10	R	S	S	S	S	R	R	R
ACI10	S	S	S	S	S	R	R	S
ACO3	R	S	S	S	S	R	R	S
ACO10	R	R	S	S	S	R	R	S
GDE2	I	I	S	S	S	R	R	S
ADE13	R	S	S	S	R	R	R	I
GCI19	S	S	R	S	S	R	R	S
ACI2	R	S	S	S	S	R	I	S

3.5. EPS production

Table 5 shows the milligrams of EPS produced by each strain isolated from guinea pig intestines.

3.6. Effect of NaCl

Fig. 2 shows the average percentage growth values of the isolated strains at NaCl concentrations of 3.5 % and 6.5 % (w/v).

3.7. Effect of temperature

Fig. 3 shows the average LAB growth percentages after exposure to different temperatures.

Table 5
Exopolysaccharides produced by strains isolated from guinea pig intestines.

Strain	Exopolysaccharides (mg/mL)
ACI1	$1,71 \pm 0,08^{CD}$
GCI9	$1,76 \pm 0,13^{D}$
GDE10	$1,67 \pm 0,17^{CD}$
ACI10	$1,07 \pm 0,08^{A}$
ACO3	$1,25 \pm 0,16^{AB}$
ACO10	$1,16 \pm 0,12^{AB}$
GDE2	$1,35 \pm 0,06^{ABC}$
ADE13	$1,44 \pm 0,12^{BCD}$
GCI19	$1,21 \pm 0,14^{AB}$
ACI2	$1,12 \pm 0,09^{AB}$

Columns with different superscript letters indicate significant differences. Strains ACI1, GCI9, GDE10, and ADE13 produced the highest amounts of exopolysaccharides, with no statistical difference between the strains.

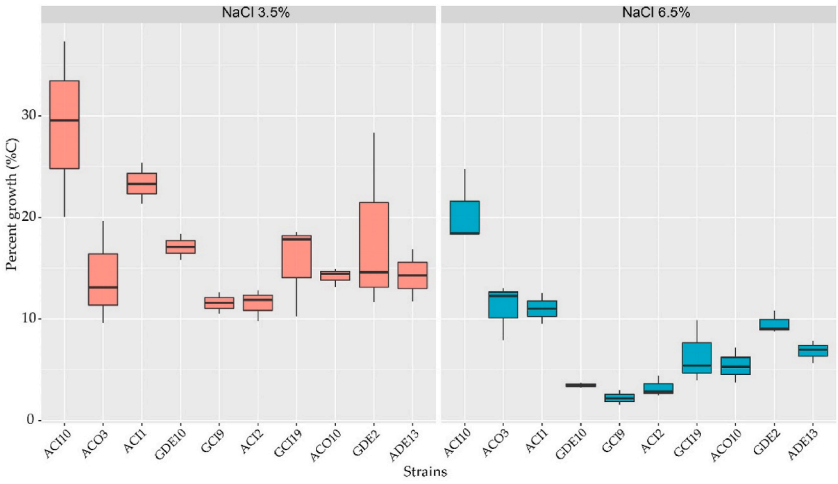

Fig. 2. Effect of NaCl on the percentage growth of lactic acid bacteria isolated from guinea pigs.

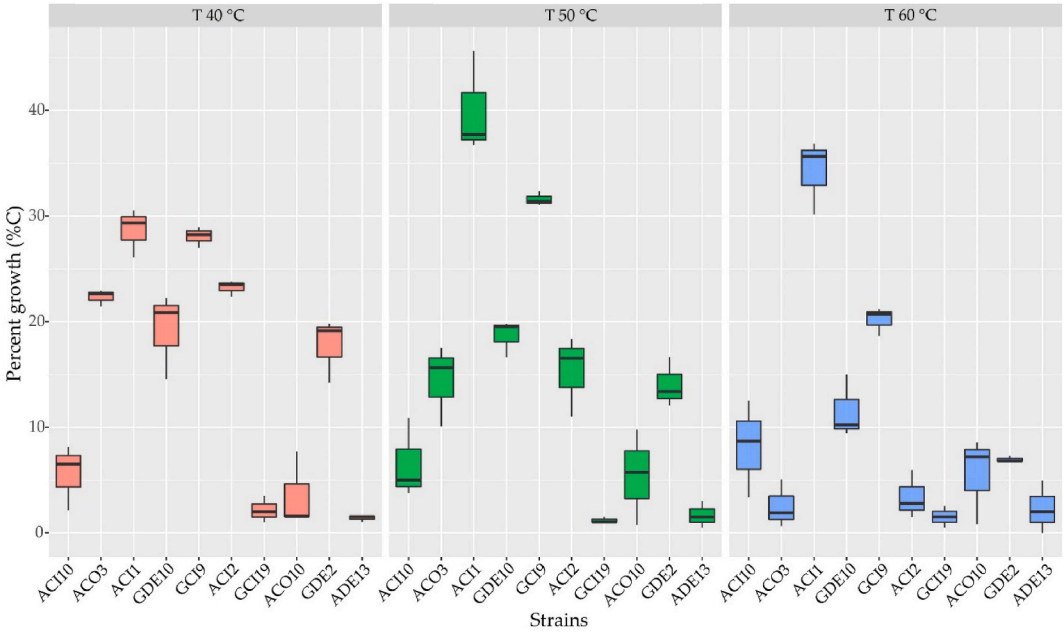

Fig. 3. Effect of temperature on the percentage of growth of isolated strains.

3.8. *Effect of pH*

Fig. 4 shows the average percentages of LAB growth at different pH.

3.9. *Effect of bile salt tolerance*

Fig. 5 shows the average growth percentages of LABs subjected to three concentrations of bile salts. For a bile salt concentration of 0.15 %, the %C ranged from 40 % to 72 %. At a concentration of 0.30 %, the %C ranged from 29 % to 61 %, and at a concentration of 0.45 %, the %C ranged from 11 % to 48 %.

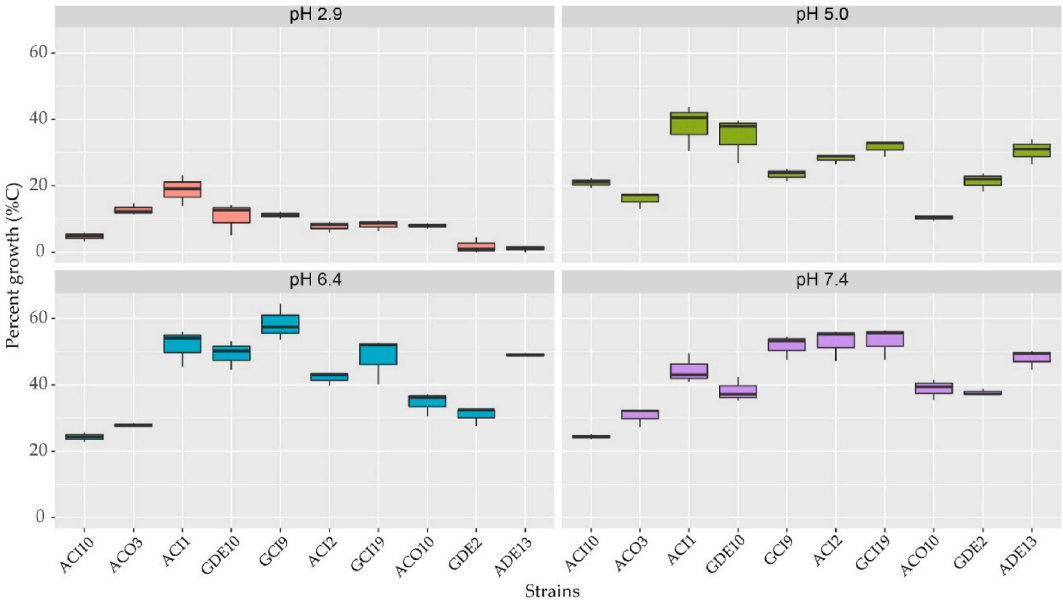

Fig. 4. Effect of pH on the percentage growth of lactic acid bacteria isolated from guinea pigs.

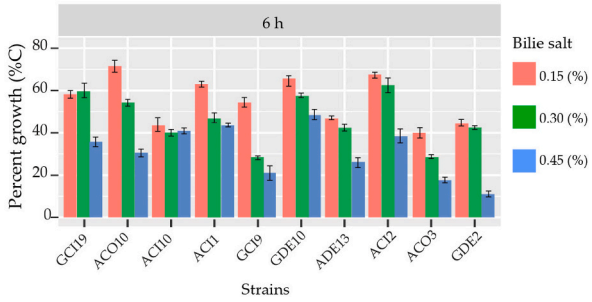

Fig. 5. Effect of bile salt on the percentage growth of lactic acid bacteria isolated from guinea pigs.

4. Discussion

4.1. Isolation and molecular identification of LAB

Strains closely related with *Ligilactobacillus salivarius* (ACI1, GCO2, ADE3, GCI4, ACI5, GCI6, ADE7, GCO8, GCI9, GDE10, ACI11, ACO12, GDE13, GCO14, and ADE15), *W. confusa* (ACI2, GCO4), *W. viridescens* (ACI8 and GDE2), *Enterococcus faecium* (GCO6), *Limosilactobacillus alvi* (ACI10), *W. paramesenteroides* (ACO3), *Limosilactobacillus reuteri* (ACI13), *E. hirae* (GCI19, GCI10, ACO7, ACO10, ADE5, and ADE6) and *W. cibaria* (ADE13) were isolated from different intestinal sections of adult and young guinea pigs. These strains, shown in Table 1, correspond to LAB that are generally recognized as safe.

Previous studies have demonstrated the presence of LAB in the intestinal tract of young and adult guinea pigs. Porturas [25] isolated lactobacilli from the intestinal mucosa of 2–6 day old guinea pigs and 2 month old adult guinea pigs. Of a total of 85.18 % of the strains, 33.33 % corresponded to LAB *Lactobacillus reuteri, frumenti, johnsoni,* sp.; 37.03 % to *E. hirae, faecalis,* sp.; 3.7 % to *Streptococcus thoraltensis*, and 11.11 % to *Bacillus pumilus and Bacillus* sp. The remaining 14.81 % corresponded to *Staphylococcus,* which is not a probiotic.

Additionally, Killer et al. [5] isolated a strain from the oral cavity of the guinea pigs (MOZM2T) that showed 98.4 % similarity to *Lactobacillus reuteri* DSM 20016^{T}. However, because of the difference in the cellular profiles of fatty acids between MOZM2T and *Lactibacillus reuteri* DSM DSM 20016^{T}, they proposed that their strain be considered a new taxon within the group of heterofermentative lactobacilli and named it *Lactobacillus caviae* sp. nov., where *caviae* is derived from the scientific name of the guinea pig.

According to the literature, the genera and species closely related with LAB in this study present certain probiotic characteristics, such as immunobiotic potential, antimicrobial activity against gram-negative intestinal pathogens, and adhesion to mucins and cells. According to several authors, these bacteria can stimulate the innate response of TLR 4 receptors responsible for the regulation of intestinal epithelial inflammatory expression [26,27]. Indo et al. [13], and Quilodrán-Vega et al. [27] demonstrated in their studies

that *Ligilactobacillus salivarius* has probiotic and immunobiotic potential, antimicrobial activity against gram-negative intestinal pathogens, and adhesion to mucins and epithelial cells. Therefore, the LAB isolates from this study are of high relevance because these probiotics may have immunostimulatory effects and antibody immune responses in guinea pigs.

4.2. Antimocrobial activity

The inhibition halos presented in Table 2 demonstrate that *Ligilactobacillus salivarius* and *E. hirae* (GCI9 and ACO10, respectively) exhibited antimicrobial activity against the three pathogens *S. typhimurium, E. coli*, and *S. aureus*. The antagonism test, utilizing only the supernatant, confirmed that the antimicrobial activity was attributable to lactic acid. This inference is supported by the findings of Serna Cock et al. [19], Zhou et al. [21], and Sobrino et al. [28], whereas *Ligilactobacillus salivarius* demonstrated inhibitory effects against various pathogens, such as *Clostridium perfringens* MP34, *Enterococcus faecalis* MP42, *S. aureus* MP83, *Streptococcus suis* MP205, and *Trueperella pyogenes* MP214, which were attributed to the production of lactic acid, specifically the L-lactic acid isomer. Additionally, a previous study has indicated that *Ligilactobacillus salivarius* can reduce *S. typhimurium* infection in an in vivo mouse model [27]. The administration of *L. salivarius* TUCO-L2 to mice resulted in a significant decrease in the number of *Salmonella* in the liver and spleen, with no pathogens detected in the blood of the animals.

4.3. Adhesion capacity

Adhesion capacity varied among the isolated strains, as shown in Table 3. Notably, *L. salivarius* (ACI1 and GCI9), *E. hirae* (ACO10), and *W. cibaria* (ADE13) exhibited the highest adherence. Specifically, *Lactobacillus salivarius* and *Ligilactobacillus salivarius* strains isolated from the cecum (strain 1 from the cecum of an adult guinea pig and strain 9 from the cecum of a young guinea pig) demonstrated substantial adherence capacity compared to the strain isolated from the small intestine. These finding aligns with that of Garcia-Gonzalez et al. [20] who suggested that lactobacilli generally possess a high adhesion capacity to gastric mucin, albeit varying among genera and species. According to Nishiyama et al. [29], LAB recognize and adhere to mucin through a carbohydrate-protein interaction mechanism involving the mucin chain and various adhesins on the bacterial cell surface. Therefore, it can be inferred that *Lactobacillus salivarius* and *Ligilactobacillus salivarius* strains employed this antimicrobial mechanism for adhesion. Additionally, LAB utilize various structures such as S-layer proteins, lipoteichoic acid, EPS, and mucus-binding proteins for adhesion [30].

Moreover, Sobrino et al. [28] and Quilodrán-Vega et al. [27] reported that strains such as *L. salivarius* TUCO-L2 can adhere to porcine mucins, Caco-2 cells, and HT29 cells. These studies also identified MucBP1, a protein associated with the adhesion of lactobacilli to the intestinal mucosa [27]. These findings underscore the safety and biotechnological significance of the *Lactobacillus salivarius* and *Ligilactobacillus salivarius* strains isolated in this study.

4.4. Antibiotic resistance

LAB exhibited resistance (R) to at least two of the investigated antibiotics. Notably, all strains showed resistance to ciprofloxacin, a broad-spectrum antibiotic effective against both gram-positive and gram-negative bacteria, including *Haemophilus* spp. and *Pseudomonas* spp. Ciprofloxacin is active against gram-positive cocci and bacilli, *Chlamydia*, *Mycoplasma*, and *Mycobacterium* spp. Similarly, resistance to enrofloxacin, which is used to treat infections caused by *Staphylococcus* spp., *E. coli, Haemophilus* spp. *Pasteurella* spp. and *Salmonella* spp., has also been reported [4]. As emphasized in the studies by Hummel et al. [31] and Giuliano et al. [32], antibiotic susceptibility is a crucial consideration when selecting probiotic strains. The antibiotic resistance demonstrated by the LAB in this study raises concerns when considering their use as probiotics. However, strains that exhibit susceptibility or resistance to specific antibiotics in vitro may not necessarily exhibit similar effects in vivo. Therefore, the results shown in Table 4 should be considered a preliminary reference, and further tests are necessary to analyze the mechanisms of resistance, strain-specific toxin production, and the pharmacokinetic and pharmacodynamic properties of antibiotics [32]. Notably, LAB may harbor antibiotic resistance genes owing to the presence of plasmids of varying sizes. However, a comprehensive understanding of the physiological and molecular characteristics of the lactobacilli in this study is essential [31].

4.5. EPS production

L. salivarius strains (ACI1, GCI9, and GDE10) exhibited the highest EPS production, with no significant differences among them. These substances play important roles in safeguarding microbial cells against various conditions including desiccation, osmotic stress, antibiotics, and toxic compounds [16]. Additionally, strains from genera such as *Streptococcus, Lactococcus, Pediococcus, Lactobacillus, Leuconostoc*, and *Weissellale* are known for their ability to produce EPS, which contribute significantly to human health [16,33]. These substances possess prebiotic properties and exhibit anticoagulant, antioxidant, anti-inflammatory, antiviral, cholesterol-lowering, and anticancer activities [34]. The promising results regarding EPS production by *Ligilactobacillus salivarius* strains in this study open avenues for the potential application of these bacteria in the development of probiotic food matrices [16].

4.6. NaCl, temperature, and pH tolerance

Upon examining LAB growth at two NaCl concentrations (Fig. 2), it was noteworthy that *Lactobacillus salivarius* (ACI1) exhibited greater NaCl tolerance, evident from a higher growth percentage at both concentrations (3.5 % and 6.5 %). In contrast, *Lactobacillus*

salivarius (GCI9) and *W. confusa* (ACI2) displayed lower NaCl tolerance. As emphasized in the studies by Vasiee et al. [9] and Body et al. [35] in industrial applications, LAB must demonstrate the ability to endure salt stress conditions, as variations in NaCl concentration can influence the production of metabolites with potential health implications.

Evaluation of the impact of heat shock stress on strains' growth rates at three temperatures reveals that *Ligilactobacillus salivarius* strains (ACI1 and GCI9) exhibit the highest growth across all treatments (40, 50, and 60 °C) (Fig. 3). Notably, these strains (ACI1 and GCI9) subjected to the 50 °C treatment achieved growth rates of 40 % and 32 %, respectively. Thermal stress resilience is crucial at the industrial level, serving as an indicator that strains can withstand temperature fluctuations during the processing of diverse products, including meat and dairy [15,34].

The strains showed growth across all evaluated pH levels, with the highest growth percentages observed at pH 6.4 and 7.4. *Lactobacillus salivarius* (ACI1) particularly stands out, exhibiting optimal growth at pH 2.9 (18.75 %) and pH 5.0 (38.22 %). *Lactobacillus salivarius* ACI1 showed superior adaptation to acidic pH conditions (2.9 and 5.0), whereas *Ligilactobacillus salivarius* (GCI9) excels at neutral pH. Increasing pH correlates with enhanced growth rates for all strains. An [23] essential probiotic trait is high resistance to gastric acidity, bile salts, pepsin, pancreatin, and other enzymes. Moreover, pH adjustment at the industrial level is employed to stress LAB, potentially simulating increased metabolite production such as EPS [34]. It is important to note that the lactobacilli exhibited varying growth rates at each pH, indicating strain-dependent characteristics (Fig. 4) [23].

4.7. Effect of bile salt tolerance

Bile salt tolerance is a crucial parameter for assessing the probiotic potential of strains because bacteria susceptible to bile salts may degrade cell wall lipids, ultimately leading to cell death [11,36]. This study evaluated the impact of three bile salt concentrations on strain growth and revealed a decrease in %C as the bile salt concentration increased (Fig. 5). Notably, the strains in this study demonstrated a high survival capacity at bile salt concentration of 0.15 %, with relatively robust survival at concentrations of 0.30 % and 0.45 %. This finding is consistent with that of Saboktakin-Rizi et al. [11], which suggests that the strains exhibited favorable survival at bile salt concentrations in the gastrointestinal tract. The observed resilience at higher bile salt concentrations is a positive indicator of the ability of the strains to withstand significant challenges in the gastrointestinal environment [11]. However, it is imperative to recognize that strain-specific variations may exist that influence their effectiveness as probiotics under diverse conditions.

5. Conclusions

In various segments of the intestines in both adult and young guinea pigs, LAB strains recognized as safe were closely related with *L. salivarius, W. confusa, W. viridescens, E. faecium, L. alvi, W. paramesenteroides, L. reuteri, E. hirae*, and *W. cibaria*. Among these, *L. salivarius* demonstrated a superior probiotic capacity in situ. The confirmed probiotic attributes of *L. salivarius* make it a promising candidate for the development of probiotic food matrices tailored for guinea pigs. Additionally, considering its antimicrobial efficacy against pathogens impacting guinea pig reproduction, robust adhesion capabilities, substantial EPS production, and resilient growth at temperatures up to 50 °C, *L. salivarius* emerges as a versatile probiotic. However, the observed resistance of this strain to certain antibiotics raises concerns that may restrict its use. Consequently, further investigations are imperative to comprehensively understand the mechanisms of antibiotic resistance, assess its potential impact in vivo, and explore strategies to mitigate or circumvent this limitation. Future research endeavors will contribute to refining the application scope of *L. salivarius* and optimizing its effectiveness as a probiotic intervention for guinea pigs.

Ethics statement

The Research Ethics Committee of the National University of Colombia Sede Palmira, meeting in session on October 11, 2019, Act No. 05 grants the Endorsement for this research.

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Data availability statement

The data associated with this study have not been deposited in a publicly available repository, because the data are included in the article.. Also, the project had the permits of the Ministerio del Medio Ambiente of Colombia, through contract Otro si 28 Code RGE0152-28.

CRediT authorship contribution statement

Yamid A. Pinchao: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Liliana Serna-Cock:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Oswaldo Osorio Mora:** Writing – review & editing, Supervision, Resources, Methodology.

Declaration of competing interest

To: Heliyon.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] midagri, En el 2020 se elevarán las ventas y consumo de cuy - Noticias - Ministerio de Desarrollo Agrario y Riego - Gobierno del Perú, Nota de Prensa, 2020.
[2] M.P. Aristizábal Bedoya, La producción de cuyes aporta a pasto $80.000 millones al año por sus ventas, Industria, 2019.
[3] C. Chuquizuta, S. Morales, Identificación de agentes bacterianos aislados de gazapos muertos de cuyes en una granja de crianza intensiva en Lima, Perú, Revista Electronica de Veterinaria 18 (2017) 1–13.
[4] N.H. Shomer, H. Holcombe, J.E. Harkness, Chapter 6 - biology and diseases of Guinea pigs. https://doi.org/10.1016/B978-0-12-409527-4.00006-7, 2015.
[5] J. Killer, R. Pechar, P. Švec, H. Salmonová, R. Švejstil, M. Geigerová, V. Rada, E. Vlková, C. Mekadim, Lactobacillus caviae sp. nov., an obligately heterofermentative bacterium isolated from the oral cavity of a Guinea pig (cavia aperea f. porcellus), Int. J. Syst. Evol. Microbiol. 67 (2017) 2903–2909, https://doi.org/10.1099/ijsem.0.002044.
[6] H. Sechovcová, J. Killer, R. Pechar, M. Geigerová, R. Švejstil, H. Salmonová, C. Mekadim, V. Rada, E. Vlková, O. Kofroňová, O. Benada, Alloscardovia venturai sp. Nov., a fructose 6-phosphate phosphoketolase-positive species isolated from the oral cavity of a Guinea-pig (Cavia aperea f. porcellus), Int. J. Syst. Evol. Microbiol. 67 (2017) 2842–2847, https://doi.org/10.1099/ijsem.0.002031.
[7] S. Palakawong Na Ayudthaya, H. van der Oost, J. van der Oost, D.M. van Vliet, C.M. Plugge, Microbial diversity and organic acid production of Guinea pig faecal samples, Curr. Microbiol. 76 (2019) 425–434, https://doi.org/10.1007/s00284-019-01630-x.
[8] S. Palakawong Na Ayudthaya, L.J. Hilderink, J. van der Oost, W.M. de Vos, C.M. Plugge, Streptococcus caviae sp. nov., isolated from Guinea pig faecal samples, Int. J. Syst. Evol. Microbiol. 67 (2017) 1551–1556, https://doi.org/10.1099/ijsem.0.001758.
[9] A. Vasiee, F. Falah, B.A. Behbahani, F. Tabatabaee-yazdi, Probiotic characterization of Pediococcus strains isolated from Iranian cereal-dairy fermented product: interaction with pathogenic bacteria and the enteric cell line Caco-2, J. Biosci. Bioeng. 130 (2020) 471–479, https://doi.org/10.1016/J.JBIOSC.2020.07.002.
[10] R.C. Reuben, P.C. Roy, S.L. Sarkar, A.S.M. Rubayet Ul Alam, I.K. Jahid, Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties, J. Dairy Sci. 103 (2020) 1223–1237, https://doi.org/10.3168/jds.2019-17092.
[11] M. Saboktakin-Rizi, B. Alizadeh Behbahani, M. Hojjati, M. Noshad, Identification of Lactobacillus plantarum TW29-1 isolated from Iranian fermented cereal-dairy product (Yellow Zabol Kashk): probiotic characteristics, antimicrobial activity and safety evaluation, J. Food Meas. Char. 15 (2021) 2615–2624, https://doi.org/10.1007/s11694-021-00846-5.
[12] R.U. Khan, S. Naz, K. Dhama, K. Karthik, R. Tiwari, M.M. Abdelrahman, Direct-fed microbial: beneficial applications, modes of action and prospects as a safe tool for enhancing ruminant production and safeguarding health, Int. J. Pol. Inf. 12 (2016) 220–231, https://doi.org/10.3923/ijp.2016.220.231.
[13] Y. Indo, S. Kitahara, M. Tomokiyo, S. Araki, M.A. Islam, B. Zhou, L. Albarracin, A. Miyazaki, W. Ikeda-Ohtsubo, T. Nochi, T. Takenouchi, H. Uenishi, H. Aso, H. Takahashi, S. Kurata, J. Villena, H. Kitazawa, Ligilactobacillus salivarius strains isolated from the porcine gut modulate innate immune responses in epithelial cells and improve protection against intestinal viral-bacterial superinfection, Front. Immunol. 12 (2021) 1–22, https://doi.org/10.3389/fimmu.2021.652923.
[14] A. Vasiee, B. Alizadeh Behbahani, F. Tabatabaei Yazdi, S.A. Mortazavi, H. Noorbakhsh, Diversity and probiotic potential of lactic acid bacteria isolated from horreh, a traditional Iranian fermented food, Probiotics Antimicrob Proteins 10 (2018) 258–268, https://doi.org/10.1007/s12602-017-9282-x.
[15] A. Abushelaibi, S. Al-Mahadin, K. El-Tarabily, N.P. Shah, M. Ayyash, Characterization of potential probiotic lactic acid bacteria isolated from camel milk, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 79 (2017) 316–325, https://doi.org/10.1016/j.lwt.2017.01.041.
[16] D. Kavitake, S. Kandasamy, P.B. Devi, P.H. Shetty, Recent developments on encapsulation of lactic acid bacteria as potential starter culture in fermented foods – a review, Food Biosci. 21 (2018) 34–44, https://doi.org/10.1016/j.fbio.2017.11.003.
[17] G. Limon, E.A. Gonzales-Gustavson, T.J. Gibson, Investigation into the humaneness of slaughter methods for Guinea pigs (Cavia porcelus) in the andean region, J. Appl. Anim. Welfare Sci. 19 (2016) 280–293, https://doi.org/10.1080/10888705.2016.1138116.
[18] R. Dowarah, A.K. Verma, N. Agarwal, P. Singh, B.R. Singh, Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets, PLoS One 13 (2018), https://doi.org/10.1371/journal.pone.0192978.
[19] L. Serna, Cock, L.J. Valencia Hernández, R. Campos Gaona, Cinética de fermentación y acción antimicrobiana de Weissella confusa contra Staphylococcus aureus y Streptococcus agalactiae, Rev. Fac. Ing. (2010) 55–65. https://www.redalyc.org/articulo.oa?id=43019328006.
[20] N. Garcia-Gonzalez, R. Prete, N. Battista, A. Corsetti, Adhesion properties of food-associated lactobacillus plantarum strains on human intestinal, Front. Microbiol. 9 (2018) 1–11, https://doi.org/10.3389/fmicb.2018.02392.
[21] K. Zhou, Y. Zeng, X. Han, Modelling growth and bacteriocin production by lactobacillus plantarum BC-25 in response to temperature and pH in batch fermentation, Appl. Biochem. Biotechnol. (2015) 1627–1637, https://doi.org/10.1007/s12010-015-1666-3.
[22] X. Song, Q. Wang, X. Xu, J. Lin, X. Wang, Y. Xue, R. Wu, Y. An, Isolation and analysis of salt response of lactobacillus plantarum FS5-5 from dajiang, Indian J. Microbiol. 56 (2016) 451–460, https://doi.org/10.1007/s12088-016-0588-2.
[23] T. Teles Santos, R.M. Santos Ornellas, L. Borges Arcucio, M. Messias Oliveira, J.R. Nicoli, C. Villela Dias, A.P. Trovatti Uetanabaro, G. Vinderola, Characterization of lactobacilli strains derived from cocoa fermentation in the south of Bahia for the development of probiotic cultures, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 73 (2016) 259–266, https://doi.org/10.1016/j.lwt.2016.06.003.
[24] Ç.B. Albayrak, M. Duran, Isolation and characterization of aroma producing lactic acid bacteria from artisanal white cheese for multifunctional properties, Lebensm. Wiss. Technol. 150 (2021), https://doi.org/10.1016/j.lwt.2021.112053.
[25] K. Porturas, Aislamiento e identificación por técnicas moleculares de aislados bacterianos pertenecientes a géneros con potential aplicación probiótica presentes en el intestino de cuyes (Cavia porcellus), Universidad Nacional Mayor de San Marcos, 2011.
[26] Y. Tuo, X. Song, Y. Song, W. Liu, Y. Tang, Y. Gao, S. Jiang, F. Qian, G. Mu, Screening probiotics from Lactobacillus strains according to their abilities to inhibit pathogen adhesion and induction of pro-inflammatory cytokine IL-8, J. Dairy Sci. (2018) 1–8, https://doi.org/10.3168/JDS.2017-13654.
[27] S. Quilodrán-Vega, L. Albarracin, F. Mansilla, L. Arce, B. Zhou, M.A. Islam, M. Tomokiyo, I. Al Kassaa, Y. Suda, H. Kitazawa, J. Villena, Functional and genomic characterization of Ligilactobacillus salivarius TUCO-L2 isolated from Lama glama milk: a promising immunobiotic strain to combat infections, Front. Microbiol. 11 (2020) 1–24, https://doi.org/10.3389/fmicb.2020.608752.
[28] O.J. Sobrino, C. Alba, R. Arroyo, I. Pérez, L. Sariego, S. Delgado, L. Fernández, J. de María, P. Fumanal, A. Fumanal, J.M. Rodríguez, Replacement of metaphylactic antimicrobial therapy by oral administration of Ligilactobacillus salivarius MP100 in a pig farm, Front. Vet. Sci. 8 (2021) 1–14, https://doi.org/10.3389/fvets.2021.666887.
[29] K. Nishiyama, M. Sugiyama, T. Mukai, Adhesion properties of lactic acid bacteria on intestinal mucin, Microorganisms 4 (2016) 1–18, https://doi.org/10.3390/microorganisms4030034.
[30] D. Alp, H. Kuleaşan, Adhesion mechanisms of lactic acid bacteria: conventional and novel approaches for testing, World J. Microbiol. Biotechnol. 35 (2019) 1–9, https://doi.org/10.1007/s11274-019-2730-x.
[31] A.S. Hummel, C. Hertel, W.H. Holzapfel, C.M.A.P. Franz, Antibiotic resistances of starter and probiotic strains of lactic acid bacteria, Appl. Environ. Microbiol. 73 (2007) 730–739, https://doi.org/10.1128/AEM.02105-06.
[32] C. Giuliano, C.R. Patel, P.B. Kale-Pradhan, A Guide to Bacterial Culture Identification and Results Interpretation, P and T, vol. 44, 2019, pp. 192–200.
[33] K. Jia, M. Wei, Y. He, Y. Wang, H. Wei, X. Tao, Characterization of novel exopolysaccharides from Enterococcus hirae WEHI01 and its immunomodulatory activity, Foods 11 (2022), https://doi.org/10.3390/foods11213538.

[34] P.T. Nguyen, T.T. Nguyen, D.C. Bui, P.T. Hong, Q.K. Hoang, H.T. Nguyen, Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications, AIMS Microbiol 6 (2020) 451–469, https://doi.org/10.3934/MICROBIOL.2020027.
[35] P. Body, G. Greif, G. Greifová, M. Sliacká, M. Greifová, Effects of cultivation media and NaCl concentration on the growth kinetics and biogenic amine production of Lactobacillus reuteri, Czech J. Food Sci. 39 (2021) 9–16, https://doi.org/10.17221/190/2020-CJFS.
[36] H. Barzegar, B. Alizadeh Behbahani, F. Falah, Safety, probiotic properties, antimicrobial activity, and technological performance of Lactobacillus strains isolated from Iranian raw milk cheeses, Food Sci. Nutr. 9 (2021) 4094–4107, https://doi.org/10.1002/fsn3.2365.