Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Phenotypic and functional screening of *Weissella ghanensis* A0210 and *Gluconobacter frateurii* MP0210 strains isolated from agro-industrial fruit waste

Triagem fenotípica e funcional de cepas de *Weissella ghanensis* A0210 e *Gluconobacter frateurii* MP0210 isoladas de resíduos agroindustriais de frutas

A. C. S. Sales[a], H. L. A. Furtado[b], R. J. P. Santos[a], R. M. Ferreira[c], C. L. Souza-Silva[d], Y. C. Mendes[a], F. Nitz[b], L. C. Nascimento-Silva[b] and A. Zagmignan[a]*

[a]Universidade CEUMA, Laboratório de Microbiologia Ambiental, São Luís, MA, Brasil
[b]Universidade CEUMA, Departamento de Biociências, Laboratório de Patogenicidade Microbiana, São Luís, MA, Brasil
[c]Laboratório de Análises Clínicas do Maranhão – LACMAR, Departamento de Microbiologia, São Luís, MA, Brasil
[d]Laboratório Cedro, Setor Núcleo de Controle, São Luís, MA, Brasil

Abstract

The growing demand for functional foods has driven research into microorganisms with probiotic potential from sustainable and unconventional sources. In this context, agro-industrial fruit byproducts represent promising matrices for exploring phenotypic screening of bacterial strains with functional relevance. This study aimed to perform an exploratory phenotypic screening of bacterial strains from agro-industrial byproducts of tropical fruits, focusing on probiotic-related and technological traits. Samples were cultured in De Man, Rogosa, and Sharpe (MRS) medium, and isolates were identified using MALDI-TOF MS mass spectrometry. *Weissella ghanensis* A0210 and *Gluconobacter frateurii* MP0210 were evaluated for tolerance to acidic pH, resistance to bile salts, hemolytic activity, biofilm formation, antimicrobial susceptibility, and antagonistic activity against pathogenic bacteria. For *W. ghanensis* A0210, titratable acidity increased from 0.4% to 1.4% within 24 hours, accompanied by a decrease in pH, confirming its fermentative profile. The strain exhibited resistance to amoxicillin, cefaclor, lincomycin, and norfloxacin, while remaining susceptible to erythromycin, rifampicin, and gentamicin. In antagonism assays, *W. ghanensis* A0210 inhibited all tested pathogens, with the highest inhibitory activity observed against *Salmonella sp*. and *Enterococcus faecalis*. In addition, the strain showed survival under resistance to simulated gastrointestinal conditions and moderate biofilm formation; however, its β-hemolytic activity restricts its direct application as a probiotic. In contrast, *G. frateurii* MP0210 showed lower tolerance and adhesion capacity, yet demonstrated potential for fermentative and biotechnological applications. Overall, the findings support the relevance of agro-industrial fruit residues as sources of microbial biodiversity and highlight the importance of initial functional assessments to guide future safety and application-oriented studies.

Keywords: *Weissella ghanensis*, *Gluconobacter frateurii*, lactic acid bacteria, phenotypic characterization, fruit residues.

Resumo

A crescente demanda por alimentos funcionais tem impulsionado a pesquisa de microrganismos com potencial probiótico provenientes de fontes sustentáveis e não convencionais. Nesse contexto, subprodutos agroindustriais de frutas representam matrizes promissoras para a triagem fenotípica de cepas bacterianas com relevância funcional. Este estudo teve como objetivo realizar uma triagem fenotípica exploratória de cepas bacterianas isoladas de subprodutos agroindustriais de frutas tropicais, com foco em características probióticas e tecnológicas. As amostras foram cultivadas em meio MRS (Man, Rogosa e Sharpe) e os isolados foram identificados por espectrometria de massa MALDI-TOF. As cepas *Weissella ghanensis* A0210 e *Gluconobacter frateurii* MP0210 foram avaliadas quanto à tolerância a pH ácido, resistência a sais biliares, atividade hemolítica, formação de biofilme suscetibilidade antimicrobiana e atividade antagonista frente a bactérias patogênicas. Para *W. ghanensis* A0210, a acidez titulável aumentou de 0,4% para 1,4% em 24 horas, acompanhada por uma diminuição do pH, confirmando seu perfil fermentativo. A cepa apresentou resistência à amoxicilina, cefaclor, lincomicina e norfloxacina, enquanto demonstrou suscetibilidade à eritromicina, rifampicina e gentamicina. Nos ensaios de antagonismo, *W. ghanensis* A0210 inibiu todos os patógenos testados, com maior atividade

*e-mail: adrielle004602@ceuma.com.br
Received: December 10, 2025 – Accepted: May 16, 2026
Editor: Takako Matsumura Tundisi

This is an Open Access article distributed under the terms of the Creative Commons Attribution license (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

observada frente a *Salmonella sp*. e *Enterococcus faecalis*. Além disso, a cepa apresentou boa resistência às condições gastrointestinais simuladas e formação moderada de biofilme; no entanto, sua atividade β-hemolítica restringe sua aplicação direta como probiótico. Em contraste, *G. frateurii* MP0210 apresentou menor tolerância e capacidade de adesão, porém demonstrou potencial para aplicações fermentativas e biotecnológicas. De modo geral, os resultados corroboram a relevância dos resíduos agroindustriais de frutas como fontes de biodiversidade microbiana e destacam a importância de avaliações funcionais iniciais para orientar futuros estudos de segurança e aplicação.

Palavras-chave: *Weissella ghanensis*, *Gluconobacter frateurii*, bactérias ácido-lácticas, caracterização fenotípica, resíduos de frutas.

1. Introduction

The growing focus on health and well-being has significantly increased the consumption of functional foods, which, in addition to providing essential nutrients, offer metabolic and physiological benefits such as immune system modulation and the maintenance of intestinal homeostasis (Alves et al., 2024). This scientific interest has stimulated the development of products enriched with bioactive compounds, particularly probiotics (Fekete et al., 2025).

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits, especially within the gastrointestinal tract by strengthening the intestinal barrier, modulating immune responses, inhibiting pathogenic microorganisms, and contributing to digestive processes (Barros et al., 2022). Additionally, these microorganisms have shown potential in preventing metabolic and inflammatory disorders, further increasing their relevance for industrial, nutritional, and clinical applications (Fehily et al., 2024; Vivek et al., 2023).

Probiotic strains must comply with regulatory requirements established by agencies such as the Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), and the Brazilian Health Regulatory Agency (ANVISA), which emphasize safety assessment and proven health benefits (FAO, 2022; Aquilina et al., 2012).

Lactic acid bacteria (LAB), particularly those of the genus *Lactobacillus*, are the main source of probiotics used in foods and supplements. This is attributed to their safety, resistance to digestive conditions, and their role in fermentation processes (Gao et al., 2022). However, the predominance of dairy-derived LAB presents limitations, especially given the growing demand for non-dairy products driven by lactose intolerance, food allergies, and the rise of vegan diets (Huang et al., 2022).

In this context, the isolation of new probiotic strains from alternative sources, such as agro-industrial waste derived from fruit processing, has gained prominence (Carvalhinho, 2024; Romero-Martínez et al., 2025). Although LAB are traditionally prioritized in probiotic prospecting studies, fruit waste constitutes a complex microbial ecosystem that also harbors species not classified as LAB, but that perform relevant biotechnological functions.

Fruit-derived agro-industrial residues harbor diverse microbial communities, including lactic acid bacteria, yeasts, and acetic acid bacteria (Pereira Barbosa et al., 2022). In tropical fruits, high moisture and sugar content favor the growth of fermentative and oxidative microorganisms, promoting microbial interactions and the selection of strains with biotechnological potential (Vuyst and Leroy, 2020; Vermote et al., 2022).

Among these microorganisms, oxidative bacteria stand out, capable of performing bioconversions of sugars and alcohols, resulting in the production of organic acids, antimicrobial metabolites, and compounds of industrial interest (Román-Camacho et al., 2023). These strains can act as functional cultures with complementary applications in fermentation, biopreservation, and the production of value-added products (Yukphan et al., 2020).

These byproducts are rich in bioactive compounds and harbor functional microorganisms that remain largely unexplored. This diversity contributes to the circular economy and drives innovation in the search for probiotics and unconventional technological cultures (Alves et al., 2024). Therefore, the investigation of microorganisms derived from agro-industrial waste integrates scientific development into sustainability, considering that frequently discarded materials may contain microorganisms with significant biotechnological potential. The selection of these strains aims to support the production of more accessible, dairy-free functional foods, with an ecological and innovative approach

Therefore, this study aimed to perform an exploratory phenotypic and functional screening of bacterial strains isolated from agro-industrial fruit waste, focusing on probiotic-related and technological traits relevant to the initial functional assessment.

2. Materials and Methods

2.1. Collection and preparation of fruit waste

Seven types of fruit residues were collected: acerola pulp (*Malpighia emarginata*), apple peel and seeds (*Annona squamosa*), pineapple crown and peel (*Ananas comosus*), Malay apple pit and biomass (*Syzygium malaccense*), sapodilla peel (*Manilkara zapota*), pequi peel and biomass (*Caryocar brasiliense*), and passion fruit peel (*Passiflora edulis*). The samples were obtained from a small agricultural property located in the peri-urban area of the metropolitan region of São Luís, Maranhão, Brazil, between July 2024 and June 2025. All samples were obtained from the same sampling site.

After collection, the residues were transported to the Environmental Microbiology Laboratory at CEUMA University, where they were subjected to a sanitization process using sterile distilled water, followed by air drying at room temperature (25 °C). The samples were then cut into

small pieces (pulp and/or peel) using sterilized instruments. The prepared samples were placed in sterile plastic bags and stored in a freezer at −20 °C until processing.

2.2. Isolation and characterization of lactic acid bacteria isolates

2.2.1. Isolation of probiotics

From each sample, 5 g were homogenized in 45 mL of sterile 0.85% saline solution (NaCl). Subsequently, tenfold serial dilutions (10^0 to 10^{-4}) were prepared, and 100 µL of each dilution were plated onto MRS agar (De Man, Rogosa & Sharpe), which is suitable for the growth of lactic acid bacteria (LAB). Inoculation was performed using a Drigalski spatula, and the plates were incubated under anaerobic conditions (AnaeroGen, Oxoid) at 37 °C for 48 hours. All strains were cultured in MRS medium under standardized conditions to allow comparative screening among isolates, despite known differences in optimal growth requirements among bacterial groups.

After the incubation period, colonies with distinct morphological characteristics (e.g., size, color, and shape) were selected from the three highest dilutions and subcultured on MRS agar using the streak plate technique, followed by incubation at 37 °C for 48 hours under anaerobic conditions. This procedure was adapted from Mulaw et al. (2019). After growth, the isolates were characterized by Gram staining (Gram-positive cocci or rods) and catalase testing (catalase-negative), according to standardized protocols. For preservation, 1,350 µL of culture was mixed with 150 µL of sterile glycerol (final concentration of 10% v/v) and stored at −20 °C as microbiological stock, following the methodology adapted from (Meruvu and Harsa, 2023).

2.2.2. Preliminary identification of isolates by mass spectrometry

The isolates were preliminarily identified using MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) with the Bruker Biotyper 3.1 system (Dušková et al., 2012). This technique enables initial identification at the genus or species level by analyzing the generated protein profiles. The identification results were compared with the Bruker Biotyper 3.1 reference database. Identification scores were interpreted according to the manufacturer's criteria (Jussiaux et al., 2021).

2.3. Safety assessment

2.3.1. Bacterial susceptibility profile

The antimicrobial susceptibility of the strains was evaluated using the disk diffusion method, performed in duplicate. Antibiotics were selected based on their clinical relevance and previous reports of resistance in lactic acid and acetic acid bacteria. The following antibiotic discs were used: erythromycin (15 µg), rifampicin (5 µg), gentamicin (10 µg), lincomycin (2 µg), cefaclor (30 µg), norfloxacin (10 µg), and amoxicillin (10 µg). Bacterial suspensions were adjusted to the 0.5 McFarland standard (1.5×10^8 CFU/mL) and plated onto MRS agar. Plates were incubated at 37 °C for 24 h under anaerobic conditions.

After incubation, antimicrobial activity was assessed by measuring inhibition zone diameters around the discs. The isolates were classified according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024). However, due to the lack of specific breakpoints for *Weissella spp*., the interpretation of inhibition zones was based on criteria adapted for lactic acid bacteria, considering halos ≥20 mm as susceptible, 15-19 mm as intermediate, and ≤14 mm as resistant.

2.3.2. Antagonist activity against pathogens

The strains *Weissella ghanensis* A0210 and *Gluconobacter frateurii* MP0210 were evaluated for antagonistic activity against the pathogenic strains *Staphylococcus aureus* 01, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Salmonella* spp. using the spot-on-lawn method (agar spot overlay).

Briefly, 10 µL of overnight cultures (10^8 CFU/mL) were inoculated as single spots onto the surface of MRS agar plates, followed by incubation under anaerobic conditions at 37 °C for 48 h. After this period, the plates were overlaid with 10 mL of Mueller-Hinton agar previously inoculated with the respective pathogenic strain (10^8 CFU/mL). The plates were then incubated at 37 °C for 24 h, and inhibition zones were measured in millimeters. The experiment was performed in four independent replicates.

2.3.3. Hemolytic activity

To evaluate hemolytic activity, a loopful of each isolate previously grown on MRS agar was inoculated into sterile MRS broth, and the bacterial suspension was standardized using the 0.5 McFarland scale, corresponding to approximately 1.5×10^8 CFU/mL. Then, 10 µL of each suspension was inoculated onto blood agar plates (base agar medium supplemented with 5% defibrinated sheep blood). The plates were incubated aerobically at 37 °C for 48 hours. Experiments were performed in triplicate. The hemolytic activity was assessed qualitatively based on colony morphology and halo formation on blood agar, in accordance with standard microbiological procedures (Asadi et al., 2022). After incubation, hemolytic activity was visually assessed and classified as:

β-hemolysis: presence of clear zones indicating complete lysis around the colonies;

α-hemolysis: presence of greenish zones indicating partial lysis;

γ-hemolysis: absence of lysis zones.

2.4. Assessment of total titratable acidity

Total titratable acidity (TTA) was determined in cultures at 0 h, 12 h, and 24 h of fermentation. The strain was cultured in MRS broth at 37 °C under agitation, and the supernatant was obtained by centrifugation. TTA was quantified according to the potentiometric method described by the Instituto Adolfo Lutz (IAL, 1985). For each analysis, 10 mL of supernatant was diluted in 100 mL of distilled water and titrated with 0.1 M NaOH until reaching pH 8.2-8.4. Acidity was expressed as the equivalent percentage of acid, calculated based on the volume of titrant consumed.

Calculation:

Use the following Formula 1 to express titratable acidity as a percentage of lactic acid (or other acid, substituting the appropriate equivalent factor):

$$\% \text{ acid (as lactic acid)} = \frac{V \times N \times F \times 100}{V_s} \quad (1)$$

where: V = volume of titrant used (mL); N = normality of titrant (eq·L^{-1}, e.g. 0.1 for 0.1 M NaOH); F = factor for the acid expressed as grams of acid per meq (for lactic acid $F = 0.09008$ g·meq^{-1}); V_s = volume of sample titrated (mL) in your protocol this is the original 10 mL

2.5. Assessment of acid pH and bile salt tolerance

2.5.1. Acid pH tolerance

The tolerance to acidic pH was assessed according to the methodology previously described by (Chen et al., 2022), with minor modifications. The strains were first cultured on MRS agar for 48 h at 37 °C. Subsequently, a loopful of growth was transferred to tubes containing sterile MRS broth, and the cell density was adjusted to an optical density (OD) at 630 nm corresponding to approximately 10^6–10^7 CFU/mL.

MRS broths were prepared with pH values adjusted to 2, 3, 5, and 7.2 using 1 M HCl or NaOH. In each well of a microplate, 100 µL of the adjusted medium and 100 µL of the bacterial inoculum were added, and the assay was performed in quadruplicate. After homogenization, 25 µL samples were collected from each well at two time points (0 and 3 h), serially diluted (up to 10^{-6}) in saline solution, and 1.5 µL of each dilution was plated onto MRS agar plates. After aerobic incubation at 37 °C for 48 h, visible colonies were counted, and the results were expressed as the logarithm of colony-forming units per milliliter (log CFU/mL).

2.5.2. Bile salt tolerance

Bile salt tolerance was evaluated following the same protocol described for the acid pH assay, with the only difference being that MRS broth was supplemented with bile salts at final concentrations of 1%, 2%, and 3% (w/v) (Oxgall, Becton, Dickinson and Company, Franklin Lakes, USA). The 96-well plate setup was performed in quadruplicate, with 100 µL of the supplemented medium and 100 µL of the standardized inoculum added to each well. At 0 and 3 hours, 25 µL samples were collected from each well, serially diluted in sterile saline, and 1.5 µL of the dilutions were plated onto MRS agar plates. The plates were incubated at 37 °C for 48 hours for viable colony enumeration. Counts obtained from wells at pH 7.2 without bile salt supplementation were used as controls (Chen et al., 2022).

2.5.3. Biofilm formation assessment

The biofilm-forming ability of the bacterial strains was evaluated using sterile 96-well polystyrene microplates (Corning, New York, NY, USA), following a protocol adapted from (Parolin et al., 2021). Initially, the cultures were subcultured in MRS broth and incubated at 37 °C for 24 hours. A full loop of each culture was then transferred into tubes containing 5 mL of sterile MRS broth, and the optical density was adjusted to 0.5 at 630 nm.

Subsequently, 200 µL of the standardized inoculum was added in quadruplicate to designated wells of the microplate, including negative controls (sterile MRS). The plates were incubated statically at 37 °C for 48 hours. After incubation, the supernatant was carefully removed, and the wells were washed twice with sterile saline solution (0.9% NaCl) to remove non-adherent cells.

For biofilm staining, 200 µL of 0.1% (v/v) crystal violet solution was added to each well, including controls. The plates were then shaken for 30 minutes on a microplate shaker. Excess stain was discarded, and the wells were rinsed with running water. Subsequently, the adhered stain was solubilized with 95% ethanol and homogenized for 30 minutes. The optical density was measured at 630 nm using a microplate spectrophotometer.

2.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Data were expressed as the mean ± standard deviation (SD). Parametric analysis of variance (ANOVA) was applied, followed by Dunnett's and Sidak's multiple comparison tests. In all cases, comparisons were performed using the respective control conditions as reference. A 95% confidence level was adopted, with statistical significance set at $p < 0.05$.

3. Results

3.1. Isolation of lactic acid bacteria from tropical fruit residues

The analysis of tropical fruit residues resulted in the isolation of ten bacterial colonies exhibiting distinct morphological profiles (Figure 1), distributed across different fruit components such as peel, biomass, and pulp. Among them, the colony shown in Figure 1A stands out, displaying a small, circular, transparent morphology with regular edges, whereas Figure 1B presents a larger colony with an opaque, white appearance and more extensive growth on the agar surface.

In addition to morphological differences, significant variations in microbial counts were also observed across the analyzed substrates. The highest estimated CFU/mL count was detected in the pineapple crown (1.0×10^5 CFU/mL). Conversely, substrates such as sapodilla and pequi biomass exhibited markedly low counts (1.0×10^0 CFU/mL). Most isolates were Gram-positive and catalase-negative, consistent with the typical characteristics of lactic acid bacteria (Table 1). A total of ten bacterial isolates were obtained from various tropical fruit byproducts, including acerola pulp, sugar apple peel, pineapple peel and crown, Malay apple biomass, sapodilla peel and biomass, pequi peel and biomass, and passion fruit peel.

3.2. Identification of isolates by MALDI-TOF MS

After screening, the isolates were subjected to identification by MALDI-TOF MS to characterize the microorganisms at the genus or species level. The analysis revealed the presence of different genera and species,

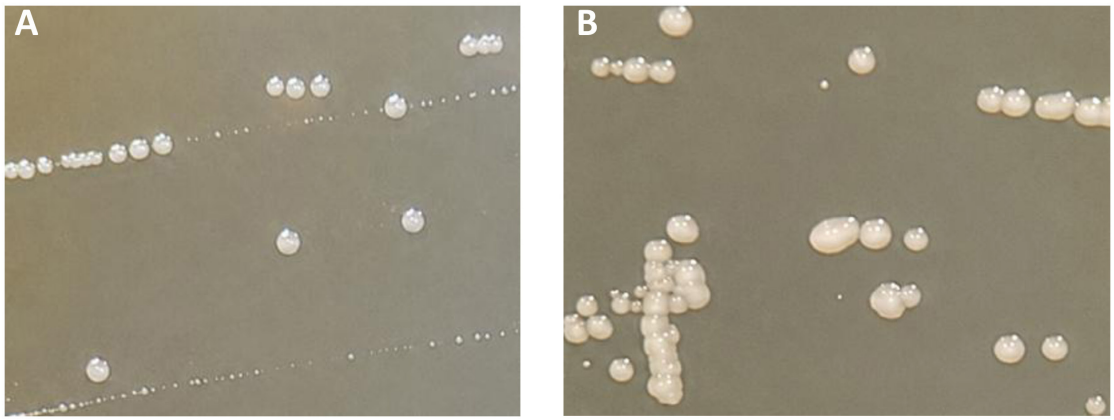


Figure 1. Bacterial colonies obtained by streaking on MRS agar. (A) *Colonies Weissella ghanensis*. (B) *Colonies de Gluconobacter frateurii*.

Table 1. Isolation of bacteria from tropical fruit residues.

Fruit	Part	Dilution	Isolated Colonies	Estimated CFU/mL	Gram	Catalase
Acerola	Pulp	10^1	1	$1{,}0 \times 10^2$	+	-
Sugar apple	Peel	10^1	1	$1{,}0 \times 10^2$	+	+
Pineapple	Peel	10^3	1	$1{,}0 \times 10^4$	+	+
Pineapple	Crown	10^4	1	$1{,}0 \times 10^5$	+	+
Malay apple	Biomass	10^1	1	$1{,}0 \times 10^2$	+	-
Sapodilla	Peel	10^3	2	2,0 × 104	+	+
Sapodilla	Biomass	10^0	2	$2{,}0 \times 10^0$	+	+
Pequi	Peel	10^0	1	$1{,}0 \times 10^0$	+	+
Pequi	Biomass	10^0	1	$1{,}0 \times 10^0$	-	+
Passion fruit	Peel	10^3	1	$1{,}0 \times 10^4$	+	+

The dilution 10^0 corresponds to the original, non-diluted sample (stock solution), while subsequent values represent serial tenfold dilutions used for microbial enumeration. Values of 1.0×10^0 CFU/mL indicate the lowest countable microbial level based on the plating method, corresponding to the detection limit of the assay.

including *Weissella ghanensis*, *Periweissella ghanensis*, *Klebsiella variicola*, *Hanseniaspora opuntiae*, *Staphylococcus* spp., and *Gluconobacter frateurii* (Table 2). Only colonies consistent with the LAB profile (Gram-positive and catalase-negative) were considered for specific probiotic analyses.

Hanseniaspora opuntiae was detected in samples of sugar apple peel, as well as sapodilla biomass and peel. Species of the genus *Staphylococcus*, such as *Staphylococcus warneri* and *Staphylococcus hominis*, were found in isolates obtained from passion fruit peel, pineapple, and sugar apple. Furthermore, *Periweissella ghanensis*, isolated from Malay apple biomass, and *Gluconobacter frateurii*, isolated from a mixture of pequi peel and biomass, were successfully identified. In contrast, two colonies remained unidentified.

3.3. Bacterial susceptibility profile by disk diffusion

The antimicrobial susceptibility profile of *W. ghanensis* A0210 revealed resistance to amoxicillin, cefaclor, lincomycin, and norfloxacin (Table 3). In contrast, the strain was susceptible to erythromycin and rifampicin. Gentamicin exhibited antimicrobial activity, with a mean inhibition halo of 20.0 ± 1.4 mm and was therefore classified as susceptible.

Additionally, the assay was performed with *G. frateurii* MP0210; however, the strain exhibited limited growth under the experimental conditions, preventing the determination of its antimicrobial susceptibility profile.

3.4. Antagonistic activity against pathogenic bacteria

The antimicrobial activity of the strains was evaluated against five indicator pathogens (Table 4). The strain *W. ghanensis* A0210 exhibited inhibitory activity against all tested microorganisms, with the highest mean inhibition zones observed against *Salmonella* sp. (13.00 ± 7.55 mm) and *E. faecalis* (9.75 ± 0.96 mm). Moderate inhibition halos were observed against *E. coli*, *S. aureus*, and *K. pneumoniae*.

In contrast, *G. frateurii* MP0210 exhibited limited growth under the experimental conditions used in the antagonism assay, which prevented the evaluation of its inhibitory potential against the tested pathogens.

Table 2. Identification of isolates by MALDI-TOF MS.

Colony Code	Fruit	Identification by MALDI-TOF MS
A0210	Acerola	*Weissella ghanensis*
CA0210	Pineapple	Not identified
COA0210	Pineapple	*Staphylococcus hominis*
CM0210	Passion fruit	*Staphylococcus warneri*
CAT0210	Custard apple	*Hanseniaspora opuntiae*
CS10210	Sapodilla	Not identified
CS20210	Sapodilla	*Hanseniaspora opuntiae*
BS20210	Sapodilla	*Hanseniaspora opuntiae*
MP0210	Pequi	*Gluconobacter frateurii*
BJ20210	Malay apple	*Periweissella ghanensis*

Confidence levels were interpreted according to the Bruker Biotyper criteria as follows: ≥2.0 indicates species-level identification, 1.7-1.99 indicates genus-level identification, and <1.7 indicates unrel.

Table 3. Antimicrobial susceptibility profile of *Weissella ghanensis* A0210 strains by disk diffusion test.

Antibiotic	Disck (μg)	Halo (mm)	Classification
		W. ghanensis	
Amoxicilina	10	0 ± 0.0	R
Erythromycin	15	40.0 ± 1.4	S
Gentamicin	10	20.0 ± 1.4	S
Rifampicin	5	38.0 ± 0.0	S
Lincomycin	2	9.5 ± 0.7	R
Cefaclor	30	0 ± 0.0	R
Norfloxacina	10	0 ± 0.0	R

Legend: Inhibition halos were measured in duplicate and expressed as mean ± standard deviation. R = resistant, S = sensitive.

Table 4. Evaluation of the antagonistic activity of *W. ghanensis* A0210 and *G. frateurii* MP0210 against pathogenic bacteria based on inhibition halos (mm).

Pathogens	Halos (mm)*	
	***W. ghanensis* A0210**	***G. frateurii* MP0210**
E. coli	9.25 ± 4.57	ND**
K. pneumoniae	5.0 ± 3.37	ND
S. aureus 01	5.90 ± 2.80	ND
E. faecalis	9.75 ± 0.96	ND
Salmonella sp.	13 ± 7.55	ND

*The values represent the Mean ± Standard Deviation (n=4). The inhibition zone was calculated by the difference between the halo diameter and the colony diameter. (*d*) Inhibition zone diameter: (+), weak (*8 ≤ d <13*); (++), medium (*13 ≤ d <18*); (+++), and high (*18 ≤ d*) antibacterial activity; **ND: Not determined due to insufficient growth under the experimental.

3.5. Hemolytic activity

W. ghanensis A0210 exhibited β-hemolytic activity, evidenced by the presence of clear zones of complete erythrocyte lysis surrounding the colonies on blood agar. In contrast, *G. frateurii* MP0210 exhibited limited growth under the experimental conditions, preventing the assessment of its hemolytic activity.

3.6. Total titratable acidity

During fermentation, a progressive decrease in pH, accompanied by an increase in total titratable acidity was observed in the culture of *W. ghanensis* A0210. At the initial time point (0 h), the pH was 6.0 ± 0.1, with an acidity of 0.4 ± 0.1%. After 12 h of fermentation, the pH decreased

to 5.3 ± 0.6 and acidity increased to 0.7 ± 0.3%. By the end of 24 h, the pH reached 4.0 ± 0.07, while titratable acidity rose to 1.4 ± 0.1%, indicating intense production of organic acids throughout the process (Table 5).

3.7. Tolerance of bacterial isolates to different acidic pH values

The evaluation of acid pH tolerance was performed to determine the ability of bacterial strains to survive under conditions similar to those of the gastrointestinal tract, particularly the gastric environment. *W. ghanensis* A0210 exhibited significant survival at pH 3.0 ($p = 0.0094$), 5.0 ($p = 0.0005$) and 7.2 ($p = 0.0004$) during the tested period, with an increase in viability observed at all these pH levels.

In contrast, *G. frateurii* MP0210 did not survive after 3 hours of exposure at pH 2.0, indicating marked sensitivity to highly acidic conditions. At pH 3.0, 5.0, and 7.2, no significant variation was detected, and viability remained relatively stable over time (Figure 2).

3.8. Survival capacity of isolates in the presence of bile salts

Bile salt tolerance was evaluated as a relevant criterion for selecting microorganisms with desirable probiotic properties, as it simulates the adverse conditions of the human small intestine. The strain *W. ghanensis* A0210 maintained stable growth across all tested concentrations, indicating high resistance to the action of bile salts. In contrast, *G. frateurii* MP0210 exhibited growth only under the control condition (0% bile salts) after 3 hours. At concentrations of 1% and 2%, growth remained stable over time; however, at 3% bile salts, the strain was unable to maintain viability, suggesting limited tolerance to simulated intestinal conditions (Figure 3).

3.9. Evaluation of the biofilm-forming ability of the isolated strains

The biofilm-forming ability of the isolated strains was assessed as an indicator of adhesion and persistence on surfaces, characteristics relevant to microorganisms with functional potential for use in functional foods. The strain *W. ghanensis* A0210 exhibited moderate biofilm formation, with values significantly higher than the negative control ($p = 0.0003$), indicating substantial adhesion capacity. In contrast, *G. frateurii* MP0210 displayed weak biofilm formation, with no significant difference compared to the control (Figure 4).

4. Discussion

4.1. Morphological characterization and microbial diversity

This study represents an exploratory phenotypic and functional screening of bacterial strains isolated from agro-industrial fruit waste, focusing on functional responses and safety-related traits assessed during early-stage strain selection rather than definitive probiotic validation.

Overall, the phenotypic profile observed among the isolates, marked by the predominance of Gram-positive and catalase-negative bacteria, reinforces the presence of LAB in the analyzed residues. This pattern is widely described in the literature, particularly in studies investigating

Table 5. Determination of total titratable acidity of *Weissella ghanensis* A0210. Values are expressed as mean ± standard deviation (n = 4).

Fermentation time (hours)	*pH*	*Titratable Acidity*
0	6.0 ± 0.1	0.4 ± 0.1
12	5.3 ± 0.6	0.7 ± 0.3
24	4.0 ± 0.07	1.4 ± 0.1

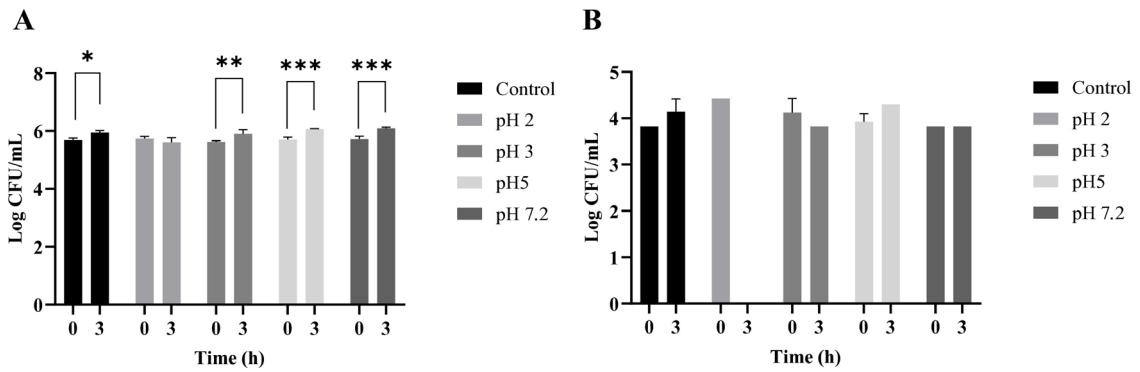


Figure 2. Acid pH tolerance assay in bacterial strains. (A) Growth of *Weissella ghanensis* A0210 at different pH levels; (B) Growth of *Gluconobacter frateurii* MP0210 at different pH levels. Results are expressed as mean ± SD (n = 4). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test multiple comparison test, using the control group as reference. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to the control (0% bile salts).

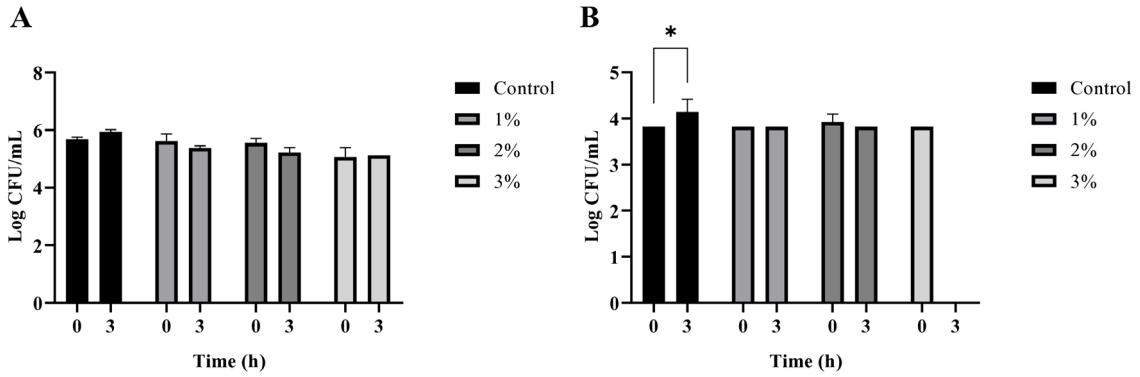


Figure 3. Bile salt tolerance assay in bacterial strains. (A) Growth of the *Weissella ghanensis* A0210 strain under different bile salt concentrations; (B) Growth of the *Gluconobacter frateurii* MP0210 strain under different bile salt concentrations. Results are expressed as mean ± SD (n = 4). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test multiple comparison test, using the control group as reference. *indicates statistically significant difference compared to the control (0% bile salts) ($p < 0.05$).

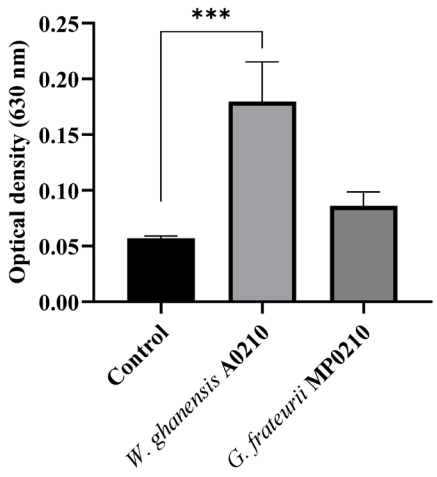


Figure 4. Biofilm formation by bacterial strains. Biofilm formation by *Weissella ghanensis* A0210 and *Gluconobacter frateurii* MP0210. Results are expressed as mean ± SD (n = 4). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, using the control as reference. ***indicates statistically significant difference compared to the control ($p < 0.001$). Biofilm formation was classified according to optical density (OD) values as follows: OD ≤ ODc (non-producer), ODc < OD ≤ 2×ODc (weak), 2×ODc < OD ≤ 4×ODc (moderate), and OD > 4×ODc (strong), where ODc represents the optical density of the negative control.

microorganisms associated with tropical fruits, probiotic formulations, and spontaneous fermentation environments (Alves et al., 2024). The absence of catalase, a common characteristic of LAB, supports this interpretation.

However, the detection of catalase-positive isolates reveals a relevant microbial diversity, possibly influenced by the food matrix and the physicochemical characteristics of the different residues, as previously reported in fermented tropical fruits (Fusco et al., 2023). Thus, in addition to confirming the presence of LAB, our results demonstrate that fruit residues constitute diverse and complex microbial niches.

4.2. MALDI-TOF MS identification

Continuing the characterization, MALDI-TOF MS analysis allowed the identification of only two colonies as LAB: *W. ghanensis* A0210 and *Periweissella ghanensis* BJ0210. The occurrence of these species is consistent with studies reporting their recurrence in tropical fruits and their high probiotic potential, including tolerance to acidity and bile salts, production of exopolysaccharides (EPS), and antimicrobial activity (Fusco et al., 2023; Rodrigues et al., 2021).

In addition, the detection of *Gluconobacter frateurii*, although not belonging to the LAB group, reinforces the biotechnological relevance of this species, which is frequently associated with organic acid production and aerobic biotransformations, as described by Adachi et al. (2020). The consistent presence of *Hanseniaspora opuntiae* in the samples also reflects its known role in spontaneous fermentations, contributing to the formation of volatile compounds and characteristic sensory profiles (Macêdo, 2023).

On the other hand, the identification of *Staphylococcus warneri* and *Staphylococcus hominis* may reflect contamination due to handling or environmental exposure, since these species are part of the human microbiota (Parlet et al., 2019). Although their presence does not necessarily represent an immediate risk, it highlights the importance of safety monitoring in food-related studies.

Furthermore, the non-identification of two colonies highlights limitations of the MALDI-TOF technique, particularly when the spectra generated do not find adequate correspondence in the database. This technique provides preliminary identification, with 16S rRNA sequencing or other markers such as *gyrB* considered the gold standard (Elbehiry and Abalkhail, 2025). Due to technical limitations, this procedure could not be performed, and therefore, our identifications should be regarded as preliminary (Bielen et al., 2024).

4.3. Bacterial susceptibility profile

Due to the "Generally Recognized as Safe" (GRAS) status granted by the Food and Drug Administration

(FDA), lactic acid bacteria (LAB) are widely used in the food industry. However, international guidelines, such as those established by the European Food Safety Authority (EFSA), recommend that newly isolated strains or those with biotechnological potential undergo rigorous safety screening before application (Haryani et al., 2023).

In this context, *W. ghanensis* A0210 exhibited a susceptibility profile consistent with what is typically reported for lactic acid bacteria. Resistance to amoxicillin, cefaclor, lincomycin, and norfloxacin is in agreement with recent studies describing intrinsic resistance in *Weissella* species, generally associated with structural features of the cell wall and reduced affinity for β-lactam antibiotics rather than the presence of transferable resistance genes, thereby lowering the risk of horizontal gene dissemination (Yilmaz et al., 2025).

On the other hand, the high susceptibility to erythromycin and rifampicin observed in this study is consistent with findings reported in the literature. Variable susceptibility to erythromycin among *Weissella* isolates has been described, with approximately 46% of the strains classified as susceptible, highlighting intra-species heterogeneity (Jamwal et al., 2024)

Gentamicin susceptibility was observed in this study, contrasting with previous findings that reported a low susceptibility rate (14.7%) among isolates from fermented food sources (Duche et al., 2023). This behavior is often attributed to low membrane permeability and limited intracellular transport of aminoglycosides (Haryani et al., 2023). Overall, the results confirm the heterogeneous susceptibility profile observed within the *Weissella* genus, characterized by intrinsic resistance to certain antibiotics and susceptibility to others.

Additionally, *G. frateurii* exhibited limited growth under the experimental conditions, which may be attributed to its strictly aerobic metabolism, potentially constrained by the use of MRS medium under anaerobic conditions. These findings underscore the importance of strain-specific evaluation, as such variability may directly influence the safe and effective application in biotechnological contexts.

4.4. Assessment of antagonistic activity

The results demonstrate that *W. ghanensis* A0210 exhibited a broad spectrum of inhibitory activity, affecting both Gram-positive bacteria (*S. aureus* and *E. faecalis*) and Gram-negative bacteria (*Salmonella* sp. and *E. coli*). The highest activity was observed against *Salmonella* sp. (13.00 ± 7.55 mm), which is consistent with recent studies highlighting the *Weissella* genus as a promising candidate for next-generation probiotics due to its ability to produce organic acids and bacteriocins, such as weissellicin (Bochgire et al., 2026).

Studies have highlighted that *Weissella* species demonstrate the ability to modulate the microbiota and inhibit intestinal pathogens (Marques et al., 2024; Singh et al., 2024). Our results are consistent with trends reported in the literature, in which *Weissella* strains isolated from fermented foods exhibited significant inhibition against *E. coli* and *Salmonella* (Liu et al., 2025). This suggests that secondary metabolites, such as lactate and acetate, may play a crucial role in destabilizing the outer membrane of these pathogens (Ji et al., 2023).

The limited growth of *G. frateurii* under the tested conditions may be attributed to its specific nutritional and metabolic requirements. Acetic acid bacteria (AAB) are obligate aerobes and may be inhibited in culture media designed for lactic acid bacteria, such as MRS, due to the presence of sodium acetate and the available carbon sources (Han et al., 2024). This condition may explain the absence of inhibition halos for this strain, highlighting the need for specific media in future evaluations.

4.5. Hemolytic activity assessment

Regarding safety aspects, the β-hemolytic activity observed in *W. ghanensis* A0210 represents a limiting factor for its application as a probiotic or starter culture. According to FAO/WHO and EFSA guidelines, only non-hemolytic microorganisms are considered safe (Icer et al., 2023; Fusco et al., 2023). Although *Weissella* species are generally classified as non-pathogenic, recent studies indicate phenotypic variation, including strains exhibiting partial or complete hemolysis (Maione et al., 2024). While most isolates exhibit γ-hemolysis, some strains have been reported to display α- or β-hemolytic activity, highlighting their strain-dependent behavior (Ma et al., 2025; Fhoula et al., 2022). In addition, comprehensive safety evaluations, including phenotypic and genomic approaches, are recommended to ensure the absence of virulence factors (Fanelli et al., 2023).

Despite this, hemolysis does not entirely preclude its biotechnological application. *Weissella* strains may play important technological roles, such as exopolysaccharide (EPS) or bacteriocin production, particularly in fermented foods where there is no direct contact with mucosal surfaces (Kavitake et al., 2020; Singh et al., 2024). Indeed, safe applications of *Weissella* strains have been demonstrated in silage, expanding the functional potential of this genus even when strains are unsuitable for direct human consumption (Zhao et al., 2024).

In the case of *G. frateurii* MP0210, the limited growth observed on blood agar and across several assays should be interpreted by considering both the experimental conditions applied and its physiological characteristics. *Gluconobacter* species are strictly aerobic microorganisms with specific nutritional requirements, and their growth may be impaired under conditions optimized for lactic acid bacteria (Yukphan et al., 2020). In the present study, standardized conditions based on MRS medium and incubation parameters were used to enable comparative screening of all isolates; however, such conditions may not be optimal for acetic acid bacteria, potentially underestimating the physiological performance of *G. frateurii.*

Despite this limitation, the reduced growth on blood agar aligns with its incomplete oxidative metabolism and high nutritional requirements, as well as the lack of hemolysins, as described by Yukphan et al. (2020). Species of the genus *Gluconobacter* are widely used in both commercial and experimental applications for the production of gluconic acid and its derivatives, as well as in oxidative biotransformations, due to the incomplete

oxidation of sugars and alcohols, which generates high-value compounds for the food and pharmaceutical industries (Ma et al., 2025; Román-Camacho et al., 2023).

Beyond these applications, *Gluconobacter* species have also been explored in the production of keto-gluconates and other value-added compounds, as well as in the bioconversion of renewable substrates. Their metabolic versatility highlights their potential in the valorization of agro-industrial residues and reinforces their relevance in sustainable biotechnological processes (Deppenmeier and Ehrenreich, 2009; Hidayatullah et al., 2025).

Moreover, recent evidence indicates that *Gluconobacter frateurii* can produce EPS and other metabolites of functional interest in fruit fermentations, highlighting its technological potential even though it is not a candidate for probiotic use (Ning et al., 2024; Lam et al., 2024). Thus, although it is not a LAB, its screening is relevant for understanding the autochthonous microbiota of fruit residues and for identifying strains with potential application in industrial bioconversion and biopreservation processes.

Overall, these findings reinforce the rationale for including non-LAB microorganisms in the initial screening, as they may exhibit valuable technological potential, and emphasize the importance of detailed phenotypic evaluations to guide the selection of strains suitable for different functional applications.

4.6. Titratable acidity

The progressive reduction in pH observed during the fermentation by *W. ghanensis* A0210 is consistent with the expected behavior of lactic acid bacteria. In the present study, the pH decreased from 6.0 to 4.0 after 24 hours, accompanied by an increase in titratable acidity from 0.4% to 1.4%. *Weissella* species isolated from cocoa fermentations also exhibited strong acidifying capacity, rapidly reducing pH during the first 24 hours of growth, which confirms the fermentative profile observed in this work (De Bruyne et al., 2008).

The increase in titratable acidity observed in this study is also consistent with previous reports, in which acidity increments between 0.7% and 0.8% were observed within 24 hours using LAB strains in plant-based media. Although the values reported are slightly lower than those obtained in the present study (1.4%), differences in the culture medium, carbohydrate source, and strain can substantially influence the final amount of acid produced, which may explain the higher acidity observed for our strain (Manhokwe et al., 2025).

Therefore, according to the literature, the results obtained for *W. ghanensis* A0210 are consistent with the expected behavior of the genus. The marked decrease in pH and the increase in titratable acidity confirm its high fermentative activity and indicate its technological potential as a starter culture in controlled fermentations.

4.7. Acid pH tolerance

Regarding the assessment of acid pH tolerance, the results showed that *W. ghanensis* A0210 exhibits a characteristic profile of microorganisms adapted to acidic environments, an essential trait for potential probiotic use. Previous studies have demonstrated that *Weissella* species activate mechanisms such as proton pumps, acid stress proteins, and membrane remodeling to survive low pH conditions (Jeong et al., 2023). In this context, the survival observed at pH 3.0 confirms the strain's potential to withstand gastrointestinal conditions.

Jeong et al. (2023) reported that *Weissella* strains adapted to acidic environments generally exhibit alterations in membrane lipid composition and increased expression of genes associated with stress resistance. Based on these observations, the tolerance of *W. ghanensis* A0210 to acidic pH suggests that its ability to survive gastric passage is a relevant trait for potential probiotic application. This characteristic may also support its application in fermented food formulations that naturally display acidity.

In contrast, *G. frateurii* exhibited low tolerance to acidic pH, a behavior expected due to its physiology and membrane characteristics, which are more adapted to mildly acidic to neutral conditions (Oliveira et al., 2010; Jackson et al., 2019). Therefore, although it is not suitable for gastrointestinal applications, this species remains relevant for technological processes associated with acetic fermentation, such as vinegar production and other aerobic fermentations (Román-Camacho et al., 2023).

The evaluation of bile salt tolerance also reinforces the probiotic potential of *W. ghanensis* A0210. Although *Weissella* strains show considerable variation in resistance, studies indicate that many tolerate physiological levels ranging from 0.3% to 1.0% (Patrone et al., 2020; Ahmed et al., 2022; Liu et al., 2025).

For example, Patrone et al. (2020) reported that *Weissella cibaria* SP7 and SP19, isolated from cattle in Kuwait, tolerated 0.3% bile salts and acidic pH, showing performance comparable to the standard probiotic *Lacticaseibacillus rhamnosus* GG. S

The present study is consistent with the results reported by Liu et al. (2025) who described that *Weissella confusa* SY628 remained viable even after three hours of exposure to bile salts at concentrations up to 1.5%. These findings highlight that the stability demonstrated by *W. ghanensis* A0210 at all tested concentrations aligns with the profile of strains previously recognized as promising probiotic candidates (Bentahar et al., 2024).

In contrast, *G. frateurii* MP0210 showed low survival at 3% bile salts. This response is expected, as Gram-negative bacteria tend to be more susceptible to the detergent action of bile salts, especially when lacking mechanisms such as bile salt hydrolase (BSH) activity (Dong et al., 2025; Yukphan et al., 2020). Although this limitation prevents its use as a probiotic, the species still holds biotechnological relevance. Its oxidative metabolism, together with its ability to convert sugars into organic acids, supports applications such as vinegar production and the bioconversion of plant residues (Adachi et al., 2020).

4.8. Biofilm formation

Finally, the biofilm-forming ability further supports the functional characteristics of *W. ghanensis* A0210. The moderate biofilm formation observed is consistent with studies showing that *Weissella* relies on exopolysaccharides

(EPS), cell aggregation, and signaling mechanisms to adhere to surfaces and establish an extracellular matrix (Kang et al., 2023; Fusco et al., 2023).

Biofilm formation is a dynamic and time-dependent process. However, several in vitro studies assess biofilm formation at a single time point using microtiter plate assays as an initial approach to compare adhesion capacity among strains (Parolin et al., 2021). In this context, the evaluation performed at 48 h allowed the identification of distinct adhesion profiles among the tested strains and should be interpreted as a preliminary indicator of biofilm-forming potential.

In contrast, *G. frateurii* MP0210 exhibited low biofilm-forming capacity, possibly due to the absence of quorum-sensing (QS) systems or reduced EPS production features commonly reported among certain Gram-negative bacteria (Zhao et al., 2023; Chiang et al., 2021). Nevertheless, its metabolic versatility suggests potential for environmental and industrial applications, including bioconversion and biodegradation of plant residues (Hidayatullah et al., 2025).

Although several functional traits commonly associated with probiotic screening were evaluated, the present study was not intended to provide a comprehensive validation of probiotic potential. The absence of reference probiotic strains as positive controls, together with the lack of sequential gastrointestinal simulation, multi-time-point biofilm analysis, and expanded safety assessment, reflects the exploratory nature of this screening approach. Additionally, the use of standardized culture conditions may not have been optimal for all isolates, particularly for non-LAB microorganisms such as *Gluconobacter frateurii*. Therefore, the results should be interpreted as preliminary, highlighting the need for further studies to better define the functional and safety profiles of the evaluated strains.

Despite these limitations, the results highlight agroindustrial fruit residues as important reservoirs of microbial diversity and reinforce the relevance of exploratory phenotypic screening as an initial step for identifying strains with functional and technological potential. This approach supports future applicationoriented and safetyfocused research.

5. Conclusion

This study demonstrates that agroindustrial fruit residues represent valuable reservoirs of microbial diversity and highlights the relevance of exploratory phenotypic and functional screening as an initial step for the identification of bacterial strains with functional and biotechnological potential. *Weissella ghanensis* A0210 and *Gluconobacter frateurii* MP0210 exhibited distinct phenotypic and functional responses, reinforcing the importance of earlystage screening for both application prospects and safetyrelated constraints. Although the results do not support definitive probiotic classification, the findings contribute to the understanding of microbial resources associated with fruit waste and support future research focused on safety validation, regulatory assessment, and applicationoriented development.

Acknowledgements

We are especially grateful to all the researchers who collaborated. We also thank FAPEMA for the financial support provided under Call 38/2022 – FAPEMA Process ACT-02510/2023. CAPES, FAPEMA and CNPq.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the
corresponding author upon reasonable request.

References

ADACHI, O., NGUYEN, T.M., HOURS, R.A., KATAOKA, N., MATSUSHITA, K., AKAKABE, Y. and YAKUSHI, T., 2020. 5-Keto-D-fructose production from sugar alcohol by isolated wild strain *Gluconobacter frateurii* CHM 43. *Bioscience, Biotechnology, and Biochemistry*, vol. 84, no. 8, pp. 1745-1747. https://doi.org/10.1080/09168451.2020.1767500. PMid:32427050.

AHMED, S., SINGH, S., SINGH, V., ROBERTS, K.D., ZAIDI, A. and RODRIGUEZ-PALACIOS, A., 2022. The Weissella Genus: clinically treatable bacteria with antimicrobial/probiotic effects on inflammation and cancer. *Microorganisms*, vol. 10, no. 12, pp. 2427. https://doi.org/10.3390/microorganisms10122427. PMid:36557680.

ALVES, G.C., SILVA, D., OLIVEIRA, R., MARTIN, R., TERRA, R., SOUTO FILHO, S., SOUZA, C. and MIRANDA, L., 2024 [viewed 26 April 2026]. Ação funcional das catequinas presentes no chá verde: uma revisão narrativa. *Revista Científica da UNIFENAS* [online], vol. 6, no. 1, pp. 6-10. Available from: https://revistas.unifenas.br/index.php/revistaunifenas/article/view/912

AQUILINA, G., BORIES, G., CHESSON, A., COCCONCELLI, P.S., DE KNECHT, J., DIERICK, N.A., GRALAK, M.A., GROPP, J., HALLE, I., HOGSTRAND, C., LENG, L., LÓPEZ PUENTE, S., LUNDEBYE HALDORSEN, A.-K., MANTOVANI, A., MARTELLI, G., MÉZES, M., RENSHAW, D., SAARELA, M., SEJRSEN, K. and WESTENDORF, J., 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*, vol. 10, no. 6, pp. 2740. https://doi.org/10.2903/j.efsa.2012.2740.

ASADI, A., LOHRASBI, V., ABDI, M., MIRKALANTARI, S., ESGHAEI, M., KASHANIAN, M., OSHAGHI, M. and TALEBI, M., 2022. The probiotic properties and potential of vaginal Lactobacillus spp. isolated from healthy women against some vaginal pathogens. *Letters in Applied Microbiology*, vol. 74, no. 5, pp. 752-764. https://doi.org/10.1111/lam.13660. PMid:35080271.

BARROS, D.M., SILVA, J.H.L., LEITE, A.R.F., FREITAS, T.S., SILVA, M.M., COSTA, M.P., DINIZ, M.A., FERREIRA, S.A.O., MELO, N.S., RIBEIRO, A.N.S., ROCHA, T.A., BARBOSA, T.S.L., MELO, C.C., MACHADO, E.K.S., SANTOS, J.A.S., SANTOS, J.M.S., PONTES, A.K.A., SENA, B.S., SOUTO, B.O., OLIVEIRA, F.H.P.C. and MOURA, D.F., 2022. A importância do consumo de probióticos e prebióticos para a saúde: uma revisão. *Brazilian Applied Science Review*, vol. 6, no. 1, pp. 54-63. https://doi.org/10.34115/basrv6n1-004.

BENTAHAR, M.C., BENABDELMOUMENE, D., ROBERT, V., DAHMOUNI, S., QADI, W.S.M., BENGHARBI, Z., LANGELLA, P., BENBOUZIANE, B., AL-OLAYAN, E., DAWOUD, E.A.D. and MEDIANI, A., 2024. Evaluation of probiotic potential and functional properties of *Lactobacillus strains* isolated from Dhan, traditional Algerian Goat Milk Butter. *Foods*, vol. 13, no. 23, pp. 3781. https://doi.org/10.3390/foods13233781. PMid:39682853.

BIELEN, A., BABIĆ, I., VUK SURJAN, M., KAZAZIĆ, S., ŠIMATOVIĆ, A., LAJTNER, J., UDIKOVIĆ-KOLIĆ, N., MESIĆ, Z. and HUDINA, S., 2024. Comparison of MALDI-TOF mass spectrometry and 16S rDNA sequencing for identification of environmental bacteria: a case study of cave mussel-associated culturable microorganisms. *Environmental Science and Pollution Research International*, vol. 31, no. 14, pp. 21752-21764. https://doi.org/10.1007/s11356-024-32537-1. PMid:38393570.

BOCHGIRE, M., PINGALE, S., KAUL-GHANEKAR, R., ANGADI, K. and JADHAV, A., 2026. Weissella: A multifaceted genus making the way for next-gen biotherapeutics? *Antonie van Leeuwenhoek*, vol. 119, no. 5, pp. 96. https://doi.org/10.1007/s10482-026-02307-9. PMid:41979733.

CARVALHINHO, C.F.M.L., 2024 [viewed 26 April 2026]. *Produção de invertase de Bacillus tequilensis isolada do fruto pupunha: propriedades bioquímicas e produção de fruto-oligossacarídeos* [online]. Palmas: Universidade Federal do Tocantins, 117 p. Dissertação de Mestrado em Ciência e Tecnologia de Alimentos. Available from: http://repositorio.uft.edu.br/handle/11612/6939

CHEN, Z., LENG, X., ZHOU, F., SHEN, W., ZHANG, H., YU, Q., MENG, X., FAN, H. and QIN, M., 2022. Screening and identification of probiotic Lactobacilli from the infant gut microbiota to alleviate lead toxicity. *Probiotics and Antimicrobial Proteins*, vol. 15, no. 4, pp. 821-831. https://doi.org/10.1007/s12602-021-09895-0. PMid:35060081.

CHIANG, I.L., WANG, Y., FUJII, S., MUEGGE, B.D., LU, Q., TARR, P.I. and STAPPENBECK, T.S., 2021. Biofilm formation and virulence of *Shigella flexneri* are modulated by pH of gastrointestinal tract. *Infection and Immunity*, vol. 89, no. 11, e0038721. https://doi.org/10.1128/IAI.00387-21. PMid:34424745.

CLINICAL AND LABORATORY STANDARDS INSTITUTE – CLSI 2024. *M100: performance standards for antimicrobial susceptibility testing*. Wayne: CLSI.

DE BRUYNE, K., CAMU, N., LEFEBVRE, K., DE VUYST, L. and VANDAMME, P., 2008. Weissella ghanensis sp. nov., isolated from a Ghanaian cocoa fermentation. *International Journal of Systematic and Evolutionary Microbiology*, vol. 58, no. 12, pp. 2721-2725. https://doi.org/10.1099/ijs.0.65853-0. PMid:19060047.

DEPPENMEIER, U. and EHRENREICH, A., 2009. Physiology of acetic acid bacteria in light of the genome sequence of Gluconobacter oxydans. *Journal of Molecular Microbiology and Biotechnology*, vol. 16, no. 1–2, pp. 69-80. https://doi.org/10.1159/000142895. PMid:18957863.

DONG, Z., YANG, S., TANG, C., LI, D., KAN, Y. and YAO, L., 2025. New insights into microbial bile salt hydrolases: from physiological roles to potential applications. *Frontiers in Microbiology*, vol. 16, pp. 1513541. https://doi.org/10.3389/fmicb.2025.1513541. PMid:40012771.

DUCHE, R.T., SINGH, A., WANDHARE, A.G., SANGWAN, V., SIHAG, M.K., NWAGU, T.N.T., PANWAR, H. and EZEOGU, L.I., 2023. Antibiotic resistance in potential probiotic lactic acid bacteria of fermented foods and human origin from Nigeria. *BMC Microbiology*, vol. 23, no. 1, pp. 142. https://doi.org/10.1186/s12866-023-02883-0. PMid:37208603.

DUŠKOVÁ, M., ŠEDO, O., KŠICOVÁ, K., ZDRÁHAL, Z. and KARPÍŠKOVÁ, R., 2012. Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. *International Journal of Food Microbiology*, vol. 159, no. 2, pp. 107-114. https://doi.org/10.1016/j.ijfoodmicro.2012.07.029. PMid:23072695.

ELBEHIRY, A. and ABALKHAIL, A., 2025. Spectral precision: recent advances in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for pathogen detection and resistance profiling. *Microorganisms*, vol. 13, no. 7, pp. 1473. https://doi.org/10.3390/microorganisms13071473. PMid:40731982.

FANELLI, F., MONTEMURRO, M., VERNI, M., GARBETTA, A., BAVARO, A.R., CHIEFFI, D., CHO, G.S., FRANZ, C.M.A.P., RIZZELLO, C.G. and FUSCO, V., 2023. Probiotic potential and safety assessment of type strains of Weissella and Periweissella Species. *Microbiology Spectrum*, vol. 11, no. 2, e0304722. https://doi.org/10.1128/spectrum.03047-22. PMid:36847557.

FEHILY, S.R., BASNAYAKE, C., WRIGHT, E.K., YAO, C.K., GODSELL, J., GIBSON, P.R. and KAMM, M.A., 2024. Probiotics: are they beneficial? *Internal Medicine Journal*, vol. 54, no. 6, pp. 861-870. https://doi.org/10.1111/imj.16388. PMid:38717051.

FEKETE, M., LEHOCZKI, A., KRYCZYK-POPRAWA, A., ZÁBÓ, V., VARGA, J.T., BÁLINT, M., FAZEKAS-PONGOR, V., CSÍPŐ, T., RZĄSA-DURAN, E. and VARGA, P., 2025. Functional foods in modern nutrition science: mechanisms, evidence, and public health implications. *Nutrients*, vol. 17, no. 13, pp. 2153. https://doi.org/10.3390/nu17132153. PMid:40647258.

FHOULA, I., BOUMAIZA, M., TAYH, G., REHAIEM, A., KLIBI, N. and OUZARI, I.H., 2022. Antimicrobial activity and safety features assessment of *Weissella* spp. from environmental sources. *Food Science & Nutrition*, vol. 10, no. 9, pp. 2896-2910. https://doi.org/10.1002/fsn3.2885. PMid:36171785.

FOOD AND AGRICULTURE ORGANIZATION – FAO. World Health Organization – WHO, 2022 [viewed 25 April 2026]. *Guidelines for the evaluation of probiotics in food* [online]. Paris. Available from: https://isappscience.org/wp-content/uploads/2019/04/probiotic_guidelines.pdf

FUSCO, V., CHIEFFI, D., FANELLI, F., MONTEMURRO, M., RIZZELLO, C.G. and FRANZ, C.M.A.P., 2023. The Weissella and Periweissella genera: up-to-date taxonomy, ecology, safety, biotechnological, and probiotic potential. *Frontiers in Microbiology*, vol. 14, pp. 1289937. https://doi.org/10.3389/fmicb.2023.1289937. PMid:38169702.

GAO, H., LI, X., CHEN, X., HAI, D., WEI, C., ZHANG, L. and LI, P., 2022. The Functional roles of *Lactobacillus acidophilus* in different physiological and pathological processes. *Journal of Microbiology and Biotechnology*, vol. 32, no. 10, pp. 1226-1233. https://doi.org/10.4014/jmb.2205.05041. PMid:36196014.

HAN, D., YANG, Y., GUO, Z., DAI, S., JIANG, M., ZHU, Y., WANG, Y., YU, Z., WANG, K., RONG, C. and YU, Y., 2024. A review on the interaction of acetic acid bacteria and microbes in food fermentation: a microbial ecology perspective. *Foods*, vol. 13, no. 16, pp. 2534. https://doi.org/10.3390/foods13162534. PMid:39200461.

HARYANI, Y., HALID, N.A., GUAT, G.S., NOR-KHAIZURA, M.A.R., HATTA, M.A.M., SABRI, S., RADU, S. and HASAN, H., 2023. High prevalence of multiple antibiotic resistance in fermented food-associated lactic acid bacteria in Malaysia. *Food Control*, vol. 147, pp. 109558. https://doi.org/10.1016/j.foodcont.2022.109558.

HIDAYATULLAH, I.M., MARITZA, D.A., YOHDA, M., SAHLAN, M., KUSMAYADI, A., LEONG, Y.K. and HERMANSYAH, H., 2025. Fermentative synthesis of gluconic and xylonic acids from hydrolyzed palm fronds using *Gluconobacter oxydans*. *Bioengineering*, vol. 12, no. 8, pp. 801. https://doi.org/10.3390/bioengineering12080801. PMid:40868314.

HUANG, R., WU, F., ZHOU, Q., WEI, W., YUE, J., XIAO, B. and LUO, Z., 2022. Lactobacillus and intestinal diseases: mechanisms of action and clinical applications. *Microbiological Research*, vol. 260, pp. 127019. https://doi.org/10.1016/j.micres.2022.127019. PMid:35421680.

ICER, M.A., ÖZBAY, S., AĞAGÜNDÜZ, D., KELLE, B., BARTKIENE, E., ROCHA, J.M.F. and OZOGUL, F., 2023. The Impacts of acidophilic lactic acid bacteria on food and human health: a review of the current knowledge. *Foods*, vol. 12, no. 15, pp. 2965. https://doi.org/10.3390/foods12152965. PMid:37569234.

INSTITUTO ADOLFO LUTZ – IAL, 1985 [viewed 26 April 2026]. *Padrões analíticos do Instituto Adolfo Lutz: métodos químicos e físicos para análise de alimentos* [online]. São Paulo: IAL. Available from: https://wp.ufpel.edu.br/nutricaobromatologia/files/2013/07/NormasADOLFOLUTZ.pdf

JACKSON, E., RIPOLL, M. and BETANCOR, L., 2019. Efficient glycerol transformation by resting *Gluconobacter* cells. *MicrobiologyOpen*, vol. 8, no. 12, e926. https://doi.org/10.1002/mbo3.926. PMid:31532065.

JAMWAL, A., VARGHESE, G., SARAWAT, D., TEJAN, N., PATEL, S.S. and SAHU, C., 2024. Characterization of *Weissella* species during a 3-year observational study: an emerging threat. *The American Journal of Tropical Medicine and Hygiene*, vol. 110, no. 5, pp. 1006-1009. https://doi.org/10.4269/ajtmh.23-0713. PMid:38507800.

JEONG, S., KWON, A., JEONG, H. and PARK, Y.S., 2023. Synergistic immunostimulatory activities of probiotic strains, *Leuconostoc lactis* and *Weissella cibaria*, and the prebiotic oligosaccharides they produce. *Microorganisms*, vol. 11, no. 5, pp. 1354. https://doi.org/10.3390/microorganisms11051354. PMid:37317327.

JI, Q.Y., WANG, W., YAN, H., QU, H., LIU, Y., QIAN, Y. and GU, R., 2023. The effect of different organic acids and their combination on the cell barrier and biofilm of *Escherichia coli. Foods*, vol. 12, no. 16, pp. 3011. https://doi.org/10.3390/foods12163011. PMid:37628010.

JUSSIAUX, F., MIOT-SERTIER, C., NGUYEN-LOPEZ, D., BADET, C. and SAMOT, J., 2021. Reliability of MALDI-TOF mass spectrometry to identify oral isolates of *Streptococcus salivarius* and *Lactobacillus* spp. *Archives of Oral Biology*, vol. 121, pp. 104983. https://doi.org/10.1016/j.archoralbio.2020.104983. PMid:33242690.

KANG, C.E., PARK, Y.J., KIM, J.H., LEE, N.K. and PAIK, H.D., 2023. Probiotic *Weissella cibaria* displays antibacterial and anti-biofilm effect against cavity-causing *Streptococcus mutans. Microbial Pathogenesis*, vol. 180, pp. 106151. https://doi.org/10.1016/j.micpath.2023.106151. PMid:37172659.

KAVITAKE, D., DEVI, P.B. and SHETTY, P.H., 2020. Overview of exopolysaccharides produced by *Weissella* genus: a review. *International Journal of Biological Macromolecules*, vol. 164, pp. 2964-2973. https://doi.org/10.1016/j.ijbiomac.2020.08.185. PMid:32853618.

LAM, M., LEUNG, K.M., LAI, G.K.K., LEUNG, F.C.C. and GRIFFIN, S.D.J., 2024. Complete genome sequence of *Gluconobacter frateurii* ML. ISBL3, an endophytic strain isolated from aerial roots of *Syngonium podophyllum. Microbiology Resource Announcements*, vol. 13, no. 4, e0110623. https://doi.org/10.1128/mra.01106-23. PMid:38470266.

LIU, M., LI, X., YE, T., ZHAO, L. and ZHANG, X., 2025. Safety evaluation of *Weissella confusa* SY628 and the effect of its fermentation on the taste and quality of soy yogurt. *Frontiers in Microbiology*, vol. 16, pp. 1567399. https://doi.org/10.3389/fmicb.2025.1567399. PMid:40438204.

MA, W., LIU, X., JING, Y., ZHANG, M., ZHANG, X., WANG, C., KHAN, M.Z. and ZHU, M., 2025. *Weissella*: from beneficial probiotics to opportunistic pathogens - a review. *Nutrients*, vol. 17, no. 19, pp. 3162. https://doi.org/10.3390/nu17193162. PMid:41097239.

MACÊDO, E., 2023. Yeasts from fermented Brazilian fruits as biotechnological tools for increasing phenolics bioaccessibility and improving the volatile profile in derived pulps. *Food Chemistry*, vol. 401, pp. 134200. https://doi.org/10.1016/j.foodchem.2022.134200. PMid:36115231.

MAIONE, A., IMPARATO, M., BUONANNO, A., SALVATORE, M.M., CARRATURO, F., DE ALTERIIS, E., GUIDA, M. and GALDIERO, E., 2024. Evaluation of potential probiotic properties and in vivo safety of lactic acid bacteria and yeast strains isolated from traditional Home-Made Kefir. *Foods*, vol. 13, no. 7, pp. 1013. https://doi.org/10.3390/foods13071013. PMid:38611319.

MANHOKWE, S., MUSARURWA, T., JOMBO, T.Z., MUGADZA, D.T., MUGARI, A., BARE, J., MGUNI, S., CHIGONDO, F. and MUCHEKEZA, J.T., 2025. Development of a Quinoa-Based fermentation medium for propagation of *Lactobacillus plantarum* and *Weissella confusa* in opaque beer production. *International Journal of Microbiology*, vol. 2025, no. 1, pp. 5745539. https://doi.org/10.1155/ijm/5745539. PMid:39963294.

MARQUES, P.H., JAISWAL, A.K., DE ALMEIDA, F.A., PINTO, U.M., FERREIRA-MACHADO, A.B., TIWARI, S., SOARES, S.C. and PAIVA, A.D., 2024. Lactic acid bacteria secreted proteins as potential *Listeria monocytogenes* quorum sensing inhibitors. *Molecular Diversity*, vol. 28, no. 5, pp. 2897-2912. https://doi.org/10.1007/s11030-023-10722-7. PMid:37658910.

MERUVU, H. and HARSA, S.T., 2023. Lactic acid bacteria: isolation-characterization approaches and industrial applications. *Critical Reviews in Food Science and Nutrition*, vol. 63, no. 26, pp. 8337-8356. https://doi.org/10.1080/10408398.2022.2054936. PMid:35348017.

MULAW, G., SISAY TESSEMA, T., MULETA, D. and TESFAYE, A., 2019. In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented ethiopian food products. *International Journal of Microbiology*, vol. 2019, no. 1, pp. 7179514. https://doi.org/10.1155/2019/7179514. PMid:31534458.

NING, Y., CAO, H., ZHAO, S., GAO, D. and ZHAO, D., 2024. Structure and properties of exopolysaccharide produced by *Gluconobacter frateurii* and its potential applications. *Polymers*, vol. 16, no. 7, pp. 1004. https://doi.org/10.3390/polym16071004. PMid:38611262.

OLIVEIRA, A.L.D., SANTOS JUNIOR, V., LIOTTI, R.G., ZILIOLI, E., SPINOSA, W.A. and RIBEIRO-PAES, J.T., 2010. Study of bacteria *Gluconobacter* sp.: isolation, purification, phenotypic and molecular identification. *Food Science and Technology*, vol. 30, no. 1, pp. 106-112. https://doi.org/10.1590/S0101-20612010000100016.

PARLET, C.P., BROWN, M.M. and HORSWILL, A.R., 2019. Commensal staphylococci influence *Staphylococcus aureus* skin colonization and disease. *Trends in Microbiology*, vol. 27, no. 6, pp. 497-507. https://doi.org/10.1016/j.tim.2019.01.008. PMid:30846311.

PAROLIN, C., CROATTI, V., LAGHI, L., GIORDANI, B., TONDI, M.R., DE GREGORIO, P.R., FOSCHI, C. and VITALI, B., 2021. *Lactobacillus* biofilms influence anti-candida activity. *Frontiers in Microbiology*, vol. 12, pp. 750368. https://doi.org/10.3389/fmicb.2021.750368. PMid:34777300.

PATRONE, V., AL-SURRAYAI, T., ROMANIELLO, F., FONTANA, A., MILANI, G., SAGHEDDU, V., PUGLISI, E., CALLEGARI, M.L., AL-MANSOUR, H., KISHK, M.W. and MORELLI, L., 2020. Integrated phenotypic-genotypic analysis of candidate probiotic *Weissella cibaria* strains isolated from dairy cows in Kuwait. *Probiotics and Antimicrobial Proteins*, vol. 13, no. 3, pp. 809-823. https://doi.org/10.1007/s12602-020-09715-x. PMid:33085038.

PEREIRA BARBOSA, J., PIRES DE AMORIM TRINDADE, D., MAURÍCIO FURTADO MARTINS, E. and AMARAL SOUZA TETTE, P., 2022. Lactic acid bacteria isolated from fruits: a review on methods for evaluation of probiotic potential. *Journal of Food and Nutrition Research (Newark, Del.)*, vol. 10, no. 10, pp. 711-726. https://doi.org/10.12691/jfnr-10-10-9.

RODRIGUES, N.P.A., GARCIA, E.F. and DE SOUZA, E.L., 2021. Selection of lactic acid bacteria with promising probiotic aptitudes from fruit and ability to survive in different food matrices. *Brazilian Journal of Microbiology : Publication of the Brazilian Society for Microbiology*, vol. 52, no. 4, pp. 2257-2269. https://doi.org/10.1007/s42770-021-00543-x. PMid:34125419.

ROMÁN-CAMACHO, J.J., GARCÍA-GARCÍA, I., SANTOS-DUEÑAS, I.M., GARCÍA-MARTÍNEZ, T. and MAURICIO, J.C., 2023. Latest

trends in industrial vinegar production and the role of acetic acid bacteria: classification, metabolism, and applications - a comprehensive review. *Foods*, vol. 12, no. 19, pp. 3705. https://doi.org/10.3390/foods12193705. PMid:37835358.

ROMERO-MARTÍNEZ, M., ANDRADE-PIZARRO, R. and DE PAULA, C., 2025. Functional compounds in tropical fruit processing by-products and intrinsic factors affecting their composition: A review. *Current Research in Food Science*, vol. 10, pp. 101028. https://doi.org/10.1016/j.crfs.2025.101028. PMid:40190386.

SINGH, J.K., DEVI, P.B., REDDY, G.B., JAISWAL, A.K., KAVITAKE, D. and SHETTY, P.H., 2024. Biosynthesis, classification, properties, and applications of *Weissella bacteriocins. Frontiers in Microbiology*, vol. 15, pp. 1406904. https://doi.org/10.3389/fmicb.2024.1406904. PMid:38939182.

VERMOTE, L., VERCE, M., MOZZI, F., DE VUYST, L. and WECKX, S., 2022. Microbiomes associated with the surfaces of northern Argentinian fruits show a wide species diversity. *Frontiers in Microbiology*, vol. 13, pp. 872281. https://doi.org/10.3389/fmicb.2022.872281. PMid:35898900.

VIVEK, K., MISHRA, S., PRADHAN, R.C., NAGARAJAN, M., KUMAR, P.K., SINGH, S.S., MANVI, D. and GOWDA, N.A.N., 2023. A comprehensive review on microencapsulation of probiotics: technology, carriers and current trends. *Applied Food Research*, vol. 3, no. 1, pp. 100248. https://doi.org/10.1016/j.afres.2022.100248.

VUYST, L. and LEROY, F., 2020. Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. *FEMS Microbiology Reviews*, vol. 44, no. 4, pp. 432-453. https://doi.org/10.1093/femsre/fuaa014. PMid:32420601.

YILMAZ, N., OZOGUL, Y., COŞKUN DAĞGEÇEN, E., AKYOL, İ., BHOJRAJ RATHOD, N., REDDY SURASANI, V.K. and OZOGUL, F., 2025. Isolation, characterization and antibiotic resistance of lactic acid bacteria from dairy and seafood sources. *Food Bioscience*, vol. 64, pp. 105895. https://doi.org/10.1016/j.fbio.2025.105895.

YUKPHAN, P., CHAROENYINGCHAROEN, P., MALIMAS, S., MURAMATSU, Y., NAKAGAWA, Y., TANASUPAWAT, S. and YAMADA, Y., 2020. *Gluconobacter aidae* sp. Nov., an acetic acid bacteria isolated from tropical fruits in Thailand. *International Journal of Systematic and Evolutionary Microbiology*, vol. 70, no. 7, pp. 4351-4357. https://doi.org/10.1099/ijsem.0.004292. PMid:32584749.

ZHAO, A., SUN, J. and LIU, Y., 2023. Understanding bacterial biofilms: from definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*, vol. 13, pp. 1137947. https://doi.org/10.3389/fcimb.2023.1137947. PMid:37091673.

ZHAO, J., LI, X., LIU, H., JING, Z., YIN, X., LI, J., DONG, Z. and SHAO, T., 2024. Ensilage using Leuconostoc lactis and *Weissella confusa* reduces microbial risk and enhances hygienic quality of whole-crop corn. *Chemical and Biological Technologies in Agriculture*, vol. 11, no. 1, pp. 44. https://doi.org/10.1186/s40538-024-00566-0.