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In Vitro Preliminary Characterization of *Lactiplantibacillus plantarum* BG112 for Use as a Starter Culture for Industrial Dry-Fermented Meats

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Abstract

The objective of this study was to perform a preliminary in vitro characterization of *Lactiplantibacillus plantarum* BG112, assessing its safety and technological features for potential application as a culture starter for an industrial fermented dry meat product. In vitro assays assessed its viability, probiotic properties, and safety for use in food formulations. The strain was characterized through morphological and biochemical tests, carbohydrate fermentation profiling, and various in vitro assays based on FAO/WHO criteria for probiotic selection. These included proteolytic activity, auto-aggregation capacity, tolerance to simulated gastric juice and bile salts, antimicrobial activity, and resistance to sodium chloride, nitrite, and low pH. Safety evaluations were also performed by testing antibiotic susceptibility, hemolytic activity, and DNAse production. The results showed that *L. plantarum* BG112 exhibited strong tolerance to adverse environmental conditions typically found during sausage fermentation and ripening, along with significant inhibitory activity against pathogenic bacteria, such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Staphylococcus aureus*. The strain also demonstrated no hemolytic or DNAse activity and presented a favorable antibiotic sensitivity profile, meeting key safety requirements for probiotic use. Further studies using meat matrices and in vivo models are needed to validate these findings. This study contributes to the early-stage selection of safe and technologically suitable strains for use in fermented meat products. These findings support the potential application of *L. plantarum* BG112 as a safe and effective starter culture in the development of high-value, premium fermented meat products, aligned with current consumer demand for health-enhancing and natural foods.

Keywords: dry-fermented sausages; probiotic property; safety; fermented foods

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1. Introduction

Lactic acid bacteria (LAB) constitute a diverse group of Gram-positive, catalase-negative bacteria that produce lactic acid as the main end product of carbohydrate fermentation [1]. Many of these bacteria possess probiotic properties and have been successfully introduced into a wide variety of carrier food products as health-promoting functional foods, enhancing gut microbiota balance and supporting immune functions [1,2]. According to Bağdatli and Kundakci [3], meat products have proven to be an excellent vehicle

for probiotics. However, the commercial application of probiotic bacteria in fermented meat products, such as salami, is not yet common [4–6]. Considering that the population's behavior is shifting towards adopting healthier lifestyles, there is an increasing preference for natural products or those that enhance health, such as food with probiotics and postbiotic compounds. Consequently, meat product research groups and industry have together studied reformulation methods for traditional formulations, targeting this market niche.

Meat products, particularly fermented sausages, such as salami, offer an advantageous environment for probiotics because they are not subjected to heat treatment during production or consumption [7]. However, this matrix presents technological challenges, including low pH, high salt concentrations, and the presence of curing agents, such as sodium nitrite, which can negatively affect bacterial viability [7,8]. Despite these limitations, the successful introduction of probiotics into fermented sausages could provide a novel functional food, combining traditional flavor with added health benefits. This makes them a promising platform for the development of innovative functional meat products.

Salami-type cured meat products represent an important sector of meat product manufacturing in Argentina, both for domestic consumption and export. Salamis are prepared using raw meat (80%) and bacon (pork fatty tissue) (20%), with the addition of starter cultures, sodium chloride, sucrose, curing agents (sodium nitrite and nitrate), and spices. The ingredients are mixed and stuffed into casings, followed by a drying and curing period that controls temperature, relative humidity, and air circulation. The addition of probiotics in foods is a key goal of public health programs in this country and a trend in the food industry. For probiotics to be truly effective in fermented meat products, it is essential that they remain viable throughout the entire fermentation and drying process [9,10]. During maturation, these products tend to lose moisture, while the concentration of sodium chloride increases, and the pH drops. Because of this, it becomes especially important to evaluate how well probiotics can survive within the meat matrix under such conditions. An initial screening step is crucial to identify strains that are suitable for industrial use, particularly those that can maintain high cell viability throughout processing. In recent years, there has been growing interest in using starter cultures that not only support fermentation but also offer added health benefits [11,12]. The objective of this study was to assess the potential of *L. plantarum* as a probiotic starter culture for an industrial fermented dry meat product. In vitro assays assessed its viability, probiotic properties, and safety for use in food formulations. Our findings contribute to the development of a high-value, local, functional food product that aligns with current health trends and consumer expectations.

2. Materials and Methods

2.1. Strain

Research was conducted to explore the possible application of a commercial Lactobacilli strain, *L. plantarum* BG112 (Clerici-Sacco, Cadorago, Italy), as a probiotic in fermented meat products. The lactobacilli strain was selected for its safety, technological, and probiotic properties [13–16].

2.2. Cell Morphology and Biochemical Tests

LAB are characterized as being Gram-positive, catalase-negative, and anaerobic-facultative bacteria. A high dilution was made, and the colonies were selected from countable MRS agar (Britania, Argentina) plates. Isolates were tested for Gram reaction, catalase, oxidase, and cell morphology. Bacterial cultures in the stationary phase grown on MRS agar were mounted on microscopic slides and examined under a light microscope

using oil immersion objectives. Isolates were conserved frozen at −80 °C in MRS broth and 20% glycerol.

2.3. Carbohydrate Fermentation Profile

The carbohydrate fermentation profile was determined using the API50CHL® enzymatic gallery and API50CH® medium (bioMérieux, Inc., Marcy-l'Etoile, France), following the manufacturer's protocol. A suspension of *L. plantarum* BG112, adjusted to match tube 2 on the McFarland scale, was prepared and added to 5 mL of API50CH® medium. Sterile water was used to distribute in the panel components, and each microtube was then filled with 100 µL of the bacterial suspensions and sealed with sterile Vaseline to prevent evaporation. The gallery was incubated at 37 °C for 72 h. Results were considered positive fermentation (+) when the medium changed from bromocresol purple to yellow. The first microtube without substrate was used as a negative control (bromocresol purple). The results were recorded, and species designation was carried out using the ABIS online database (https://www.tgw1916.net/bacteria_abis.html, accessed on 3 December 2024).

2.4. Probiotic Characterization of Pure Culture

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established selection criteria for probiotic microorganisms in 2002 in the report of the working group on the drafting of guidelines for the evaluation of probiotics in foods [17]. Taking this into account, *L. plantarum* BG112 was tested for proteolytic capacity, auto-aggregation, tolerance to simulated gastric conditions and bile salts, antimicrobial activity, tolerance to low pH and different sodium chloride concentrations, sodium nitrite resistance, antibiotic susceptibility, hemolytic activity, and DNAse activity. All experiments were performed in triplicate.

2.4.1. Proteolytic Capacity

Strains of *L. plantarum* BG112, previously cultured in MRS broth at 37 °C for 48 h, were plated onto milk agar plates and incubated at the same temperature for 72 h. A clear halo around the colonies indicated the presence of protease-producing organisms [18].

2.4.2. Auto-Aggregation Test

The auto-aggregation test was carried out following the methodology described by Del Re et al. [19], with slight modifications. The *L. plantarum* strain was cultured in MRS broth for 48 h at 37 °C under microaerobic conditions. After incubation, the bacterial cells were harvested by centrifugation at 10,000 rpm for 10 min and washed three times with sterile phosphate-buffered saline (PBS). The cell pellet was then suspended in sterile PBS to achieve a final concentration of 10^8 CFU/mL. An aliquot was vortexed for 10 s and left undisturbed at room temperature for 24 h. Finally, the optical density (OD) at 600 nm was measured.

The percentage of auto-aggregation was expressed as follows:

$$Equation\ (1) = Auto\text{-}aggregation\ (\%) = \left[\frac{ODi - ODf}{ODi}\right] \times 100 \quad (1)$$

where *ODi* and *ODf* are the optical densities at the initial time and after 24 h (final time), respectively. All experiments were performed in triplicate.

2.4.3. Tolerance to Simulated Gastric Secretion Conditions

According to Villareal [20], a gastric solution (GS) was prepared. First, 1 ml of the overnight culture of *L. plantarum* BG112 was centrifuged and washed in duplicate, obtaining two pellets per strain for each repetition. A buffer was added to one of them and used as a control group (C), and to the other, the GS was added. Decimal dilutions were made,

incubated at 37 °C for 3 h, plated on M-17 agar (Biokar), and then incubated at 37 °C for 72 h. The results were expressed as viable counts ($\log_{10}$ CFU/mL) of LB strains under optimal growth conditions (C) and under GS conditions.

2.4.4. Tolerance to Bile Resistance

The bile resistance of the *L. plantarum* BG112 strain was studied using the agar diffusion test. A volume of 20 mL of M-17 agar was dispensed into tubes, and each was inoculated with 200 µL of an overnight culture. The inoculated medium was gently mixed and maintained at 45 °C before being poured into sterile Petri dishes. Once the agar solidified, wells measuring 10 mm in diameter were bored into the surface.

Sterile ox bile solutions at concentrations of 0.15%, 0.3%, 0.6%, and 1.0% (*w*/*v*) (Britania) were added to the wells in 180 µL aliquots and left to diffuse at room temperature. The plates were then incubated at 37 °C for 24 h under microaerobic conditions. Based on the resulting inhibition zones, bile tolerance was categorized as follows: total inhibition (clear zones indicating no bacterial growth), partial inhibition (areas with reduced turbidity suggesting limited or weak growth), and no inhibition (no visible zone, indicating full resistance).

2.4.5. Antimicrobial Activity

The agar overlay method was used to evaluate *L. plantarum* BG112's ability to inhibit bacterial pathogens. The antimicrobial activity was evaluated against three pathogenic bacteria: *Escherichia coli* STEC O157:H7 (EDL933), *Salmonella* Typhimurium (isolated from a pig farm) [21], and *Staphylococcus aureus* (isolated from a pork meat market) [22] at the Laboratorio de Inmunoquímica y Biotecnología of the Faculty of Veterinary Sciences. *L. plantarum* BG112 was cultured on MRS agar for 48 h at 37 °C under microaerobic conditions. From a pure single colony, the strain was inoculated onto MRS agar plates and incubated under the same conditions. After incubation, the plates were exposed to chloroform vapors for 2 h to inactivate the grown bacteria. Then, the plates were covered with Trypticase Soy Soft Agar (TSA) (0.75% agar) containing the pathogenic bacteria. The plates were incubated aerobically for 24 h at 37 °C, and the inhibition zones were subsequently measured in millimeters. The observation of translucent circular areas around the colonies was considered indicative of the inhibition of the growth of pathogenic bacteria by the action of the test strain. The presence of inhibition zones greater than 1 mm was considered a positive result for antimicrobial activity [23].

2.4.6. Evaluation of Growth Kinetics Under Conditions of Fermented Dry-Cured Sausage Processing: Tolerance to Low pH and Resistance to Sodium Chloride and Sodium Nitrite

The acid tolerance of the study strain was evaluated by inoculating active cultures in MRS broth, with the pH adjusted to 5.5, 5.0, 4.5, and 4.0 with 1N HCl. Additionally, different sodium salt concentrations were tested, including 2.5% *w*/*v*, 3% *w*/*v*, and 3.5% *w*/*v* NaCl. The resistance to sodium nitrite studied was considered to be the maximum value allowed by national legislation [24]. MRS broth medium with 150 ppm of sodium nitrite was used.

To determine the effect of the fermenting temperature on the applicability of *L. plantarum* as a starter culture, the OD (600 nm), pH value, and cell viability counts were measured initially and at 2, 4, 6, 7, and 24 h of incubation at two different temperatures, i.e., 25 °C and 15 °C, under microaerobic conditions [11]. Considering that the ripening process of fermented meat products is carried out at two different temperatures, these variables were taken into account in this study (25 °C and 15 °C). At each time point, decimal dilutions were plated on MRS agar to determine the colony-forming units per mL (CFU/mL).

2.5. Safety Evaluation of Selected Strain

2.5.1. Antibiotic Susceptibility Assay

The susceptibility of *L. plantarum* to antibiotics was determined using the microdilution test (10) to establish the minimum inhibitory concentrations (MICs) of 5 antibiotics following the method described by Klare et al. [25] and the Guidance on the assessment of bacterial antimicrobial susceptibility [26]. The antibiotics tested (with ranges in µg/mL noted in parentheses) were streptomycin (0.5–56), gentamicin (0.5–256), ciprofloxacin (0.5–256), ampicillin (0.032 to 16), and vancomycin (8–4096). The antibiotics were dissolved in diluents to create 10X stock solutions. *L. plantarum* inoculum was prepared by suspending isolated colonies in lactic acid bacteria susceptibility test medium (LSM). This medium consisted of 90% Iso-Sensitest (IST) broth and 10% MRS broth and was adjusted to reach an OD equivalent to 0.5 on the McFarland scale. Each well was inoculated to reach a final concentration equivalent to 10^8 bacteria/mL. The plates were incubated for 48 h at 37 °C. Minimum inhibitory concentration (MIC) assays were conducted in 96-well plates with LSM and different antibiotic concentrations. The MIC was determined as the lowest concentration with no visible bacterial growth. The antibiotic response was categorized as resistant (R), moderately sensitive (MS), or susceptible (S) based on growth levels.

2.5.2. Hemolytic Activity

The hemolytic activity test was carried out following the methodology described by Iraporda et al. [27]. To assess hemolytic activity, fresh overnight bacterial cultures were streaked onto blood agar plates and incubated aerobically at 37 °C for 72 h. The type of hemolysis was identified based on the appearance of zones surrounding the colonies: green discoloration indicated alpha-hemolysis, transparent halos were indicative of beta-hemolysis, and the absence of any visible zone was considered non-hemolytic. *Staphylococcus aureus* ATCC strain was included as a positive control.

2.5.3. DNAse Activity

DNAse activity was studied to test the production of the DNAse enzyme by inoculating a fresh overnight broth culture on Deoxyribonuclease (DNAse) agar medium according to Shuhadha et al. [28]. The plates were incubated at 37 °C for 48 h and flooded with 1N HCl. After 5 min, the agar plates were observed for a halo appearance surrounding the strains. The presence of a clear halo was interpreted as positive (+) DNase activity. *Staphylococcus aureus* DNAse-positive (isolated from home cooking) was used as the positive control [29]. The assay was performed in triplicate.

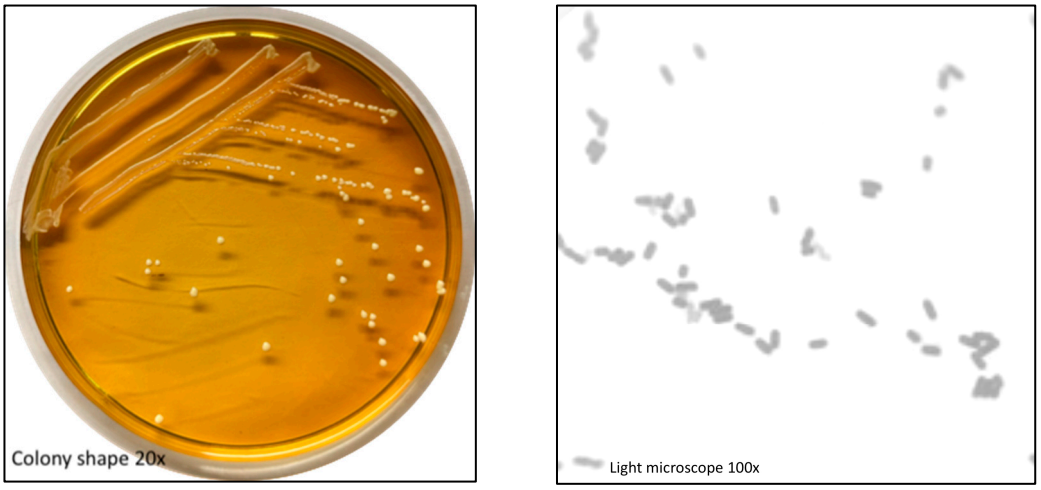
2.6. Statistical Analysis

The statistical analysis (ANOVA and Tukey tests) was carried out using Infostat software version 2014. A significant level of $p < 0.05$ was set to determine significant differences. Different letters were used to label values with statistically significant differences among them.

3. Results and Discussion

3.1. L. Plantarum BG112

The colony's macroscopic aspect of *L. plantarum* BG112 and its microscopic morphology, subjected to a Gram stain and grown on MRS agar at 37 °C under microaerobic conditions, are shown in Figure 1. The colonies were round, entire, and yellow, and the cell morphology consisted of bacilli that were Gram-positive, catalase-negative, and oxidase-negative. The study aligns with previous research, describing *L. plantarum* KLDS1.0391 isolated from traditional fermented dairy products as a rod-shaped, Gram-positive LB with probiotic characteristics [30].



(**a**) (**b**)

Figure 1. Phenotypic characterization of *Lactiplantibacillus plantarum* BG112 grown on MRS agar medium for 48 h at 37 °C under microaerobic conditions. Colony aspects (**a**) and Gram stain (**b**) of isolated selected strains.

3.2. Carbohydrate Fermentation

The carbohydrate fermentation profile of *L. plantarum* BG112 is shown in Table 1. The biochemical identification API 50 CHL® results were entered into the online ABIS software. Both expressed phenotypes allowed the identification of *L. plantarum*, identified with 96.6% similarity and 97.2% probability.

Table 1. Fermentation of carbohydrates by *L. plantarum* BG112 in the API 50 CHL® enzymatic gallery.

	Carbohydrate	***L. plantarum* BG112**		
		24 h	**48 h**	**72 h**
1	CONTROL	–	–	–
2	GLYcerol	–	–	–
3	ERYthrol	–	–	–
4	D-ARAbinose	–	–	–
5	L-ARAbinose	–	–	–
6	RIBose	d	+	+
7	D-XYLose	–	d	d
8	L-XILose	–	–	–
9	ADOnitol	–	–	–
10	β-Methyl-D-Xyloside	–	–	–
11	GALactose	d	+	+
12	GLUcose	+	+	+
13	FRUctose	+	+	+
14	MaNnosE	+	+	+
15	SorBosE	–	–	–
16	RHAmnose	d	d	d
17	DULcitol	–	–	–
18	INOsitol	–	–	–
19	MANnitol	+	+	+
20	SORbitol	+	+	+
21	1-Methyl-D-Mannoside	–	d	+
22	1-Methyl-D-Glucoside	–	–	–
23	N-Acetyl-Glucosamine	d	+	+

Table 1. *Cont.*

	Carbohydrate	*L. plantarum* BG112		
		24 h	**48 h**	**72 h**
24	AMYgdaline	d	+	+
25	ARButin	d	+	+
26	ESCulin	+	+	+
27	SALicin	+	+	+
28	CELlobiose	d	+	+
29	MALtose	d	+	+
30	LACtose	d	+	+
31	MELibiose	+	+	+
32	SAC sucrose	+	+	+
33	TREhalose	d	+	+
34	INUlin	−	−	−
35	MeLeZitose	+	+	+
36	RAFfinose	−	−	d
37	AMD starch	−	−	−
38	GLYcoGen	−	−	−
39	XyLiTol	−	−	−
40	GENtiobiose	d	+	+
41	D-TURanose	d	+	+
42	D-LYXose	−	−	−
43	D-TAGatose	d	+	+
44	D-FUCose	−	−	−
45	L-FUCose	−	−	−
46	D-ARabitoL	−	−	−
47	L-ARabitoL	−	−	−
48	GlucoNaTe	−	−	d
49	2-Keto-Gluconate	−	−	−
50	5-Keto-Gluconate	−	−	−

(+): Turbidity presence and yellow color of the broth medium, (−): turbidity absence and purple color of the broth medium, (d): weak turbidity (confirmed by plate counting) with a minimal change in the color of the broth medium.

The strain under study demonstrated the fermentation of galactose, fructose, raffinose, glucose, lactose, and fructose after 72 h of incubation. This behavior is valued in potential probiotic microorganisms because of the interest in fructooligosaccharides and galactooligosaccharides as prebiotics [31,32]. Additionally, inulin was not fermented by this strain. In this respect, and in agreement with our study, a study by Anella and Pérez-Díaz [33] showed that *L. plantarum* does not ferment inulin as a carbohydrate source.

3.3. Proteolytic Capacity and Auto-Aggregation Test

The study strain showed no casein degradation during aerobic growth on milk agar plates and exhibited an auto-aggregation capacity of 72.95% ± 3.97 after 24 h. This high auto-aggregation percentage indicates its potential for survival in challenging conditions and favorable intestinal adhesion capacity, making it a desirable candidate for a potential probiotic.

The ability of bacteria to auto-aggregate can promote their adhesion to intestinal epithelial cells, allowing bacteria to create a protective barrier and prevent the adhesion of undesirable microorganisms [33]. Auto-aggregation tests were used to assess the physical interactions between bacterial cells and the substrate. These interactions promoted the development of cell clusters that gradually settled at the bottom under static conditions [34]. The extent of aggregation was determined by measuring the sedimentation of non-motile

bacterial suspensions through changes in OD600. Bacteria that auto-aggregate exhibit a decrease in OD600 after 24 h.

The percentages of auto-aggregation obtained for the study strain were high (>70%). Janković, Ferec, Abram, and Gobin [35] reported similar results for auto-aggregation percentage, including three potential probiotic strains of *L. plantarum* (≥80%).

3.4. Tolerance to Simulated Gastric Conditions and Bile Salt Resistance

Potential probiotic candidates must have the ability to survive in gastric conditions. Acidity is considered an important destructive factor affecting the viability of lactobacilli. Table 2 shows that *L. plantarum* BG112 exhibits a high survival rate in the presence of the GS. Although there were significant differences ($p < 0.05$) between the counts in the GS and in the C at 2 h (p-value 0.0109), the counts at the final time, 3 h, between both groups did not show significant differences (p-value 0.0793). The strain survived after the simulated sequential gastric treatment (Table 2). After 1, 2, and 3 h of exposure to the gastric treatment, the percentages of survival were 92.2%, 93.06%, and 93.27%, respectively. At the final time point (3 h), no significant differences in survival percentages were observed between the C and GS groups, and the counts (CFU/mL) of *L. plantarum* BG112 increased, demonstrating the ability to grow in simulated gastric conditions. Other authors have reported similar results for *L. plantarum* strains isolated from various other sources [34,36].

Table 2. Viable counts ($\log_{10}$ CFU/mL) of bacterial survival after simulated gastric treatment and percentage of bacterial survival after bile salt exposure.

Gastric Treatment (CFU/mL)							
0 h		1 h		2 h		3 h	
C	GS	C	GS	C	GS	C	GS
9.32 ± 0.35 a	9.36 ± 0.40 a	9.08 ± 0.33 a	8.63 ± 0.25 a	9.47 ± 0.35 a	8.71 ± 0.23 b	9.11 ± 0.34 a	8.73 ± 0.10 a
Bile salts treatment							
0.15% *w*/*v*		0.30% *w*/*v*		0.6% *w*/*v*		1.0% *w*/*v*	
Negative		Negative		Positive		Positive	

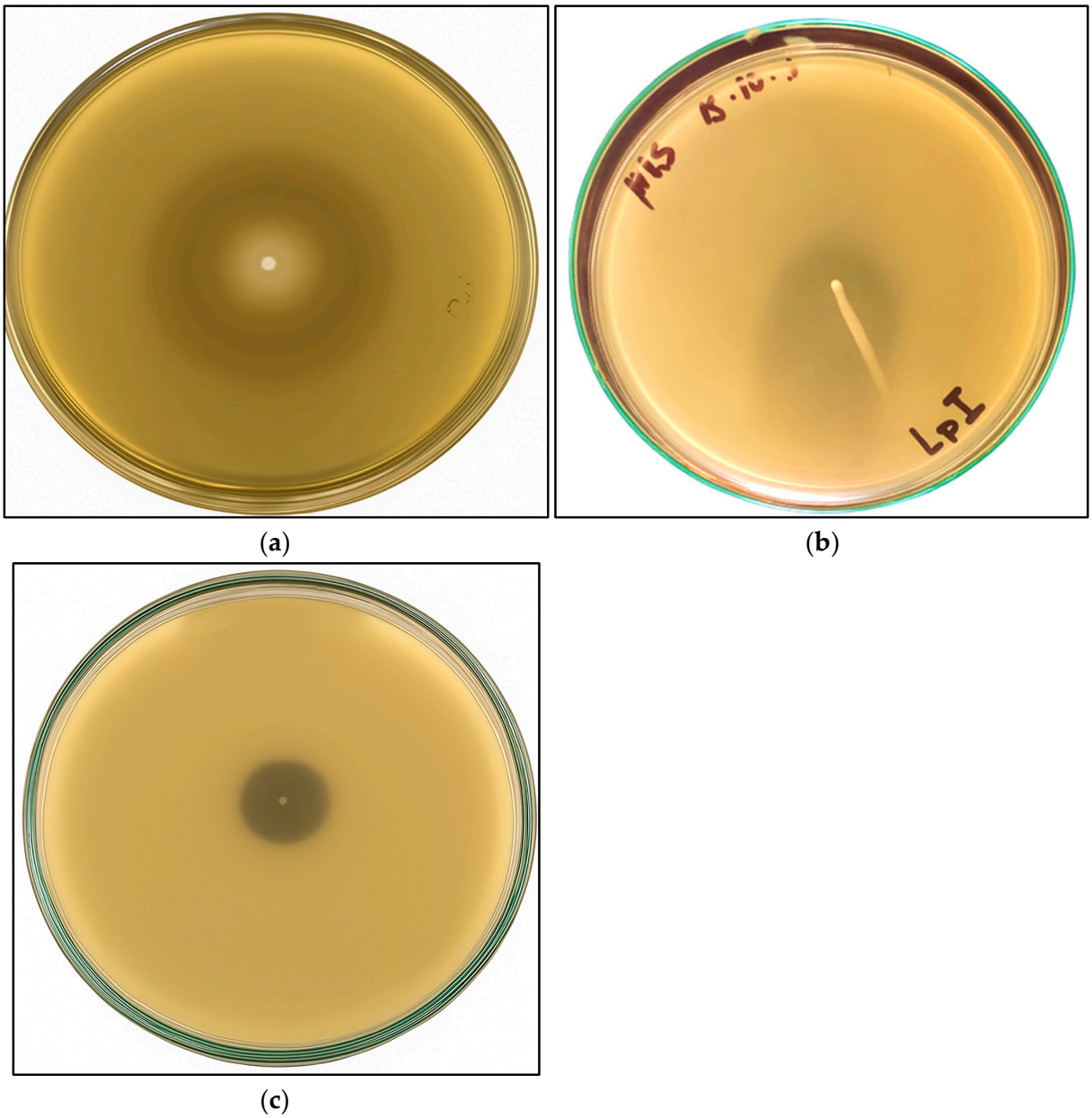
(C): control, (GS): gastric solution. Values are represented as mean ± SD of three independent replicates.

Bile salt tolerance is a fundamental trait for probiotic strains, as it directly impacts their persistence in the intestinal tract and subsequent functionality [37]. Potential probiotic candidates must survive the bile salts present in the duodenum in order to deliver health benefits to the host [38]. The concentration of bile salts within the intestinal environment fluctuates, typically averaging around 0.3% (*w*/*v*), with food remaining in the small intestine for approximately 4 to 6 h [38,39]. In the present study, *L. plantarum* BG112 demonstrated tolerance to bile salt concentrations of 0.15% and 0.30%. When cultured in a medium containing 0.6% ox bile salts, the strain exhibited partial growth inhibition, whereas complete inhibition was observed at a bile salt concentration of 1% (Table 2).

3.5. Antimicrobial Activity

The selected strain exhibited an inhibitory effect on the pathogenic microorganisms *Escherichia coli* STEC O157:H7, *Salmonella* Typhimurium, and *Staphylococcus aureus*. The strain showed an inhibitory effect with diameters of inhibition of 20.0 ± 1.0 mm, 18.3 ± 0.7 mm, and 10.2 ± 0.6 mm, respectively (Figure 2). These values indicate a high level of antimicrobial activity, particularly against Gram-negative pathogens. This antagonistic behavior suggests the production of inhibitory compounds, such as organic acids, hydrogen peroxide, or potentially bacteriocin-like substances. Notably, the persistence of inhibition reported for other *L. plantarum* strains has been associated with the production of specific antimicrobial metabolites beyond acidification, including plantaricins and phenolic compounds. Similar

findings were described by Ruiz et al. [40], who demonstrated that both cell-free extracts and their neutralized versions from *L. plantarum* LP5 retained inhibitory activity against multiple *Campylobacter coli* strains. This highlights the presence of non-acidic antimicrobial molecules with significant technological relevance.



(**a**) (**b**) (**c**)

Figure 2. Antimicrobial activity of *L. plantarum* BG112 against *E. coli* STEC O157:H7 (**a**), *Salmonella* Typhimurium (**b**), and *S. aureus* (**c**).

The ability to inhibit a broad spectrum of pathogens reinforces the strain's potential for application in fermented meat systems, where control of microbial contaminants is crucial for safety and shelf life. As noted by Sahu [41], lactic acid bacteria produce bioactive compounds capable of inhibiting the proliferation of a wide range of pathogenic and spoilage microorganisms. These results are consistent with previous research on the antimicrobial activity of *L. plantarum* and various LAB isolates [41–43].

Considering these results, future research should focus on identifying and characterizing the nature of the antimicrobial agents produced by strain *L. plantarum* BG112,

particularly under meat fermentation conditions. This could support its selection as a multifunctional starter culture with both fermentative and protective roles in food systems.

3.6. Tolerance to Low pH, Sodium Chloride Concentrations, and Sodium Nitrite

Considering the objective of this work, the viability study of the *L. plantarum* strain under conditions similar to those that will occur in a product subjected to fermentation and dehydration, such as different pH values, an increasing range of sodium chloride, and some concentration of sodium nitrite (a permitted preservative for raw cured sausages), is of vital importance to propose a microorganism as a possible starter culture in a fermented product.

All variables were studied at two different incubation temperatures, i.e., 15 °C and 25 °C, as these are the typical temperatures to which a fermented meat product is exposed during the maturation and drying process. The results are shown in separate graphs for each type of stress condition (pH, sodium chloride, and sodium nitrite) in Figures 3–6, and in Table 3.

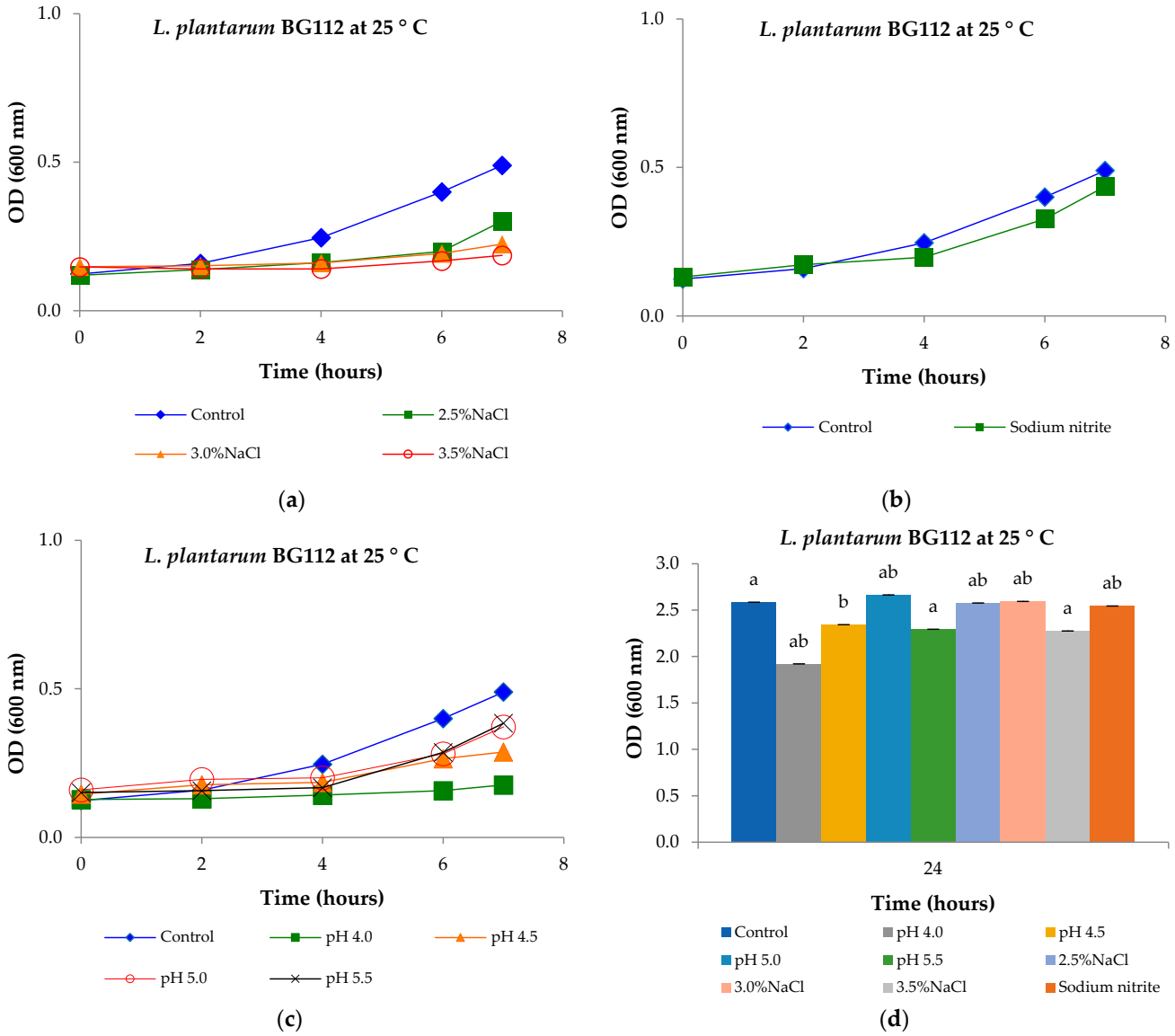


Figure 3. Averages of optical density (600 nm) values (means ± SDs), $n = 3$, of *L. plantarum* BG112 in MRS broth at 25 °C (**a**–**d**). Bars sharing the same letters indicate no significant differences between stress variables at the same time under the same temperature incubation ($p > 0.05$). Error bars represent SDs.

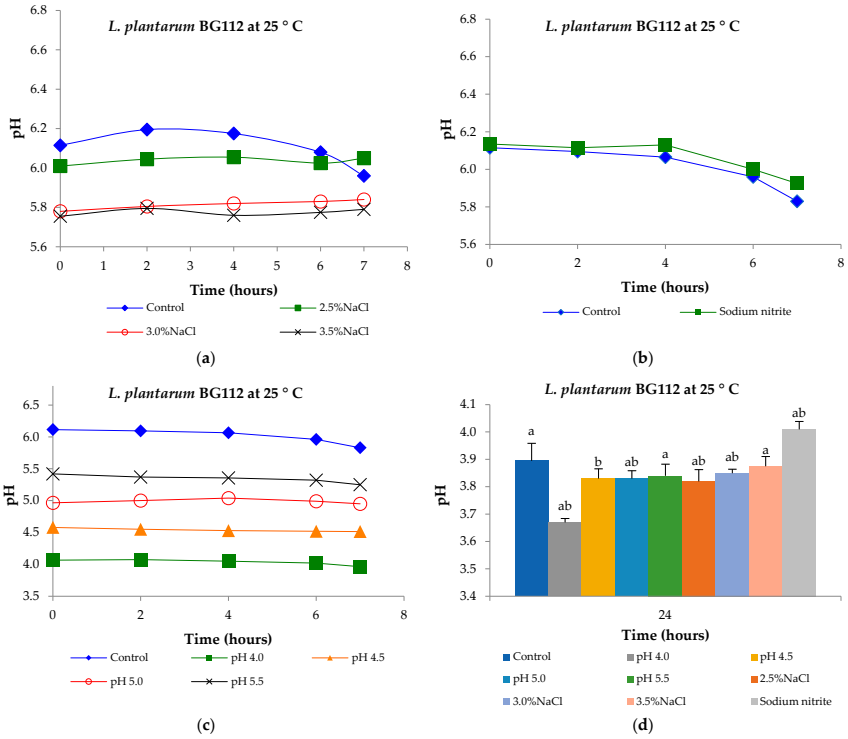


Figure 4. Averages of pH values (means ± SDs), *n* = 3, of *L. plantarum* BG112 in MRS broth at 25 °C (**a**–**d**). Bars sharing the same letters indicate no significant differences between stress variables at the same time under the same temperature incubation ($p > 0.05$). Error bars represent SDs.

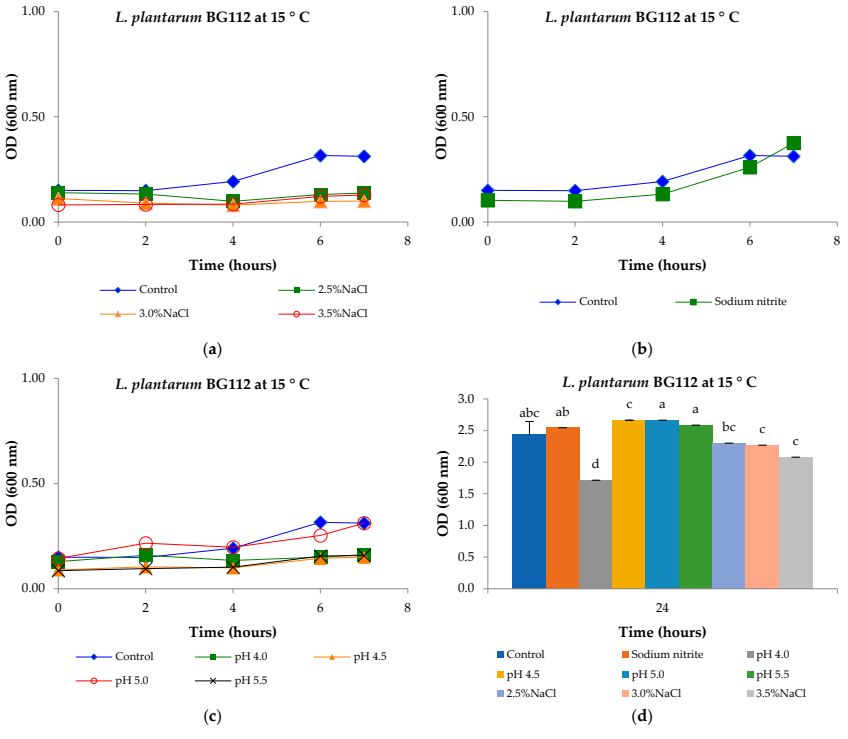


Figure 5. Averages of optical density (600 nm) values (means ± SDs), *n* = 3, of *L. plantarum* BG112 in MRS broth at 15 °C (**a**–**d**). Bars sharing the same letters indicate no significant differences between stress variables at the same time under the same temperature incubation ($p > 0.05$). Error bars represent SDs.

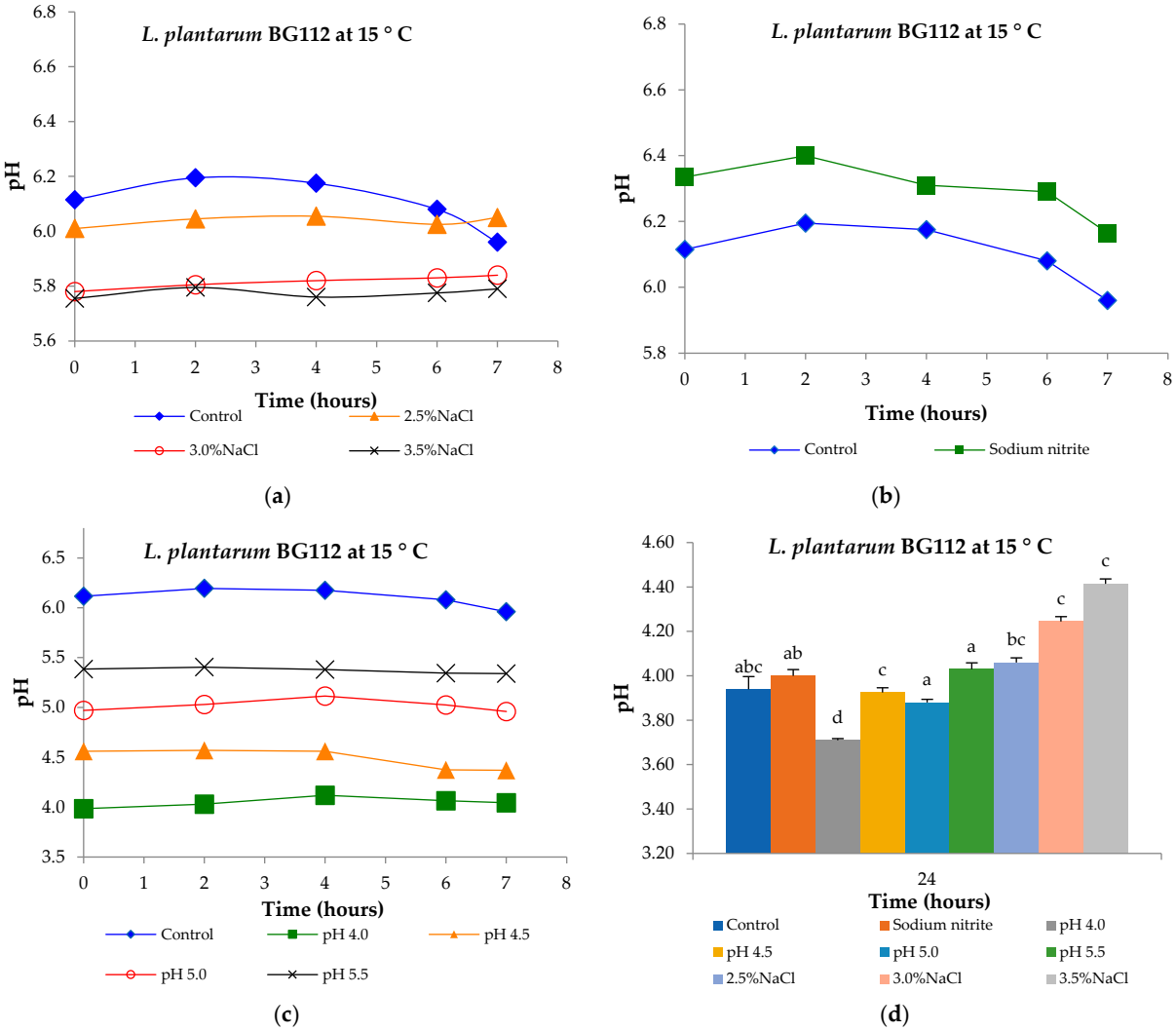


Figure 6. Averages of pH values (means ± SDs), $n = 3$, of *L. plantarum* BG112 in MRS broth at 15 °C (**a**–**d**). Bars sharing the same letters indicate no significant differences between stress variables at the same time under the same temperature incubation ($p > 0.05$). Error bars represent SDs.

Table 3. Comparison of the growth of *L. plantarum* (log CFU/mL) in MRS modified for 24 h at 25 and 15 degrees Celsius.

25 °C	Average ± SD (log_{10} UFC/mL)					
	0 h	**2 h**	**4 h**	**6 h**	**7 h**	**24 h**
Control	7.79 ± 0.03 a	7.90 ± 0.00 b	8.09 ± 0.00 a	8.30 ± 0.01 a	8.39 ± 0.00 a	9.11 ± 0.00 a
Sodium nitrite [1]	7.82 ± 0.01 a	7.94 ± 0.01 a	7.99 ± 0.00 b	8.21 ± 0.00 b	8.34 ± 0.00 b	9.08 ± 0.04 ab
pH 4.0	7.80 ± 0.01 a	7.81 ± 0.01 d	7.85 ± 0.03 d	7.89 ± 0.03 f	7.95 ± 0.01 h	8.98 ± 0.00 b
pH 4.5	7.75 ± 0.00 a	7.73 ± 0.00 e	7.78 ± 0.01 e	7.90 ± 0.00 ef	8.16 ± 0.00 f	9.07 ± 0.00 ab
pH 5.0	7.64 ± 0.02 b	7.74 ± 0.00 e	7.80 ± 0.01 e	8.00 ± 0.01 d	8.30 ± 0.00 d	9.12 ± 0.00 a
pH 5.5	7.88 ± 0.00 a	7.90 ± 0.01 b	7.92 ± 0.00 c	8.20 ± 0.00 c	8.28 ± 0.00 c	9.06 ± 0.00 ab
2.5%NaCl	7.78 ± 0.01 a	7.84 ± 0.01 c	7.91 ± 0.00 c	8.00 ± 0.00 d	8.18 ± 0.00 e	9.07 ± 0.07 ab
3.0%NaCl	7.90 ± 0.00 a	7.90 ± 0.01 b	7.90 ± 0.00 c	7.90 ± 0.00 d	8.05 ± 0.00 g	9.11 ± 0.00 a
3.5%NaCl	7.87 ± 0.02 a	7.85 ± 0.00 c	7.85 ± 0.00 d	7.92 ± 0.00 e	7.98 ± 0.00 h	9.06 ± 0.00 ab

Table 3. *Cont.*

15 °C	Average ± SD (log_{10} UFC/mL)					
	0 h	**2 h**	**4 h**	**6 h**	**7 h**	**24 h**
Control	7.88 ± 0.06 a	7.87 ± 0.01 b	7.98 ± 0.00 a	8.12 ± 0.01 a	8.19 ± 0.00 a	9.08 ± 0.04 abc
Sodium nitrite [1]	7.71 ± 0.02 a	7.69 ± 0.02 de	7.82 ± 0.00 b	8.11 ± 0.00 b	8.27 ± 0.00 b	9.10 ± 0.00 ab
pH 4.0	7.81 ± 0.01 a	7.90 ± 0.01 b	7.83 ± 0.00 b	7.88 ± 0.01 cd	7.91 ± 0.01 e	8.93 ± 0.00 d
pH 4.5	7.65 ± 0.02 a	7.72 ± 0.01 d	7.70 ± 0.01 c	7.86 ± 0.00 d	7.96 ± 0.00 c	9.03 ± 0.00 c
pH 5.0	7.86 ± 0.00 a	8.03 ± 0.02 a	7.80 ± 0.00 a	8.10 ± 0.00 b	8.19 ± 0.00 a	9.12 ± 0.00 a
pH 5.5	7.63 ± 0.10 a	7.68 ± 0.00 de	7.71 ± 0.01 c	7.89 ± 0.00 c	7.95 ± 0.01 d	9.11 ± 0.00 a
2.5%NaCl	7.84 ± 0.00 a	7.82 ± 0.01 c	7.69 ± 0.00 c	7.82 ± 0.00 e	8.07 ± 0.01 b	9.06 0.00 bc
3.0%NaCl	7.75 ± 0.09 a	7.65 ± 0.00 ef	7.60 ± 0.01 d	7.69 ± 0.01 g	7.79 ± 0.02 f	9.06 ± 0.00 c
3.5%NaCl	7.61 ± 0.12 a	7.62 ± 0.01 f	7.63 ± 0.01 d	7.78 ± 0.01 f	7.84 ± 0.01 d	9.02 ± 0.00 c

[1] 150 ppm of sodium nitrite, according to the maximum value allowed by national legislation. The same letters indicate no significant differences between stress variables at the same time under the same temperature incubation ($p > 0.05$).

L. plantarum BG112 demonstrated robust growth behavior in the face of various stresses simulating fermentation and ripening conditions for dry sausages. At temperatures of both 15 °C and 25 °C, the strain was able to maintain viability and even increase its cell concentration under adverse conditions, such as reduced pH (up to 4.0), high NaCl concentrations (up to 3.5%), and exposure to 150 ppm sodium nitrite (Figures 3–6).

The strain maintained growth of more than 2 log CFU/mL under most conditions, except at higher acidity concentration values (pH 4.0), where growth was limited but still positive (1 log cycle CFU/mL) (Table 3). Significant differences were recorded in the final time for all variables in this study compared to the control (p-value < 0.0001). The *L. plantarum* strain exhibited remarkable resilience under all the variables studied throughout the entire incubation period. This tolerance profile indicates significant metabolic adaptability and suggests that *L. plantarum* BG112 may be suitable for addressing the typical technological challenges of salami production, where decreasing pH and salinity are unavoidable conditions during ripening.

These results agree with the research carried out by Abdel-motaal et al. [44], who determined that six *Lactiplantibacillus plantarum* strains isolated from fermented dairy and non-dairy products, fermented vegetables, and fermented soybean curd showed good growth in NaCl concentrations of 4%, 6%, and 8% w/v. Although such high levels were not evaluated in this study, the data obtained support the possibility of using this strain in conventional fermented meat products.

The OD600, pH, and viable cell count profiles indicated that bacterial growth was associated with a marked acidification of the medium, confirming the acidifying capacity of the strain under microaerobic conditions. This trait is critical for the biopreservation of dry-fermented meats, as it helps inhibit the growth of pathogenic and spoilage microorganisms, while also contributing to the development of the product's characteristic sensory properties.

Significant differences ($p < 0.05$) were observed in the growth curves under different stress conditions, highlighting the importance of conducting further trials in meat-based systems. Such studies are essential to verify whether the physiological responses observed under laboratory conditions are maintained in complex food matrices. In situ evaluations would help determine the suitability of *L. plantarum* BG112 as a starter culture, not only for its fermentative performance but also for its resilience to commonly used additives in the meat industry.

3.7. Safety Aspects

When selecting probiotic candidates for use in food applications, it is crucial to consider safety aspects to confirm their potential and ensure they are suitable for human consumption. From a food safety perspective, *L. plantarum* BG112 displayed a highly favorable profile. *L. plantarum* BG112 exhibited no hemolytic capacity or DNAse activity, which is considered a safety characteristic of probiotic bacteria (Table 4). Strains of *Staphylococcus aureus* were used as a positive control. Moreover, the present strain was sensitive to the antibiotics ampicillin, streptomycin, gentamicin, ciprofloxacin, and vancomycin, at concentrations below the cut-off limit established by the EFSA [26] (Table 4). These characteristics strongly support its safety, indicating that *L. plantarum* BG112 poses no detectable risk of harboring transferable antibiotic resistance genes or virulence factors, making it a suitable candidate for incorporation into food products.

Table 4. Safety evaluation aspects of the *L. plantarum* BG112 strain.

Antibiotic Sensibility					Hemolytic Capacity	DNAse Activity
AMP	**STR**	**GEN**	**CIP**	**VAN**		
S	S	S	S	S	–	–

(S): sensitive, (+): positive, (−): negative.

3.8. Implications for Application in Traditional Meat Products and Comparison with Commercial and Autochthonous Cultures

Recent advances in the development of functional dry-fermented meats have explored the incorporation of probiotic strains directly into meat matrices under industrial conditions. Rubio et al. [7] successfully formulated nutritionally enhanced fermented sausages using *L. plantarum* and *L. rhamnosus* strains, which remained viable above 6 log CFU/g at the end of the ripening process. These strains not only demonstrated adequate fermentative behavior but also maintained the sensory acceptability of the final product. Their work highlights the feasibility of using probiotic cultures in dry-cured meats without compromising product quality. Although the current study focused on the in vitro performance of *L. plantarum* BG112, the observed stress tolerance, antimicrobial activity, and safety traits indicate its suitability for in situ applications. Its ability to withstand acidic pH, high NaCl, and nitrite concentrations (common during the fermentation and ripening of dry-cured sausages) suggests compatibility with traditional salami-type products, particularly within Argentine processing conditions.

A comparable approach was taken by Ruiz-Moyano et al. [45], who evaluated the integration of *L. fermentum* HL57 and *P. acidilactici* SP979 into traditional Iberian dry-fermented sausages. Their study confirmed the survival of these strains through fermentation and ripening while maintaining acceptable sensory profiles. Notably, both strains exhibited acidifying activity, salt tolerance, and antimicrobial potential, including bacteriocin production. These findings reinforce the importance of evaluating starter cultures not only for their in vitro performance but also for their real-world behavior in complex food matrices. Although our study focused on the preliminary characterization of *L. plantarum* BG112, the observed stress tolerance and antimicrobial capacity are in line with the traits described by Ruiz-Moyano et al. [45]. This supports the rationale for conducting follow-up trials with *L. plantarum* BG112 in fermented meat products to verify its functional properties and influence on sensory and microbial safety outcomes.

Compared to commercial starter cultures, such as *L. sakei* CTC494 or *P. acidilactici*, which are widely adopted for their predictable acidification and antimicrobial properties, *L. plantarum* BG112 offers similar technological advantages. Moreover, several autochthonous strains isolated from traditional products, like *L. fermentum* HL57 or *P. acidilactici* SP979,

have been reported to enhance microbial safety and retain sensory profiles in Iberian sausages. These strains often exhibit better adaptation to the native meat microbiota.

In this comparative context, *L. plantarum* BG112 demonstrates a promising profile, including high auto-aggregation capacity and antimicrobial activity, essential for colonization and pathogen exclusion. Its versatility may become an asset in designing multifunctional starter cultures adaptable to various fermented foods.

To confirm its application potential, further in situ trials in meat matrices are required, including physicochemical impact and consumer acceptability.

4. Conclusions

In summary, this study provides a preliminary in vitro characterization of *L. plantarum* BG112, evaluating safety and technological properties relevant to its potential use as a starter culture in dry-fermented meat products at 15 and 25 °C. The strain demonstrated favorable survival under stress conditions simulating the fermentation and ripening processes, along with desirable safety traits, such as the absence of hemolytic and DNase activity and sensitivity to clinically relevant antibiotics. Additionally, it exhibited antimicrobial activity and tolerance to low pH, sodium nitrite, and bile salts. Although some functional attributes associated with probiotics were observed, these results are part of an initial screening and are not sufficient to support the designation of the strain as a probiotic or potentially probiotic. Microbiological analyses suggest that the strain has promising properties for use in fermented meat products, such as salami, highlighting its tolerance and viability. Further studies involving the use of a meat matrix are necessary to advance this research. Substituting the MRS broth medium with a meat matrix in "in situ" assays is the next step to validate the "in vitro" results. These findings contribute to the early-stage selection of strains with promising technological and safety profiles for use in the development of innovative fermented meat products, contributing both to the diversification of traditional products and to the development of nutritionally enhanced alternatives.

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Abbreviations

The following abbreviations are used in this manuscript:

AAC	Argentinean Alimentarius Codex
C	control group
CFUs	colony-forming units
DNAse	Deoxyribonuclease
FAO	Agriculture Organization of the United Nations
GS	gastric solution
IST	Iso-Sensitest
LAB	lactic acid bacteria
MICs	minimum inhibitory concentrations
MS	moderately sensitive
PBS	phosphate-saline buffer
R	resistant
S	susceptible
TSA	Trypticase Soy Agar
WHO	World Health Organization

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