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Antagonistic Activity of Bacteriocin-like Inhibitory Substances from *Enterococcus lactis* Isolated from the Surface of Jalapeno Pepper against Foodborne Pathogens

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Abstract: Lactic acid bacteria (LAB) can produce peptides known as bacteriocins with antagonistic activity against foodborne pathogens. The potential of LAB isolated from the surface of jalapeno peppers to produce bacteriocins with antagonistic activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium was evaluated. Previously isolated LAB strains were reactivated, and their cell-free supernatants (CFSs) were evaluated. Out of 390 reactivated strains, 60 produced bacteriocin-like inhibitory substances (BLIS) since their antagonistic activity was lost after proteases addition. Subsequently, 16 BLIS showed heat resistance (HR-BLIS), retaining their bioactivity after heat treatment (121 °C for 15 min). By 16S rRNA gene sequencing and antibiotic susceptibility tests, LAB strains producing HR-BLIS were identified as *Enterococcus lactis*. Four HR-BLIS exhibited a minimum inhibitory concentration (MIC) of 80 mg/mL against *L. monocytogenes*. MIC and minimum bactericidal concentration (MBC) of HR-BLIS-67 for *S. aureus* (MIC = 80 mg/mL; MBC = 320 mg/mL), *S.* Typhimurium (MIC = 150 mg/mL; MBC = 250 mg/mL), and *E. coli* O157:H7 (MIC = 250 mg/mL; MBC = 400 mg/mL) were determined. LAB isolated from the surface of jalapeno pepper produced HR-BLIS (possibly enterocin) that exhibited broad-spectrum antagonistic activity against foodborne pathogens; therefore, they are a promising source of natural antimicrobials to ensure food safety.

Keywords: enterocin; biopreservative; *L. monocytogenes*; *S. aureus*; *E. coli* O157:H7; Salmonella

Citation: Hernandez-Mendoza, E.; Peña-Ramos, E.A.; Juneja, V.K.; Martínez-Téllez, M.Á.; González-Ríos, H.; Paredes-Aguilar, M.d.l.C.; Valenzuela-Melendres, M.; Aispuro-Hernández, E. Antagonistic Activity of Bacteriocin-like Inhibitory Substances from *Enterococcus lactis* Isolated from the Surface of Jalapeno Pepper against Foodborne Pathogens. *Microbiol. Res.* **2024**, *15*, 889–899. https://doi.org/10.3390/microbiolres15020058

Academic Editor: Yiannis Kourkoutas

Received: 5 April 2024
Revised: 1 May 2024
Accepted: 13 May 2024
Published: 22 May 2024

1. Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive microorganisms widely distributed in nature, so they can be isolated from different food sources, including fruits and vegetables like cantaloupe, pickles, tomato, and peppers [1–3]. Native to Mexico, jalapeno peppers (*Capsicum annuum*) are grown throughout much of the country and account for 31% of all pepper production in Sonora, having an important impact on local economy. Within its microbiome, LAB are present, which may play a protective role against the spoilage of this type of pepper [4]. Among the most representative genera are *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Enterococcus*. In addition, LAB have been used in the food industry as natural biopreservatives due to the production of inhibition agents with antimicrobial activity, known as bacteriocins [5].

Bacteriocins are bioactive peptides with the ability to inhibit the growth of pathogenic and spoilage microorganisms, with a bactericidal and/or bacteriostatic effect [6]. Some bacteriocins are heat resistant and effective across a broad pH range, particularly in acidic media [7]; these characteristics make them ideal for use as biopreservatives in foods. Nisin and pediocin are the most studied and commercially available bacteriocins that have been approved for use as food additives by the food and drug administration. Nisin is produced by *Lactococcus lactis*, and pediocin PA-1/AcH from *Pediococcus acidilactici*. Both possess antagonistic activity against Gram-positive bacteria; however, they are not effective against Gram-negative bacteria when used alone, limiting their applications [8,9].

There are various criteria to consider when classifying bacteriocins, such as their genetic and molecular weight, as well as their physical, chemical, and phenotypic properties [10]. Bacteriocins produced by the genus *Enterococci* are named enterocins [11]. Enterocins act by inhibition of the synthesis of the cell membrane components or by pore formation leading to cell lysis [12]. In general, bacteriocins are more effective against Gram-positive bacteria than Gram-negative bacteria. However, new LAB-derived bacteriocins, including those from vegetable sources, have demonstrated antimicrobial activity against several pathogens of both types of bacteria [1–3,13,14]. Although these studies confirm the antimicrobial effects of LAB-bacteriocins from vegetables, there are vegetable sources that are yet to be studied, such as jalapeno peppers, that may also have the potential to contain bacteriocin-LAB producers with antagonistic effects that could unveil natural alternatives for food preservation.

Among the main pathogenic bacteria that compromise food safety are *L. monocytogenes*, *S. aureus*, *S.* Typhimurium, and *E. coli* O157:H7 [15–18]. Outbreaks of infections caused by these pathogens [19] have been reported to cost hundreds of billions of dollars per year in productivity losses and medical expenses related to contaminated food consumption [20]. *L. monocytogenes* stands out for its ability to persist in ready-to-eat products after surviving multiple hurdles like heat treatment, low pH, refrigeration conditions, and high salt concentrations [21]. Therefore, it is an enormous challenge for the food industry to control and ensure the safety of their products against all pathogens, especially *L. monocytogenes*. Hence, the search for antimicrobial alternatives, preferably of natural origin with broad-spectrum antimicrobial bioactivity is very important. For these reasons, the objective of this investigation was to identify LAB isolated from the surface of jalapeno peppers with the ability to produce bacteriocins with antagonistic effect against foodborne pathogens.

2. Materials and Methods

2.1. Bacteria Strains: Conditions and Activation

A total of 390 LAB strains previously isolated from jalapeno pepper collected from commercial fields in Sonora, Mexico, belonging to the strain collection of the Molecular Plant Physiology Laboratory of the Research Center for Food and Development (CIAD) were used during this experiment. LAB strains were cryopreserved at −80 °C and were reactivated by inoculation in Man, Rogosa, and Sharpe (MRS) broth (DIFCO, Detroit, MI, USA) pH 7.0 ± 0.2 at 37 °C for 18 h. Two transfers were made under the same conditions as the previous inoculum and used in further experiments. All LAB strains were numerically labelled consecutively from 1 to 390.

Cryopreserved pathogenic bacteria (Table 1) at −80 °C in Brain-Heart Infusion (BHI) Broth (DIFCO, USA) with 15% glycerol were reactivated by transferring 0.1 mL of the inoculum to BHI broth and incubated at 37 °C for 20 h. Cultures were maintained in BHI broth at 8 °C throughout the study with monthly transfers. One day before the experiment, the inoculum of each strain was prepared by transferring 0.1 mL of the stock culture to 35 mL of BHI broth in 50 mL conical centrifuge tubes and incubated at 37 °C for 18 h with constant stirring. Overnight cultures were centrifuged (10,000× *g*, 10 min, 4 °C), and the resulting pellet of each strain was washed with 20 mM sodium phosphate buffer pH 6.5 ± 0.2, adjusted to an optical density at 600 nm of 0.1 and used during experiments.

Table 1. Pathogenic strains used with their designation.

Strains	Designation
Listeria monocytogenes	ATCC 7644
Staphylococcus aureus	ATCC 6538
Escherichia coli O157:H7	K3999 (FDA/CFSAN)
Salmonella Typhimurium	ATCC 14028

2.2. Screening for LAB from Jalapeno Pepper with Antagonistic Activity against L. monocytogenes

The antagonistic activity of the reactivated LAB isolates from jalapeno peppers was tested against *L. monocytogenes* using the spot-on-lawn method described by Hilal Cadi and Citak (2005) [22]. A volume of 20 µL of each LAB was spotted on the surface of MRS agar plates and allowed to dry. Next, the microbial inoculum (O.D. 0.1) was mixed in BHI (DIFCO, NJ, USA) soft agar medium and overlayed onto the MRS plates which contained the LAB strains. After solidification, the plates were incubated at 37 °C for 24 h. Positive results were those LAB strains which presented a clear inhibition zone around the LAB culture.

2.3. Preparation and Antagonistic Activity Evaluation of Cell-Free Supernatants (CFSs)

Cell-free supernatants of selected LAB were obtained from fresh overnight (18 h growth) cultures, centrifuged (10,000× *g*, 10 min, 4 °C), frozen at −80 °C, and freeze-dried (Labconco Freezone 4.5, Kansas City, MO, USA) in order to concentrate the bioactive compounds in the supernatants. Freeze-dried CFSs were resuspended (1:10 *w*:*v*) in 20 mM sodium phosphate buffer pH 7.0 ± 0.2, sterilized by microfiltration (Durapore®, 0.22 µm size; Millipore Co., St. Louis, MO, USA), and used for subsequent analyses. The antagonistic activity against *L. monocytogenes*, *S. aureus*, *S.* Typhimurium, and *E. coli* O157:H7 was tested by the spot-on-lawn test. After incubation at 37 °C for 24 h, clear growth zones around the CFS drop were visually detected.

2.4. Identification of the Antagonistic Compound in CFSs and Heat Stability Test

Using the method described by Cruz-Guerrero et al. (2014) [23], the chemical nature of antimicrobial compounds within each CFS was identified. Briefly, this technique consisted of first adding NaOH (0.1 M) to adjust pH to 7 to neutralize the effect of CFSs' organic acids (lactic acid); then adding 1 mg/mL of catalase (90 min at 25 °C and 10 min at 65 °C) to prevent the bactericide effect of H_2O_2; and finally, to confirm the production of protein compounds, a pool of proteases (protease, proteinase K, and trypsin at 1 mg/mL) was added, incubated for 2 h at 37 °C, and inactivated at 65 °C for 10 min, followed by the antagonistic test against *L. monocytogenes* between each step. For further discrimination, the thermal stability evaluation was performed on those CFSs containing antibacterial protein compounds by submitting them to different thermal treatments (80 or 100 °C for 10 min or 121 °C for 15 min), followed by the inhibition test against *L. monocytogenes*.

2.5. LAB 16S rRNA Sequence Analysis and Identification

For molecular identification, genomic DNA from selected LAB, based on their ability to produce heat-resistant CFS (HR-CFS) were extracted by alkaline lysis according to the Molecular Cloning Laboratory Manual 2012 [24] and used as template for standard PCR reactions using GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA). Universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1301R (5′-TACTAGCGATTCCGACTTC-3′) were used. The PCR conditions were as follows: initial denaturation at 95 °C for 10 min; 30 denaturation cycles at 95 °C for 1 min each; primer alignment at 55 °C for 1 min and primer extension at 72 °C for 2 min; and a final extension step at 72 °C for 10 min. Next, for the sequence analysis of the gene 16S rRNA, the PCR products were purified with GFX columns (Amersham Bio-sciences, Piscataway, NJ, USA) and Sanger sequenced at Macrogen (Seoul, Korea) using the 518F universal primer (5′-CCAGCAGCCGCGGTAATACG-3′). Sequences were analyzed

against the GenBank database with the Blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed on 10 October 2023), and the DNA sequences of the top hit matches were used as reference organisms for phylogenetic analysis. DNA sequence alignments were performed with the Clustal W function, and a phylogenetic tree was constructed with MEGA-X software v. 10.2.6. using the maximum-likelihood method and the general time-reversible model with gamma distribution to estimate the evolutionary distances (1000 bootstrap replicates).

Furthermore, to discriminate between LAB strains, antibiotics susceptibility of identified isolates was carried out by advanced colorimetric tests using the VITEK® 2 Compact equipment. The equipment applied a total of 43 biochemical tests, including 17 enzymatic tests for up to 8 h [25].

2.6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Antimicrobial activity of freeze-dried HR-CFSs was evaluated following the method described by Morales-Figueroa et al. (2022) [26] using the broth microdilution technique in a 96-well microplate (COSTAR). Each well was inoculated with 5 µL of adjusted microbial inoculum (O.D. 0.1. approximately 1×10^8 CFU/mL) and mixed with 295 µL of diluted filter-sterilized freeze-dried HR-CFSs (10–400 mg/mL). Furthermore, three wells with only BHI broth and the other three with pathogen + BHI were used as negative and positive controls, respectively. Microplates were incubated for 24 h at 37 °C and the next day, 20 µL from each well were plated into TSA and incubated under the same conditions. MIC was established as the lowest concentration of each resuspended HR-CFS where growth inhibition was unveiled; MBC, as the lowest concentration required to inactivate 99.9% of the pathogen where no visible growth was detected in TSA plates. Experiments were executed in triplicates, and the results were expressed as mg/mL. HR-CFSs with the lowest MICs for *L. monocytogenes* were selected for further experiments.

2.7. Growth Inhibition Assay against L. monocytogenes

The effect of different concentrations (40, 80, 160, and 320 mg/mL) of selected HR-CFSs against *L. monocytogenes* growth was carried out using a 96-well microplate. Bacteria (5 µL; O.D. 0.1) were treated with different concentrations of HR-CFSs in BHI broth, achieving a final volume of 300 µL per well. The microplate was placed into a FLUOstar Omega microplate reader (BMG LabTech, Ortenberg, Germany) for 18 h at 37 °C. Optical density (600 nm) was recorded every 30 min. Positive and negative controls were prepared using BHI broth with and without *L. monocytogenes*, respectively. Subsequently, the most effective HR-CFS, with the longest lag phase and lowest growth rate of *L. monocytogenes* at the lowest concentration, was selected and its MIC and MBC against *S. aureus*, *S.* Typhimurium, and *E. coli* O157:H7 were established following the procedure described in the previous section.

2.8. Statistical Analysis

To estimate lag time (h) and maximal growth rate (µmax; OD/h), optical density values of selected HR-CFSs against *L. monocytogenes* growth were plotted against time. Growth curves were fitted using DMFit add-in version 3.5 (Baranyi and Roberts, 1994) in Excel (Microsoft Office excel 2019). The most appropriate model was selected considering the determination coefficient or R^2.

3. Results

3.1. Jalapeno Pepper's LAB Antagonistic Activity against L. monocytogenes

Out of 390 LAB isolates from jalapeno pepper collected from Sonora, Mexico, a total of 72 isolates showed antimicrobial activity against *L. monocytogenes*, evidenced by their clear inhibition zones after the spot-on-lawn assay (Figure 1). The CFSs of these bioactive LAB isolates were freeze-dried for further in vitro inhibition assays.

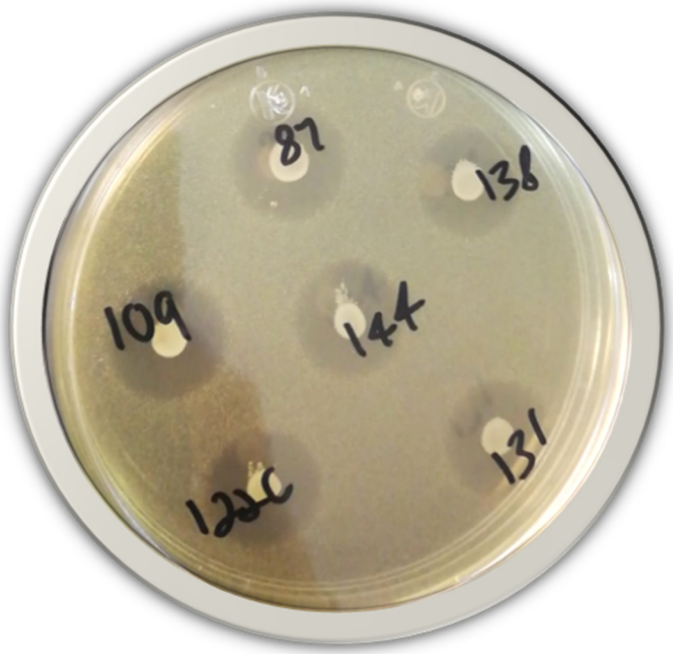

Figure 1. Representative plate showing the inhibition zones of LAB strains 87, 109, 122C, 131, 138, and 144.

3.2. In Vitro Assays of CFSs against Pathogens

Freeze-dried CFSs obtained from the 72 LAB isolates with antagonistic effect against *L. monocytogenes* were tested against different Gram-positive and Gram-negative pathogens. After adjusting the pH to 7 and adding catalase to the media, with the purpose of searching for the nature of the antagonistic compound, a total of 60 CFSs retained their antimicrobial activity against *L. monocytogenes* and *S. aureus* with clear inhibition zones, while no antimicrobial activity was detected for *S*. Typhimurium or *E. coli* O157:H7 at the evaluated concentration (100 mg/mL). The inhibitory effect of these 60 bioactive CFSs can be attributed to bacteriocin-like inhibitory substances (BLIS), since its antagonistic activity was completely inactivated after treating bioactive CFSs with different proteolytic enzymes (protease, trypsin, and proteinase K). These 60 BLIS were subjected to a thermal evaluation in order to select those able to resist heat, and 16 BLIS retained their antagonistic activity against the Gram-positive bacteria after being treated at sterilization conditions of 121 °C for 15 min.

The MIC values of the 16 heat-resistant BLIS (HR-BLIS) against *L. monocytogenes* ranged between 80 and >100 mg/mL. Samples from LAB strains 67, 144, 172, and 205 showed the lowest MIC values of 80 mg/mL. The ability of different concentrations of these four samples to inhibit the growth of *L. monocytogenes* is depicted in Figure 2. Control samples without HR-BLIS showed a normal growth curve for *L. monocytogenes* with characteristic lag, exponential, and stationary phases. Then, by treating *L. monocytogenes* with HR-BLIS at 80, 160, or 320 mg/mL, their inhibitory and bactericide effects can be noticed by prolonging the lag phase, preventing the beginning of the exponential phase for over 18 h. Figure 2 also depicts that the growth of *L monocytogenes* was affected depending on the source of the HR-BLIS, when samples were added at only 40 mg/mL (0.5 MIC). Therefore, growth parameters were determined in order to establish which HR-BLIS was the most effective (Table 2). In general, the estimated maximum growth rate of *L. monocytogenes* decreased by 83, 78, 61, and 70% when HR-BLIS from LAB strains 67, 144, 172, and 205, respectively, were added at 40 mg/mL. HR-BLIS of strain 67 (HR-BLIS-67) achieved the highest estimated lag phase time of 13 h, in comparison to the other evaluated HR-BLIS, and it was selected for further inhibition assays (MIC and MBC) against other pathogens (*S. aureus*, *S*. Typhimurium, and *E. coli* O157:H7).

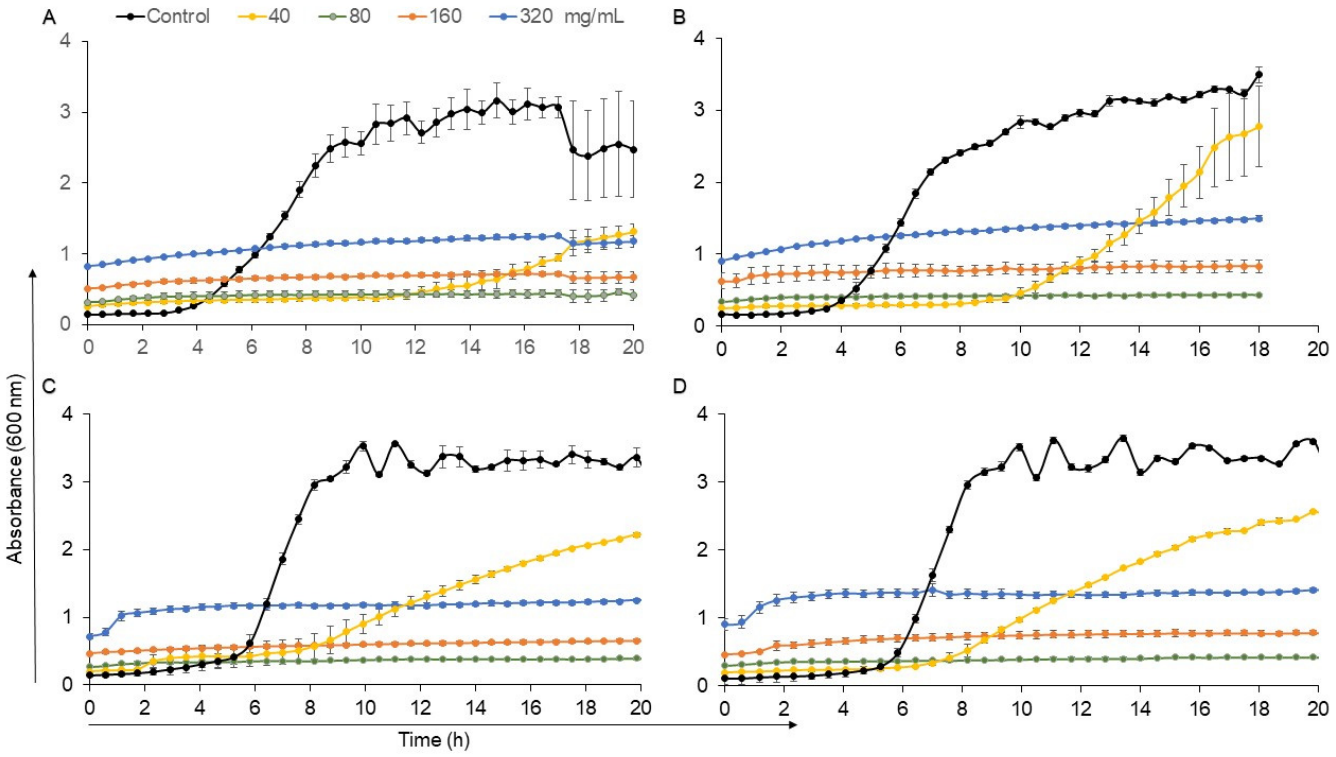

Figure 2. Growth curves of *L. monocytogenes* with different amounts (40–320 mg/mL) of heat-resistant bacteriocin-like inhibitory substances. Strains (**A**) 67, (**B**) 172, (**C**) 144, and (**D**) 205, isolated from the surface of jalapeno peppers. Data are expressed as means ± SE (n = 3).

Table 2. Kinetic parameters of *L. monocytogenes* with 40 mg/mL of heat-resistant bacteriocin-like inhibitory substances from *Enterococcus* spp.

Enterococcus	Growth Rate (OD/h)	Lag (h)	R^2
67	0.1256	12.99	0.99
144	0.1616	6.26	0.99
172	0.2891	12.17	0.99
205	0.2212	6.59	0.99
L. monocytogenes	0.7355	5.15	0.99

MIC and MBC of HR-BLIS-67 for *L. monocytogenes*, *S. aureus*, *S*. Typhimurium, and *E. coli* O157:H7 are shown in Table 3. Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) had an MIC of 80 mg/mL and an MBC of 320 mg/mL to exert a bactericide effect. However, for Gram-negative bacteria (*S*. Typhimurium and *E. coli* O157:H7), the MIC required for an inhibitory effect was 150 and 250 mg/mL, and to achieve the MBC, it was 250 and 400 mg/mL for *S*. Typhimurium and *E. coli* O157:H7, respectively.

Table 3. Minimum inhibitory concentration (MIC) and bactericide concentration (MBC) of heat-resistant bacteriocin-like inhibitory substances from strain 67.

Pathogen	MIC (mg/mL)	MBC (mg/mL)
L. monocytogenes	80	320
S. aureus	80	320
S. Typhimurium	150	250
E. coli O157:H7	250	400

3.3. LAB 16S rRNA Sequencing and Identification

The alignment of genomic sequences allowed the classification of 16 HR-BLIS-producer LAB as members of the genus *Enterococcus* spp., with high similarity scores to *E. lactis*, *E. faecium*, *E. durans*, and *E. faecalis* according to the BLAST algorithm. Further, the phylogenetic analysis showed that three isolates (67, 87, and 172) were more related (53%) to the *Enterococcus lactis* IS05 strain. However, for the remaining LAB isolates (12, 13, 64, 78, 109, 122, 131, 138, 144, 155, 205, 220, and 166), this molecular approach was not enough to discern between *E. lactis* and *E. faecium* (Figure 3), which are closely related species with a high 16S rRNA gene sequence identity. Therefore, after analyzing the antibiograms of these LAB strains, samples were categorized into five groups according to their antibiotic susceptibility profiles (Table 4). Samples 64, 12, 144, and 109 have unique profiles, while samples 13, 67, 78, 87, 122, 131, 138, 155, 166, 172, 205, and 220 all share the same profile.

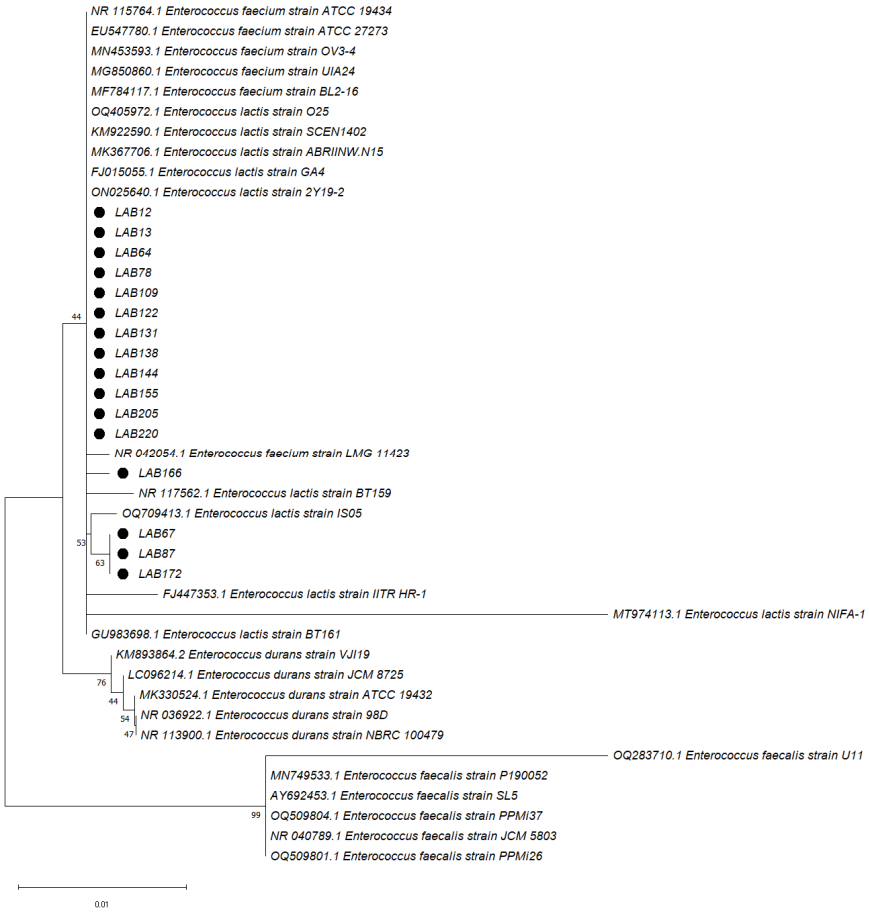

Figure 3. Tree phylogenetic relationship amongst LAB isolated from jalapeno peppers. Maximum likelihood tree based on 16S rRNA gene sequences. Bootstrap support values are displayed at the three nodes, and the branch lengths and scale bar represent the number of substitutions per site. • LAB isolates from Sonora, Mexico.

Table 4. Antibiotic susceptibility profiles of *Enterococcus* spp. isolated from the surface of jalapeno peppers.

Enterococcus	Antibiotic											
	AMP	**GEN**	**STR**	**CIP**	**LEX**	**ERI**	**LZD**	**VAN**	**DOX**	**TCY**	**TIG**	**NIT**
12	S	S	S	S	S	I	S	S	I	I	S	I
13	S	S	S	S	S	I	S	S	S	S	S	I
64	S	S	S	S	S	I	S	S	S	S	S	S
67	S	S	S	S	S	I	S	S	S	S	S	I
78	S	S	S	S	S	I	S	S	S	S	S	I

Table 4. *Cont.*

Enterococcus	Antibiotic											
	AMP	**GEN**	**STR**	**CIP**	**LEX**	**ERI**	**LZD**	**VAN**	**DOX**	**TCY**	**TIG**	**NIT**
87	S	S	S	S	S	I	S	S	S	S	S	I
109	S	S	S	S	S	I	R	R	R	I	S	I
122	S	S	S	S	S	I	S	S	S	S	S	I
131	S	S	S	S	S	I	S	S	S	S	S	I
138	S	S	S	S	S	I	S	S	S	S	S	I
144	S	R	S	S	S	I	R	S	R	I	S	I
155	S	S	S	S	S	I	S	S	S	S	S	I
166	S	S	S	S	S	I	S	S	S	S	S	I
172	S	S	S	S	S	I	S	S	S	S	S	I
205	S	S	S	S	S	I	S	S	S	S	S	I
220	S	S	S	S	S	I	S	S	S	S	S	I

S: Sensitive, I: Intermedium, R: Resistant. AMP: Ampicillin, GEN: Gentamicin, STR: Streptomycin, CIP: Ciprofloxacin, LEX: Levofloxacin, ERI: Erythromycin, LZD: Linezolid, VAN: Vancomycin, DOX: Doxycycline, TCY: Tetracycline, TIG: Tigecycline, NIT: Nitrofurantoin.

4. Discussion

The analysis performed to identify LAB in jalapeno pepper suggested different *Enterococcus* spp., specifically *E. lactis* and *E. faecium*, as the LAB responsible for producing the antimicrobial compounds (Figure 3). The lack of distinction between these two species might be caused by the similarity in their nucleotide sequences in the analyzed gene (16S rRNA). Currently, there is controversy between the classification of *E. faecium* and *E. lactis* species and how to distinguish between these two bacteria. Li and Gu (2021) [27] confirmed that *E. faecium* and *E. lactis* were different species. Conversely, other reports found that some *E. faecium* strains belong, in fact, to *E. lactis* species [28,29]. Nevertheless, in an earlier study by Morandi et al., [30] *Enterococcus lactis* was introduced in 2012 after sequencing specific genes and applying biochemical and antibiotic tests. They showed that *E. lactis*, unlike *E. faecium*, was susceptible to vancomycin, an important antibiotic effective against a broad spectrum of multi-drug-resistant pathogens. Susceptibility to this antibiotic is an important feature for LAB strains with the potential to be used as biopreservatives. Table 4 shows that, with the exception of strain 109, 15 of the 16 bioactive strains isolated from jalapeno peppers were susceptible to vancomycin; therefore, they could be classified as *E. lactis* strains. These strains may play a protective role against human foodborne pathogens by competition for space and nutrients and preventing pathogen adhesion due to the production of stable inhibitory compounds, such as bacteriocins [31].

Since the antagonistic effect shown by the CFSs from *E. lactis* isolated from jalapeño pepper was lost after adding proteases to the media, their CFSs' bioactivity can be attributed to bacteriocin-like inhibitory substances (BLIS), possibly enterocins [32]. Various studies have shown the ability of *Enterococcus* strains to produce different kinds of enterocins, including enterocins A, B, and/or P with antagonistic activity against pathogenic bacteria and fungi [33,34]. BLIS from *Enterococcus* spp., including those isolated from vegetables sources, possess a higher in vitro spectrum activity against pathogenic bacteria (including Gram-negative bacteria) in comparison to BLIS reported from other LAB from other food sources, such as dairy and meat products [35,36].

Thermostability is an important and desirable property for new food biopreservatives intended to be used in the food industry, especially if they are going to be used as part of a hurdle system where a thermal processing is commonly required. A total of 16 CFSs of *Enterococcus lactis* from jalapeno peppers were identified as HR-BLIS (121 °C for 15 min) since they were able to maintain their ability to inhibit the growth of pathogenic bacteria. This thermoresistant property has also been reported for a few other enterocins (As-48 and Gr17, LD3, and mudticin) produced by *E. faecalis, E. hirae,* and *E. mundtii* [37,38] and some *E. faecium* subspecies isolated from different vegetable sources, such as Chinese pickles [39] and black olives [40].

It is worth pointing out that an MIC of 80 mg/mL of HR-BLIS from selected *Enterococcus* isolated from jalapeno peppers for *L. monocytogenes* is significantly lower than previously reported MICs for nisin of 740 or 14,800 mg/mL against *L. monocytogenes* ATCC 7644 and ATCC 7644K, respectively [41]. The bactericide effect shown by HR-BLIS against Gram-positive bacteria has also been reported for other enterocins [37,42,43]. Enterocins' function by destabilizing the bacterial cell wall or the cytoplasmatic membrane, causing leakage of intracellular content or by inhibiting gene expression, leading to cell death [44].

The addition of different concentrations of HR-BLIS (equal and above the MIC) into the growth media of *L. monocytogenes* confirmed their inhibitory effects (Figure 2). Furthermore, evaluating the addition of only 0.5 MIC (40 mg/mL) helped to identify HR-BLIS-67 as the most effective sample since it was more efficient in increasing the lag phase and decreasing the growth of *L. monocytogenes* in comparison with the other HR-BLIS evaluated. The antagonistic activity of HR-BLIS-67 against Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) was expected, since bacteriocins are reported to be more effective against this type of bacteria [45].

Conversely, bactericide and inhibitory effects against Gram-negative bacteria (*S*. Typhimurium and *E. coli* O157:H7) (Table 3) have only been reported for a few other bacteriocins from LAB isolated from vegetable sources. However, MICs of 150 and 250 mg/mL of HR-BLIS-67 for *Salmonella* and *E. coli* O157:H7, respectively, are considerably higher when compared to those reported for CFS from *Pediococcus pentosaceus* (CM175) isolated from cantaloupe [1], crude bacteriocin from *Lactobacillus plantarum* from molasses [13], or *L. pentosus* DZ35 from pickles [14] where they use concentrations as low as µg/mL to inhibit the growth of Gram-negative bacteria (*S.* Typhimurium, *S.* Saintpaul, and/or *E. coli* O157:H7). Differences found between HR-BLIS-67 and these previous reports regarding their ability to inhibit Gram-negative bacteria may be due to differences of several environmental factors, which may impact their native microbiome [4]. As a response to harsh environmental factors, LAB can generate different antagonistic compounds, including BLIS, which can be more bioactive when compared with the BLIS obtained in the present study from *E. lactis*. These bioactivity differences enhance the necessity to continue the screening, isolation, and BLIS characterization of LAB from different food sources and to study their antagonistic capabilities against foodborne pathogens.

5. Conclusions

Enterococcus lactis strain 67 isolated from the surface of jalapeno peppers from Sonora, Mexico possess the ability to produce HR-BLIS (possibly enterocin) that exhibit broad-spectrum antagonistic activity against *L. monocytogenes*, *S. aureus*, *S.* Typhimurium, and *E. coli* O157:H7. Therefore HR-BLIS-67, is a promising natural antimicrobial alternative for the control of foodborne pathogens and promote food safety. Further analysis to purify and characterize HR-BLIS-67 should be performed to elucidate its stability and mechanism of action, as well as to perform in situ assays to confirm that its bioactivity is maintained once used in a food matrix, such as meat.

Author Contributions: Conceptualization, E.A.P.-R. and M.Á.M.-T.; methodology, E.H.-M. and E.A.-H.; software, E.A.-H.; formal analysis, M.V.-M. and E.A.-H.; investigation, E.H.-M.; resources, M.d.l.C.P.-A.; writing—original draft preparation, E.H.-M. and E.A.P.-R.; writing—review and editing, H.G.-R. and V.K.J.; visualization, E.H.-M., E.A.P.-R., M.Á.M.-T., H.G.-R. and E.A.-H.; supervision, E.A.P.-R. and V.K.J.; project administration, E.A.P.-R.; funding acquisition, M.Á.M.-T. and M.d.l.C.P.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are available upon request.

Acknowledgments: Author Hernandez-Mendoza wants to acknowledge CONAHCYT for the granted doctoral fellowship.

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

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