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Research Article

Inhibitory Substances Produced by Native *Lactobacillus plantarum* UTNCys5-4 Control Microbial Population Growth in Meat

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Microbial contamination is the cause of extensive economic loss in the food sector. Previously, the wide-range antimicrobial capacity of inhibitory substances secreted by the *Lactobacillus plantarum* UTNCys5-4 strain was demonstrated *in vitro*; however, its mechanism of action in the food matrix remains unclear. This study was aimed to evaluate the effect of antimicrobials produced by the Cys5-4 strain in raw meat applied as pure cell cultures, cell-free supernatant (CFS), and partially purified peptides. The bacteriological results indicated the presence of commensal microbes exhibiting resistance to several antibiotics in meat samples purchased from the local market. Dipping solutions containing antimicrobial substances produced by Cys5-4 resulted in a decrease by 1.91, 1.69, and 1.55 $\log_{10}$ in cell counts upon addition of CFS, peptides and respectively pure cell culture in raw meat at day 9 of storage with refrigeration. The microbial population was maintained in the untreated meat samples during storage. An increase in pH and a concentration of released ammonia was detected in nontreated meat, indicating protein degradation. The Cys5-4 peptides exerted their bacteriolytic mode of action inducing damage in the cell membrane of the target bacteria, allowing the leaching of DNA/RNA content. The results indicate that coating meat with CFS containing Cys5-4 is a promising approach to protect against further contamination by microorganism spoilage, as well as an alternative for increasing the shelf life of raw meat.

1. Introduction

The contamination of food with microorganisms, their persistence, growth, multiplication, and/or toxin production has emerged as an important public health concern [1]. Meat remains one of the most consumed products, but it is very perishable and rapidly deteriorated [2]. In Ecuador, artisanal meat-based foods are mainly sold on the street; therefore, they are prone to contamination. Despite the growth of the food sector and the harmonization of national food regulations with international standards, there has been no improvement in the food manipulation or personal hygiene control. Since foodborne pathogens do not give an organoleptic indication of their presence, there is no warning to the consumer about the quality of meat-based foods; thus, considerable human illnesses related to food adulteration have been reported [3]. Conventionally, meat products are preserved using nitrites to inhibit mostly *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium,* and *E. coli* [4, 5]. Knowing that these chemical methods contribute to the reduction of the nutritional value of food, the challenge is to provide natural ingredients with a larger spectrum of inhibition and which are less toxic to the consumer [6]. Thus, antimicrobial peptides (i.e., bacteriocins) produced by some lactic acid bacteria (LAB) species have become more attractive for food preservation [7]. In meat, only recently, the use of bacteriocinogenic LAB for the purpose of preservation was reported [8–11]. Nonetheless, nisin remains the only additive used for commercial purposes [12]. In general, the efficacy of bacteriocins to control pathogen growth depends on the producer strain's capacity to compete with other bacteria in the same microenvironment; therefore, the selection of strains with

wide-ranging antimicrobial activity is of interest. We previously reported the antimicrobial potential of several LAB species originating from extreme microenvironments such as tropical wild-type fruits of the Amazon [13]. Among them, a bacteriocinogenic *Lactobacillus plantarum* UTNCys5-4 producing plantaricin W showed elevated potential to inhibit *E. coli* and *Salmonella* in orange juice when applied as cell-free supernatant [14]. Consequently, the antimicrobials produced by this strain were considered as suitable candidates to be evaluated in a solid matrix such as beef meat for the purpose of conservation. In this study, the effect of inhibitory substances produced by Cys5-4 applied as cell culture, cell-free supernatant, and partial purified peptides, on diminishing or controlling the growth of spoilage bacteria in raw meat was evaluated. In addition, the pH and released ammonia were measured to examine meat quality upon bio-preservative addition. Moreover, we expected to understand the mechanism by which these compounds act within a bacterial system.

2. Material and Methods

2.1. Bacterial Strains and Preparation of Antimicrobial Compounds. L. plantarum UTNCys5-4 (GenBank No. KY041686.1) isolated from tropical Amazonian wild-type fruits of *Malus* sp. (Sucumbíos Provence) and *L. plantarum* ATCC 8014 (LP) was used to prepare the pure cell cultures, cell-free supernatant (CFS), and precipitated peptides. Briefly, the LAB strains grown in MRS broth at 37°C for 24 h were used to extract both CC and CFS by centrifugation at 13,000 ×g for 30 min (4°C). The supernatant was filtered using a 0.22 μm porosity syringe filter (#STF020025H, Chemlab Group, USA). To obtain PP, 60% ammonium sulphate solution was added to CFS followed by overnight incubation with refrigeration without stirring and centrifuged at 8,000 ×g for 30 min at 4°C. The PP were recovered in 25 mM ammonium acetate (pH 6.5), desalted by using a midi dialysis kit (cat # PURD10005-1KT, Sigma-Aldrich, USA) pre-equilibrated with phosphate buffer (pH 7.0) and stored at (−) 20°C before use in antimicrobial assays. The antimicrobial activity *in vitro* of CFS and peptides was evaluated as previously reported [13].

2.2. Bacteriological Analysis of Meat after Purchasing. Meat (approximately 650 g/batch) consisting of bovine muscle was purchased from an ambulatory local vender and kept in refrigeration for about 1 h until used in the experiment. The bacteriological analysis was performed in concordance with the Ecuadorian Normative [15, 16]. Briefly, 25 g of meat was inoculated for pre-enrichment in buffered peptone water (1%), homogenized, and incubated for 4–6 h at 37°C; decimal dilutions made with sterile water were inoculated on Plate Count Agar (Difco) to determine the growth of mesophilic aerobic and psychrotrophic aerobic bacteria (above 20°C, 48 h); moreover, aliquots (100 μL) were plated on SS (Shigella-Salmonella, Difco) and incubated for 48 h at 37°C–40°C to determine the presence of both *Salmonella* and *Shigella*. In addition, to confirm the presence of *Salmonella* (according to ISO 6579-2:2017), a selective enrichment of 0.1 mL of pre-enriched culture in 10 mL on Rappaport–Vassiliadis Soy (RVS, Sigma) broth for 24 h at 42°C and 1 mL of pre-enriched culture in 10 mL of Muller–Kauffmann Tetrathionate-Novobiocin (MKTTn, Merck) broth for 24 h, at 37°C was used; followed by selective isolation streaking 10 μL of RVS and 10 μL of MKTTn on plates of XLD agar and brilliant green agar for 18–24 h at 37°C; and for confirmation of *Salmonella*, the Latex agglutination test (Oxoid, Italy) was performed. In independent experiments, aliquots (100 μL) were placed on chromocult agar (Merck) to determine the total coliforms and eosin methylene blue (Difco), for the presence of *E. coli*; likewise, DRBC agar plates (Difco), for the enumeration of yeasts and moulds (incubation at 25°C for 7 days) were used.

2.3. Antibiotic Susceptibility of Selected Contaminants from Meat. Susceptibility to several antibiotics was determined using commercial discs of Amoxicillin (25 μg), Ampicillin (10 μg) Gentamicin (10 μg), Kanamycin (30 μg), Amoxicillin/Clavulanic Acid (20/10 μg), Tetracycline (30 μg), and Cefuroxime (30 μg) at the concentrations recommended by the Scientific Committee on Animal Nutrition (discs provided by Merck) by the disk diffusion assay [17]. Briefly, 25 colonies/meat batch of either *E. coli*, *Salmonella,* or *Shigella* were randomly picked, grown overnight, and streaked on Müller-Hinton agar plates to form a growth lawn; the antibiotic disks were placed at an appropriate distance and incubated for 48 h at 37°C. The diameter of the inhibition zone was measured after 24–48 h of incubation. The plates were evaluated according with the size of the inhibition zone values and classified as resistant, intermediate resistant and susceptible according to the Clinical Laboratory Standards Institute (CLSI, 2017). For quality control, *Escherichia coli* ATCC 25922 was used.

2.4. Application of Inhibitory Substances on Meat. The meat filets were divided in nine portions (150 g meat/portion/each treatment) and treated as following: (a) portions 1 and 2 were immersed in pure cell culture suspensions of Cys5-4 and LP independently at the final concentration of 8.97 and 9.03 log CFU/mL, respectively; (b) portions 3 and 4 were immersed in CFS of Cys5-4 and LP suspension at the final concentration of 6400 AU/mL (arbitrary units were determined as previously described by Garzón et al. [13]); (c) portions 5 and 6 were immersed in Cys5-4 and LP peptides, independently, at the final concentration of 6400 AU/mL; (d) portion 7 was immersed in nitrite at 200 mg/kg according with the INEN Normative for meat preservation [6]; (e) portion 8 was immersed in commercial nisin from *Lactococcus lactis* 2.5% (balance sodium chloride) at the final concentration 200 mg/mg; and (e) portion 9 was immersed in sterile water (control). Briefly, the immersed samples were maintained for 10–15 min in individual sterile trays containing the inhibitory substances as described above, followed by 30 min under the laminar bench to avoid any cross-contamination and stored in the refrigeration for 9 days in polystyrene food delivery boxes wrapped with sterile plastic

bag (Ziploc). The experiments were performed in triplicates starting with different batches of meat.

2.5. pH and Ammonia Determination. The pH values were monitored at intervals of 1, 3, 6, and 9 days of storage. Meat samples (5 g) were homogenized with 50 mL distilled water cooled at 25°C. The mixture was stirred for 30 min and decanted. The pH value was measured in the supernatant, using a pH meter (RoSH, Balance Instrument Co., Ltd). Determination of ammonia in filtrate samples of meat was performed based on a previously described method [18]. Briefly, the meat samples individually treated with Cys5-4, LP peptides, nitrite, and no treatment (control) were placed in sterile beakers, treated with solution of 6N NaOH for 15 min; aliquots of 300 μL were transferred in the 10 mL balloons, and 100 μL of EDTA was added (to avoid precipitation). Moreover, the samples were treated with 100 μL of the Nessler reagent for 10 min followed by calibration with sterile distilled water, and immediately, the absorbance at 450 nm was determined using a spectrophotometer (Nova60, Millipore) with previously determined ammonia standard curve (90–200 ppm). The addition of the Nessler reagent will produce a yellow to brown color dependent on the concentration of ammonia found in the sample. The color change was monitored at 400–450 nm using the spectrophotometer (Nova60, Millipore, Merck). A value greater than 120 ppm being associated with spoilage contamination [19]. To determine the ammonia background value, an uncontaminated meat muscle sample was used.

2.6. Bacteriological Analysis during Storage. Bacterial counts in meat filets treated and untreated with the antimicrobial substances were determined at different time intervals (1, 3, 6, and 9 days) using the agar plate method [20].

2.7. Effect of Bacteriocin Cys5-4 on the Pathogenic Membrane Cell Integrity In Vitro. If the bacterial membrane is compromised, release of inner cellular constituents such as DNA/RNA can be monitored. The bacterial suspensions consisting of *E. coli* ATCC 25922, *Salmonella enterica* ATCC 51741, and *Shigella sonnei* ATCC 25931 were grown overnight in appropriate broth culture media, harvested by centrifugation, and washed twice with 1 X PBS (phosphate-buffered saline, pH 7.5). The bacterial cells were treated independently with Cys5-4 peptides at a final concentration of 6400 AU/mL and incubated at 30°C. Cell culture aliquots were removed at 1, 2, and 4 h, centrifuged, the supernatants were filtered, and optical density was measured using the spectrophotometer (Nova60, Millipore). Moreover, the supernatants were precipitated with isopropanol and ammonium acetate (3 M), washed with 75% ethanol, then the DNA/RNA molecules were visualized on electrophoresis in 1% agarose gels with ethidium bromide running in 1X TBE (Tris-borate EDTA, pH 8.0) buffer (Sigma, New York, USA).

2.8. Statistical Analysis. The effect of antimicrobial components was evaluated using ANOVA with split-split-plot experimental design. Then, Duncan's multiples range tests and LSD (Least Significant Difference with Bonferroni correction) were applied to determine significant differences between the means. The statistical significance used was $p < 0.05$ (SPSS version 10.0.6, USA and Microsoft excel).

3. Results and Discussion

3.1. Bacteriologic Analysis Indicated the Presence of Antibiotic Resistant Microorganisms in Commercialized Raw Meat. Control of microorganism's growth in food products is essential to ensure the human health. Contamination with antibiotic-resistant strains is of concern, as food may act as a vector for the transfer of antimicrobial resistant bacteria and antimicrobial resistance genes to humans [21]. The bacteriologic analysis indicated the presence of coliforms (average of 8.81 ± 0.13 log CFU/g) along with yeasts and molds in all meat batches (9) purchased at the intervals of three weeks from the same vender. The results reveal the presence of *Shigella* sp., *E. coli*, and *Salmonella* sp. in meat samples; thus no compliance with the guidelines [16]. The contamination by microorganisms might occur at the slaughterhouse, the meat being stored up to 48–72 h at room temperature before dispatching to the retailers; during animal evisceration as result of inappropriate manipulation (e.g. cutting the product without wearing gloves, failure to wash hands between food and money transactions and restroom breaks); or environmental storage conditions (yearly average of about 14 degrees). When examined the antibiotic susceptibility, the results indicated that *Shigella* and *E. coli* isolates exhibited high resistance to all antibiotics tested except gentamycin, while *Salmonella* was sensible to kanamycin (see Table 1). Above 53% of *Shigella* isolates were resistant to ampicillin and amoxicillin/clavulanic acid and cefuroxime, while 44% of *Salmonella* isolates were tetracycline and amoxicillin resistant. The data showed that over 13% of *E. coli* isolates were resistant to all antibiotics tested. A recent study on *E. coli* isolates recovered from pork meat sold in China indicated that the isolates exhibited resistance to old antibiotics, such as tetracycline, ampicillin, and amoxicillin [22]. Furthermore, the food industry argues with the fact that the population knows how to prepare the meat; thus, contamination of raw meat with bacteria is not of concern [2], but, this is not always verifiable truth as we identified contaminants in several ready-to-eat products (personal communication). Therefore, to satisfy consumer demand, extensive attention must be given to food manipulation and consequent protection of consumers against possible severe illness.

3.2. Bacteriocins Produced by Cys5-4 Reduced the Viability of Microbes Contaminating Meat. Application of cell-free supernatant or partial purified bacteriocins to foods, or of LAB strains capable of producing bacteriocins *in situ*, may be a suitable alternative to improve food safety. The effect of inhibitory substances produced by Cys5-4, LP, commercial nitrite and nisin towards microbial population in contaminated meat was investigated. The initial cell density of

Table 1: Antibiotic susceptibility of bacteria originated from raw beef meat.

Antimicrobials		Breakpoints (CLSI, 2017) R/I/S (mm)	% of resistance		
			E. coli ($n = 225$)	*Salmonella* sp. ($n = 150$)	*Shigella* sp. ($n = 225$)
Tetracyclines	Tetracycline (30 μg)	≤11/12–14/≥15	17.78	44.44	44.44
β-Lactam-lactamase inhibitor combinations	Ampicillin (10 μg)	≤13/14–16/≥17	44.44	44.44	55.56
	Amoxicillin (25 μg)	≤13/14–17/≥18	13.33	33.33	44.44
	Amoxicillin/clavulanic acid (20/10 μg)	≤19/-/≥20	33.33	33.33	53.33
Aminoglycoside	Kanamycin (30 μg)	≤13/14–17/≥18	22.22	0	33.33
	Gentamycin (10 μg)	≤12/13–14/≥15	17.78	11.11	0
Cephalosporins	Cefuroxime (30 μg)	≤16/72–19/≥20	55.56	55.56	55.56

% was calculated as number of resistant bacteria/number of isolates. R = resistant; I = intermediate resistant; S = susceptible.

microorganisms detected in meat was 8.81 ± 0.13 log CFU/g (after purchase), which decreased to 6.09 ± 0.21–6.34 ± 0.18 log CFU/g at day 9 of storage with refrigeration. The decrease of viable cell counts in the untreated meat might be associated with the experimental conditions such packing and refrigeration. A statistically significant decrease ($p < 0.05$), in the viable cell counts was registered when meat was treated with pure cell culture of Cys5-4 and LP respectively, as well as nitrite on day 9 of storage compared with untreated meat (see Figure 1(a)). Thus, the addition of pure culture dropped the counts to 4.54 ± 0.2 log CFU/g (1.55 log difference) when added Cys5-4 and 4.64 ± 0.18 log CFU/g (1.45 log difference) when LP was added, while the cell counts decreased to 4.92 ± 0.24 log CFU/g (1.19 log) when treated with nitrite. Although no significant difference was recorded between Cys5-4 and the commercial strain, the inhibitory effect of the pure culture was superior to the nitrite treatment, considering the visible changes in meat appearance (bluish color) and smell (rotten). We suggest that the inhibitory effect is a result of bacteriocin produced *in situ* by LAB strains. The initial counts of inoculated cells were of 8.97 ± 0.23 and 9.03 ± 0.18 log CFU/mL of Cys5-4 and standard strain (LP), respectively, which diminished with about 2 log units upon 9 days of storage. This decrease does not influence their capacity to adapt and produce *in situ* the inhibitory components expressing its antagonistic effect against other bacteria growing in the same microenvironment. In addition, we suggest that the producer strain should be carefully selected, as required to maintain its viability and produce antimicrobials in divergent food matrix. However, the biological activity of Cys5-4 was not inhibited by the meat composition, revealing its efficacy. Similarly, the bioproductive effect of antimicrobial components produced by pure culture mixture of *Lactococcus lactis* and *Lactobacillus sakei* demonstrated the capacity to extend the shelf life and reduce the counts of spoilage bacteria in commercial bacon [8]. Moreover, similar results were obtained when dipping the meat in the CFS suspension of Cys5-4 and LP, respectively (see Figure 1(b)). The viable cell counts reduced from $8{,}81 \pm 0.18$ log CFU/g to 6.29 ± 0.21 log CFU/g on day 9 in the untreated meat, while in the treated CFSCys5-4 samples a significant decrease ($p < 0.05$) to 4.98 ± 0.24 log CFU/g (about 1.91 log difference) was recorded upon 9 days of storage. No significant difference ($p > 0.05$) was observed between the Cys5-4, LP and nitrite upon 9 days of storage. These results were in agreement with our previous findings that Cys5-4 inhibited *in vitro* the growth of *E. coli* and *Salmonella* at both vegetative and exponential target growth [13]. These results are similar to the results obtained using the crude extract of *Lactobacillus* LAC231 on the microbiota presented in the meat packed in controlled atmospheres [19]. Comparable results were obtained using *Leuconostoc mesenteroides* ALB101 on fresh orange juice [3]. The addition of ALB101 decreased slowly the viability of the microbial population from 6.75 to 6.56 log CFU/mL until the 4th day. Likewise, a small decrease (0.5 log) in the viability of *Listeria* was observed when treated with sodium nitrite on the second day of storage [23]. Furthermore, when using precipitated peptides, a significant reduction ($p < 0.05$) by 1.69 log was registered when treated with Cys5-4 peptides and respectively, 1.76 log and 1.48 log when using LP peptides and nitrite at day 9 of storage. Although from day 1 to 6 the effect of nisin was comparable with Cys5-4 and nitrite, on day 9 a slight increase in the viable cell counts was observed (see Figure 1(c)). A recent study using bacteriocin produced by *L. curvatus* MBSa2 indicated a reduction of *L. monocytogenes* counts with 0.5 log in salami [4]. Other research using nisin adsorbed on cellophane for packaging showed a reduction of bacterial counts upon 12 days of storage [24]. The results presented pointed out that the application of Cys5-4 as peptides has a similar effect as nitrite but unlike nitrites are "amiable" in term of consumer health. Although all application forms of antimicrobials were effective in the reduction of contaminants in meat, we suggest that CFS being less sensitive to enzymes (proteases), higher solubilized, and less expensive might be a satisfactory alternative to be used as preservative in raw meat.

3.3. The Increment in pH and Ammonia Indicated Meat Deterioration. The results reveal that the pH varies from 5.69 to 5.89 in the samples treated with the antimicrobials in the three application modes, while an increase from 6.0 to 6.35 was measured in the untreated meat. The pH was maintained in acceptable range of 5.69 to 5.89 in the meat samples treated with the Cys5-4, while in the untreated samples increased by 0.35–0.5 units out of the maximum

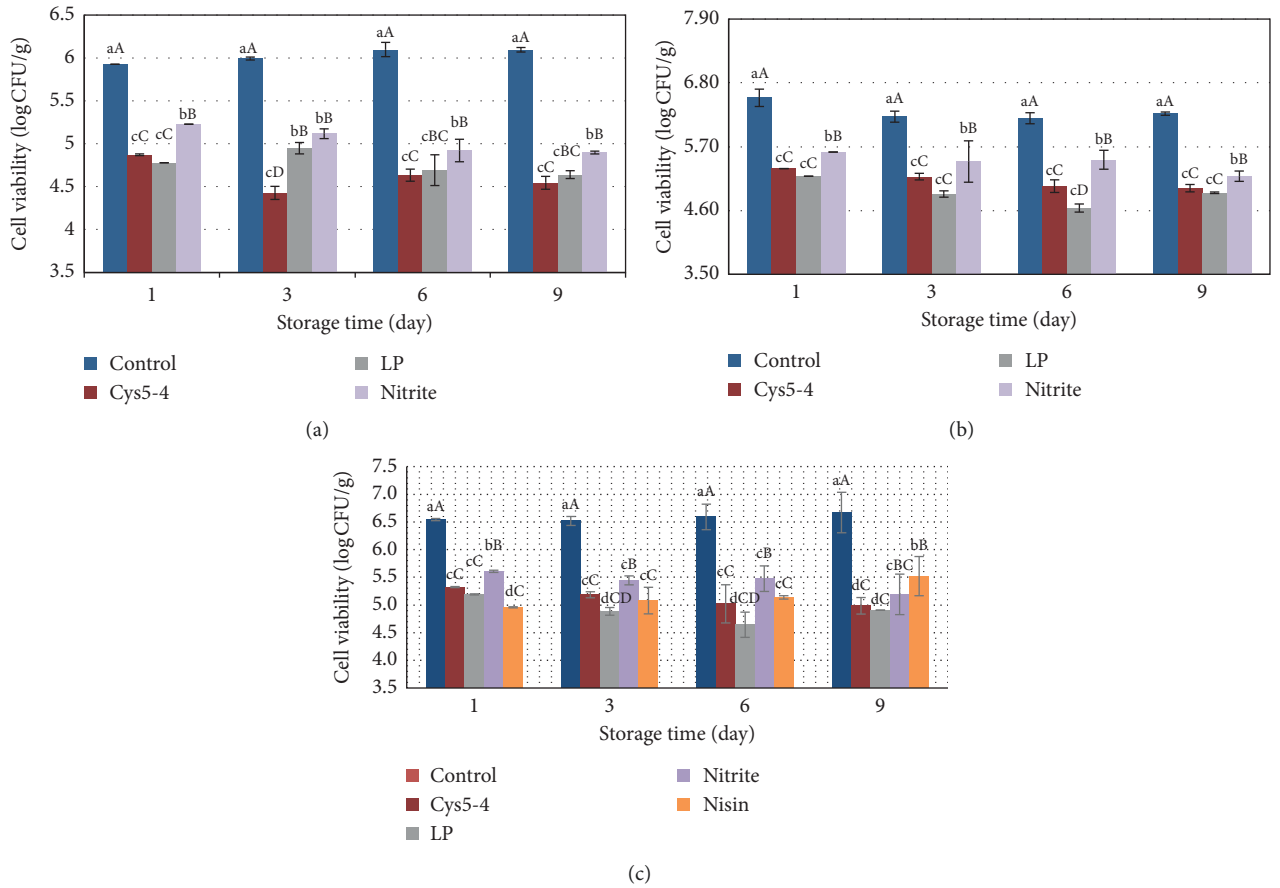

Figure 1: Average counts of microbial population in meat samples after treatment with (a) cell cultures; (b) cell-free supernatant; and (c) partial precipitated peptides, nisin and nitrite. Values with different letters are significantly different $p < 0.05$. Small letters show the difference between total contaminant-storage time and control (LSD with Bonferroni correction); capital letters indicate the differences in the cell counts at different storage time (Duncan's test). Control: total viable counts detected in untreated meat; Cys5-4: *L. plantarum* UTNCys5-4; LP: *L. plantarum* ATCC 8014.

acceptable value (5.4–6.2). In the samples treated with nitrite, the pH varies from 5.87 to 6.10, while in the samples treated with nisin, pH varies from 5.83 to 5.98. These results correlated with the decrease of the total coliforms in meat batches inoculated with the antimicrobial substances. An early study associated the increase in pH as indicator of meat deterioration through the degradation of proteins producing amino acids, giving rise to the formation of alkaline compounds [25]. When monitored, an increase in the release of ammonia during storage in the untreated control versus nitrite treated meat samples was observed (see Figure 2). A previous study indicated that the ammonia background level varies between different meat muscles, dry-aged meat carcasses, and the increment of contamination [26]. However, in the present study, the minimum ammonia value determined in the uncontaminated meat samples varied from 86 to 90 ppm, while in the contaminated samples treated with antimicrobial substances, it varied from 95 to 118 ppm; upon day 9 of storage, the ammonium released in the untreated samples already contaminated control was above 155 ppm, indicating protein degradation. Contrary to our previous study, where the effect of antimicrobial substances produced by Cys5-4 was monitored in artificially contaminated

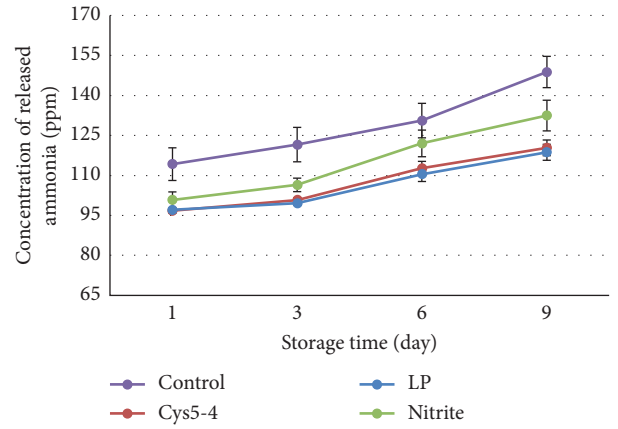

Figure 2: Changes in ammonia released during the storage time. Cys5-4: meat treated with Cys5-4; LP: meat treated with LP, nitrite: meat treated with nitrite. Control: untreated meat; Cys5-4: *L. plantarum* UTNCys5-4; LP: *L. plantarum* ATCC 8014.

samples (juice), in the present study, all batches of meat purchased from the local vender were contaminated; however, the registered reduction of cell counts of existing pathogens

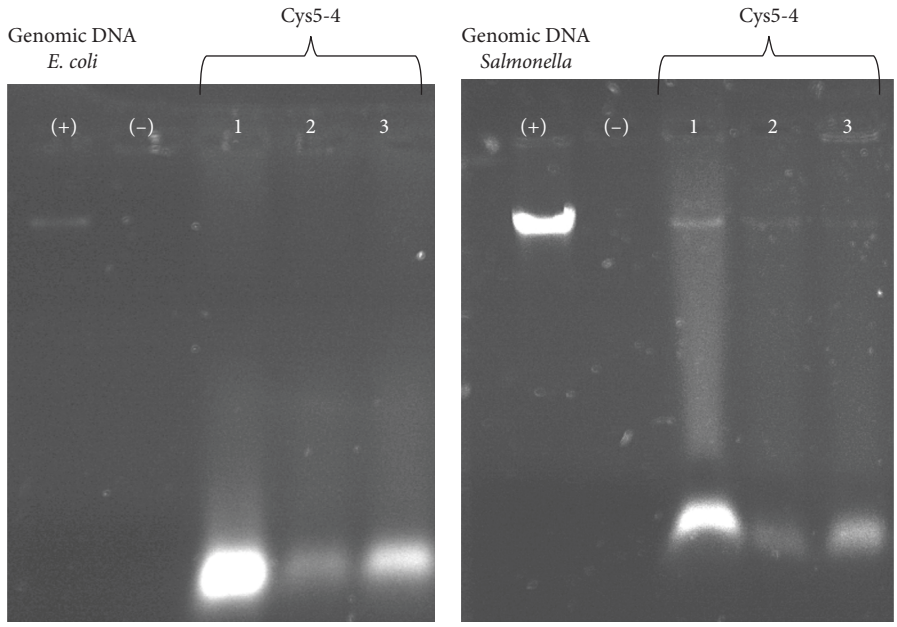

Figure 3: The effect of peptides on the target membrane integrity. (a) *E. coli* ATCC 25922. (b) *Salmonella enterica* subsp. *enterica* ATCC 51741. (−): negative control (absence of DNA/RNA molecules in untreated cells); 1–3: DNA/RNA released after treatment with Cys5-4 at different intervals of incubation (1, 2 and 4 h).

corresponded to the maintenance of pH and ammonia within acceptable ranges.

3.4. Bacteriocin Cys5-4 Induced Killing by Disrupting the Cell Membrane of Target Pathogens. The release of intracellular components with strong UV absorption (260 nm) indicates cell membrane damage [27, 28]. When *E. coli* and *Salmonella* suspension cells were treated with Cys5-4 bacteriocin in precipitated form, the optical density increased rapidly after one hour of incubation for both Cys5-4 and LP in comparison with untreated counterparts. For example, when *E. coli* was treated with Cys5-4 peptides, the sample registered A260 values varied from 2.44 to 2.67 with an increase over incubation time, while in the untreated cells, the value was 0.27. This signified the damage of the target cell membrane by Cys5-4, allowing the release of DNA/RNA molecules. Only RNA was detected in the *E. coli* samples treated with Cys5-4 peptides, while both DNA/RNA molecules were detected *Salmonella* samples treated with peptides (see Figures 3(a) and 3(b)). No DNA/RNA was detected in untreated control samples. The results suggested that Cys5-4 could interact with DNA molecules, inducing their degradation, while free RNA molecules might be protected by other mechanisms in *E. coli* samples treated with peptides. It has been shown that antimicrobials induced DNA damage and genomic instability across some microbial pathogens, including bacterial and fungal species [19]; antimicrobial binds DNA and directly induces double-strand breaks by a mechanism that is not fully understood or may interact with target proteins in a manner that directly induces DNA damage [29]. Similarly, both DNA and RNA molecules were released after the treatment of *Shigella* cells with Cys5-4 or LP. This observation is consistent with our previous findings about the bactericidal mode of action of the bacteriocin Cys5-4 [13]. In a recent paper, the molecular weight of partial purified bacteriocin from *L. plantarum* ATCC8014 was estimated at 68 kDa [30], while a previous study indicated that the protein was larger than 122 kDa [31]. Instead, the Cys5-4 produced peptides of 30, 20, 15, and 10 kDa (see Figure S1 in Supplementary Material for comprehensive image analysis). We suggest that the difference between Cys5-4 and LP might be related with the difference in their mode of action and overall inhibitory activity might be the effect of more than one peptide working together in one antimicrobial unit. A recent study illustrated the bactericidal effect of a bacteriocin produced by *Lactobacillus paracasei* subsp. *tolerans* FX-6 by disrupting the cell membrane of *Staphylococcus aureus*, followed by genomic DNA binding in cytoplasm, then cell death [32]. Cytoplasm membrane damage is a characteristic of bacteriocin being reported by nisin and pediocin [33]. In another study, the two-peptide bacteriocin PlnEF accumulates on the cell membrane of sensitive strain *L. plantarum* pl2 having a drastic impact on the structure and integrity of target cells [34]. Our results were in agreement with other research that indicated that the bacteriocin Cys5-4 damaged the cellular membrane of target bacteria leading to its cellular death.

4. Conclusions

The current study indicated the efficacy of inhibitory substances produced by *L. plantarum* Cys5-4 strain to diminish the microbial population in contaminated meat during storage with refrigeration. The Cys5-4 peptides exerted a

bacteriolytic effect on *E. coli* and *Salmonella* by disrupting their cellular membranes, leading to cell death. Thus, the application of inhibitory substances produced by the Cys5-4 strain as part of an overall best manufacturing practices program could enhance the shelf life of meat-based products and consequently reduce the need for use of chemical additives in food.

Data Availability

The data used to support the findings of this study are included within the article and the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Figure S1: SDS-PAGE analysis of precipitated peptides of Cys5-4. M: molecular marker (Takara, Clearly Protein Ladder); arrows indicated different bands. (*Supplementary Materials*)

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