Antimicrobial compounds produced by Weissella confusa Cys2-2 strain inhibit Gram-negative bacteria growth

Gabriela N. Tenea and Mauricio Israel Lara

Faculty of Engineering in Agricultural and Environmental Sciences, The Technical University of the North, Ibarra, Ecuador

ABSTRACT
Antimicrobial compounds produced by lactic acid bacteria emerged as a promising group of agents for managing the growth of pathogens in food. Previously, we have isolated a bacteriocinogenic strain, Weissella confusa Cys2-2, producing active substances with inhibitory potential, however, its antimicrobial mechanism is still undefined. This study was aimed to determine the Cys2-2 bacteriocins mechanism of action against food pathogens using: agar-well diffusion and broth assay to evaluate the spectrum of inhibition and the minimum inhibitory concentration, Tricine-SDS-PAGE analysis to estimate the molecular weight, determination of the target cell viability with the Cys2-2 compounds applied as cell-free supernatant (CFS) and precipitated peptides (PP) with or without an chelator agent (EDTA) and evaluate the effect on the membrane integrity by monitoring the release of DNA/RNA molecules. The results revealed that Cys2-2 bacteriocin exerted its bactericidal effect by weakening of membrane integrity of target cells leading to cell death.

1. Introduction
The preservation of food by natural methods such as the use of antimicrobial peptides produced by lactic acid bacteria (LAB) is an alternative to be considered (Ge, Sun, Xin, Wang, & Ping, 2016; Mokoena, 2017; Yang, Lin, Sung, & Fang, 2014). In Ecuador, despite the growth of alimentary sector, the food legislation seems to be less effective, as the artisanal ready-to-eat food products sold out in the street in very poor conditions are prone to contamination; thus, to extend their shelf-life, new methods are compulsory. Through LAB strains, Weissella species were recently investigated (Abriouel et al., 2015; Fusco et al., 2015; Goh & Philip, 2015; Nam, Ha, Bae, & Lee, 2002; Ndagano, Lamoureux, Dortu, Vandermoten, & Thonart, 2011; Pal, Ramana, & Bawa, 2010). The genome sequence of Weissella confusa MBF8-1, isolated from a homemade fermented soybean comprises a 2.2-Mbp chromosome and a 17.8-kbp bacteriocin-encoding plasmid was early published (Heng, Yeh, & Malik, 2017). The antimicrobial agents are often membrane and non-membrane disruptive molecules, and their optimum production requires specific conditions and environmental interactions (Giuliani, Pirri, & Nicoletto, 2007). Nonetheless, limited studies examine the mechanism by which Weissella antimicrobial substances inhibit Gram-negative bacteria. It has been shown that cell-free supernatant containing bacteriocin of Weissella confusa PL9001 decreased the viability of Helicobacter pylori by inducing ruptures in the cell walls (Nam et al., 2002). Although is regarded as an opportunistic pathogen causing extremely rarely sepsis and bacteremia in humans (Fairfax, Lephart, & Salimnia, 2014), a recent literature-based safety assessment concluded that Weissella confusa are generally safe (Sturino, 2018). Currently, the technological application of weissellin A and bacteriocin D1501 of W. hellerica D1501 enhancing the shelf life of in fermented sausages and tofu (Chen, Rui, Lu, Li, & Dong, 2014; Papagianni, 2012) were shown. However, along with dextran production, an important exopolysaccharide of bread (Amari et al., 2013; Katina et al., 2009; Shukla, Shi, Maina, Juvenon, & Goyal, 2014, Weissella
might be a valuable source of antimicrobial substances to be further incorporated in food/feed to prevent the growth of undesirable microorganisms.

Previously, we prospect the wild-type fruits of subtropical forest, to identify and select new bacteriocinogenic LAB strains (Garzón, Ortega, & Tenea, 2017). Among several selected lactobacilli and lactococcus species (Tenea, Hurtado, & Ortega, 2018), only one strain isolated from wild-type spiral ginger and assigned Cys2-2 was identified as *Weissella confusa*. The preliminary analysis indicated that the active compounds released in CFS of Cys2-2 are of proteinaceous nature, were heat stable and highly resistant to acidic conditions. Considering those properties, the present study was aimed to evaluate the spectrum of inhibition of bacteriocin Cys2-2, its production, molecular characterization along with the effect against different food pathogens to get insight into its possible mode of action. Thus, understanding the mechanism by which the bacteriocin-like substances exert their effect against pathogenic bacteria will further expand our knowledge on their application to control the contamination in different food or feed matrices.

2. Materials and methods

2.1. Bacterial strain and bacteriocin extraction

*Weissella confusa* isolated from Costus sp (spiral ginger), a wild-type flower of Cuyabeno, (semi-permanently inundated forests flooded by black-water river of Ecuadorian Amazon) assigned Cys2-2 was registered at GenBank with the accession number KY041684.1. The microorganism culture was preserved by deep-freezing in glycerol solution before use. Production of bacteriocin was determined by inoculating 100 mL of MRS broth (Difco) with an 18 h old culture (2%, v/v) of Cys2-2 and incubated at 37°C without agitation. Optical density (OD600) of the culture was monitored at certain intervals for 33 h. Antimicrobial activity of Cys2-2 was performed using the agar-well diffusion method (Garzón et al., 2017). As indicator strain, *E.coli* ATCC 25922 was used.

2.2. Evaluation of inhibitory spectrum against Gram-positive and Gram-negative bacteria

*Weissella confusa* Cys2-2 was cultivated in MRS broth and incubated at 37°C for 24 h. CFS collected by centrifugation at 13,000 × g for 20 min (4°C) was recovered and filtered using 0.22 μm porosity syringe filter (# STF020025H, ChemLab Group, USA). To obtain the precipitated peptides the CFS was treated with 60% ammonium sulfate, incubated overnight at 4°C and centrifuged at 8,000 × g for 30 min. The PP were recovered in ammonium acetate 25 mM (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 –80°C before use in antimicrobial assays. Antimicrobial activity was performed using the agar-well diffusion method (Garzón et al., 2017). Titer estimated as AU·mL−1 is defined as the highest dilution that inhibited the growth of the indicator strain (Ge et al., 2016). The indicator strains consisting of bacteria purchased from ATCC and laboratory source (previously isolated from local food) used are presented in Table 1.

2.3. Determination of the minimum inhibitory concentration (MIC)

The CFS and PP with a determined concentration ranged from 800 to 12,800 AU·mL−1 were added independently into broth tubes containing the pathogenic bacteria, incubated for 24 h at 37°C followed by plate agar to determine the minimum concentration that inhibit 50% of the target bacteria also considered as MIC value (Arena et al., 2016). The target bacteria were *E. coli* ATCC 25922, *Salmonella enterica* ATCC 51741 and *Shigella sonnei* ATCC 25931.

2.4. Bacteriocin molecular size approximation

The bacteriocin Cys2-2 was analyzed using Tricine-SDS-PAGE method using pre-casted acrylamide gels (4–20%) and Thermo Fisher OWL (10 × 10) vertical electrophoresis system. PPCys2-2 and the broad range protein molecular marker (cat # V8491, Promega) were run for approximate 4 h at 100 V. The gel was stained with Takara CBB Safe Stain (cat # T9320A, Takara, Bio Company) for 4 h, destained with a solution of 30% methanol (v/v) and glacial acetic acid, 10% (v/v) until the bands become clear. The peptide weight was estimated relative to molecular marker. The half of the gel was washed twice with distillate water, placed in nutrient agar plate and covered with overnight culture of *Salmonella enterica* ATCC 51741 for 4 h in refrigeration followed by 24 h at 37°C.

2.5. The activity of Cys2-2 bacteriocin against Gram-negative viability

The indicator bacteria *E. coli* ATCC 25922, *Salmonella enterica* ATCC 51741 and *Shigella sonnei* ATCC 25931, were grown

---

**Table 1. Inhibitory spectrum of bacteriocin-produced by *W. confusa* Cys2-2 strain.**

| Indicator organisms | Inhibition zone (mm) |
|---------------------|----------------------|
|                     | CFS                  | PP       |
| **Gram-negative**   |                      |          |
| *Salmonella* UTNSm2 (laboratory collection) | 17.66 ± 0.94<sup>a</sup> | 14.33 ± 0.47<sup>b</sup> |
| *Salmonella enterica* subsp. *enterica* (Kauffmann and Edwards) Le Minor and Popoff ATCC 51741 | 16.00 ± 0.00<sup>a</sup> | 14.00 ± 0.47<sup>b</sup> |
| *E. coli* ATCC 25922 | 15.66 ± 0.94<sup>a</sup> | 13.66 ± 0.94<sup>b</sup> |
| Enterobacter aerogenes UTNeag1 (laboratory collection) | 15.33 ± 0.47<sup>a</sup> | 13.66 ± 0.94<sup>b</sup> |
| *Shigella* sonnei ATCC 25931 | 14.33 ± 0.47<sup>a</sup> | 13.66 ± 0.94<sup>b</sup> |
| Shigella sp. UTN Shg1 (laboratory collection) | 14.33 ± 0.47<sup>a</sup> | 13.00 ± 0.00<sup>b</sup> |
| *Salmonella enterica* subsp. *enterica* serovar Ababetuta ATCC 35640 | 14.00 ± 0.00<sup>a</sup> | 12.66 ± 0.94<sup>b</sup> |
| *E. coli* UTN Ec1 (laboratory collection) | 14.00 ± 0.00<sup>a</sup> | 12.33 ± 0.33<sup>c</sup> |
| Streptococcus thermophilus ATCC 19258 | 13.00 ± 0.00<sup>a</sup> | 11.66 ± 0.94<sup>c</sup> |
| *E. coli* ATCC 10536 | 12.66 ± 0.47<sup>a</sup> | 11.33 ± 0.47<sup>c</sup> |
| **Gram-positive**   |                      |          |
| Bifidobacterium breve ATCC 15700 | 12.66 ± 0.47<sup>a</sup> | 11.33 ± 0.47<sup>c</sup> |
| Staphylococcus aureus ATCC 1026 | 12.33 ± 0.47<sup>a</sup> | 11.33 ± 0.47<sup>c</sup> |
| L. acidophilus ATCC 4356 | 11.33 ± 0.47<sup>a</sup> | 11.33 ± 0.82<sup>c</sup> |

*Data are means ± standard error. Values in the same column that are followed by different lowercase letters are significant different (P < 0.05). Values in the row with different capital letters indicate that are significant (P < 0.05). CFS: cell-free supernatant, PP: precipitated peptides.*
independently in tubes containing LB and respectively nutrient agar medium. The bacteriocin Cys2-2 was added as following: (a) 1 MIC CFS Cys2-2; (b) 1 MIC PPCys2-2; (c) 1 MIC CFS and PP of Cys2-2 combined with 20 mM EDTA were added to indicator bacteria (OD_{600} = 0.2) individually; (d) indicator bacteria with 20 mM EDTA; (f) control: untreated indicator cells. Incubation was performed at 37°C for 6 h and optical density (OD_{600}) was measured every hour using spectrophotometer (Nova60, Millipore, Merck) followed by plate-agar method (PCA, Difco, Fisher Scientific) to determine the viable cell counts. The results were analyzed by determining Log reduction calculated as the difference between log10 (CFU) of the untreated cells (no bacteriocin, no EDTA) and the treated cells (bacteriocin added, EDTA or a combination thereof). Log reduction of <1 was considered insignificant.

2.6. Integrity of cell membranes

If the bacterial membrane is compromised the inner cellular components such DNA/RNA molecules are released and can be monitored by determining the absorbance at 260 nm. The bacterial suspension consisting of E. coli, Salmonella and Shigella were grown overnight in appropriate culture media, harvested by centrifugation and washed twice with 1 X PBS (phosphate buffered saline, pH 7.5). The cells were treated with different concentrations (100–400 μg·mL^{−1}) of bacteriocin Cys2-2 (determined using standard Bradford method) and incubated at 30°C for 1 h. Cell culture aliquots were centrifuged, the supernatants were filtered, and optical density was determined using the spectrophotometer (Nova60, Millipore, Merck) as described by Patra et al. (2015). Additionally, the supernatants were precipitated with isopropanol and ammonium acetate (3 M), washed twice with ethanol 75%, then the DNA/RNA molecules were visualized on electrophoresis with 1% agarose gels containing ethidium bromide, in 1 X TBE (Tris-borate EDTA, pH 8.0) buffer (Sigma, New York, USA).

2.7. Statistical analysis

All experiments were performed in triplicate, repeated from three independent experiments and expressed as mean ± standard deviation. Analysis of variance was applied with Tukey to determine the significant differences between the means (SPSS version 15.0).

3. Results and discussion

3.1. Bacteriocin-like substances from Cys2-2 displayed a wide-range inhibition spectrum

The inhibitory potential of peptides produced by lactic bacteria is associated with the strain performance (Tenea et al., 2018; Ye et al., 2018). In this study, the inhibitory potential of antimicrobial substances produced by Weissella confusa Cys2-2 against several indicator strains was investigated (Table 1). The results indicated a significant inhibitory activity (P < 0.05) towards pathogenic Salmonella UTN Sm2 (isolated from cooked chicken) registered for both CFS and PP application form, suggesting that the resistant membrane of the pathogen might be depleted at the interaction with the active substances. This result was in agreement with our previous findings indicating that CFS activity was greater in the agar well diffusion test, meaning that the presence of acids along with peptides might be synergistically contributed to the overall inhibitory activity (Tenea et al., 2018).

When studying the antimicrobial activity of two Weissella viridescens strains, the results indicated that their efficiency against L. monocytogenes was strain related (Ye et al., 2018). Early study on the bacteriocin produced by Weissella confusa A3 indicated that in precipitated from does not inhibit Staphylococcus aureus RF122 (Goh & Philip, 2015). The inhibitory profile of Cys2-2 might be explained by its origin, tropical wild-type fruits of climate Amazonian region, suggesting that those strains might acquire superior characteristics such as maintaining viability under stressful environmental conditions, elevate inhibitory activity towards pathogens sharing the same microclimate, being valuable natural compounds that could be useful to industry. Therefore, to rescue and investigate the microbiome associated with wild-type fruits will contribute to broadening our understanding of its diversity, ecology and essential role in the physiology of the fruit after harvesting as well as the effects on human health. In general, the production of bacteriocins is associated with cell growth rate. The Cys2-2 bacterial cells gain a density of 1.72 (OD_{600}) within 6 h and maximum of 2.57 at 18 h of incubation suggesting a high cell division rate. The inhibitory activity of Cys2-2 was detected at 6 h of cell growth (average inhibition zone of 9.0 ± 0.0 mm) achieving the maximum concentration at the early stationary phase (average inhibition zone of 15 ± 33 mm) (Figure 1). In the study of Goh and Philip (2015) the maximum bacteriocin A3 production was registered at 24 h spanning a density of 0.9 and inhibition zone of 12 mm, followed by a decrease over 36 h of incubation. Contrary, in the present study, the bacteriocin Cys2-2 production remained stable from 18 to 33 h of growth. Although W. confusa Cys2-2 had 98% similarity with an exopolysaccharide producer W. confusa SL3 strain of milled sugarcane (Hector et al., 2015), the Cys2-2 showed superior inhibitory capacity against some harmful food microorganisms such as the commensal Shigella, Salmonella and E. coli, thus could be a promising agent to control the microorganism growth in food or feed.

3.2. Molecular weight estimation

The bacteriocin Cys2-2 molecular size was estimated at 10 kDa in the polyacrylamide gel, and its corresponding activity was visualized by the inhibition zone formed around the active band (Figure 2). The size seems larger than previously characterized Weissella bacteriocins as weissellin A3 of 2.7 kDa (Goh & Philip, 2015), weissellin A of 4.45 kDa (Papajann & Papamichael, 2011), weissellicin 110 of 3.5 kDa (Srionnual, Yanagida, Lin, Hsiao, & Chen, 2007) or weissellicin Y and M of 4.9 kDa (Masuda et al., 2012). Possibly, the binding of Cys2-2 peptides with another released compounds in the medium results in a larger or complex protein. Nonetheless, larger bacteriocins of lactic acid bacteria were previously reported (Chopra, Singh, Kumar Jena, & Sahoo, 2015; Ge et al., 2016). The results correlate with our previous data showing that the antimicrobial compounds of Cys2-2 displayed stable inhibitory activity at different range of pH (2–10) and high heat (60–121°C). In addition, the antimicrobial activity enhanced throughout time (60 min) at higher heat (100°C) suggesting that larger...
proteins might develop greater activity when binding chemical elements following a pathway similar of Maillard Reaction (Garzón et al., 2017). As such these antimicrobial substances revealed a wide activity spectrum against Gram-negative and Gram-positive bacteria, however, further analysis to clarify its amino acid sequence is required.

3.3. Peptides Cys2-2 decreased the target bacteria viability

The MIC was equivalent (6400 AU·mL⁻¹) towards E. coli, Salmonella and Shigella indicator strains. Previous study on sonorensin, a bacteriocin produced by Bacillus sonorensin MT93, indicated that the MIC values were dependent on the target bacteria (Chopra et al., 2015). The addition of CFSCys2-2 to the suspension cells of E. coli at the early logarithmic phase growth resulted in a decrease of the cell density over 6 h of incubation while untreated cells continue to increment. If the initial cell counts were 6.2 log CFU·mL⁻¹, at 6 h the microbial population diminished significantly (P < 0.05) with 2.88 log (Figure 3(a)), indicating the bactericidal mode of action of Cys2-2. The addition of PPCys2-2 resulted in a marginal reduction of cell viability (1.46 log). When combined PPCys2-2 with EDTA, the inhibitory effect restored at the same level as observed with CFS (2.86 log reduction) suggesting that the indicator cells were sensitized in the presence of a chelating agent. EDTA only had a marginal effect on cell viability (1.49 log reduction). Similarly, Cys2-2 in combination with EDTA significantly inhibit (>6.0 log reduction in growth) Salmonella upon 5 h, as no viable cells were detected (data not shown). Instead, treating Shigella suspension cells (initial density 6.3 log CFU·mL⁻¹) with either CFS or PP of Cys2-2 led to significant decrease (P < 0.05) of microbial population with 1.76 log and, respectively, 1.78 log, while adding EDTA no significant changes were observed, indicating that EDTA does not interfere with the antimicrobial activity as observed with E. coli and Salmonella (Figure 3(b)). We suggested that the variation in the inhibitory profile might be linked with the target strain resistance, including the combination of bacteriocin with an extra destabilizing membrane factor. Recent report indicated that nisin in purified form inhibited the activity of E. coli, Salmonella and Pseudomonas when cotreatment with EDTA (Branen & Davidson, 2004). Alike, the study of Bhatia and Bharti (2015) showed that nisin combined with lysozyme or EDTA enhanced the bactericidal effect towards spoilage microorganisms. Similarly, Salmonella typhimurium was sensitized by bovicin HCS when stress condition as heating and freezing were enclosed disrupting the LPS layer of the outer membrane (Galvao, Prudencio, & Vanetti, 2015). In
general, EDTA act as a destabilizing agent of the lipopolysaccharide layer of target bacteria by activating bacteriocin to exert its suppression effect (Hansen, Austin, & Gill, 2001; Martin-Visscher, Yoganathan, Sit, Lohans, & Vederas, 2011). These results corroborate with our previous findings that bacteriocin Cys2-2 was solubilized by chelating agents in vitro (Garzón et al., 2017), thus prospecting the factors that enhance their effectiveness would contribute to the overall control of microbial growth ex vitro.

3.4. Peptides Cys2-2 disrupt the cell membrane of target bacteria

When E. coli, Salmonella and Shigella cell suspension (OD605 = 1.0) were treated with different doses of Cys2-2, incubated for 1 h at 30°C and then subjected to spectrophotometric determination, an increase of the absorbance over time (A260) was recorded (Figure 4(a)), indicating that a damage of cell membrane might occur allowing the leaching of DNA and RNA molecules. The release of those molecules was confirmed by the electrophoresis gel (Figure 4(b)). It has been shown that antimicrobials can damage the outer membrane of Gram-negative bacteria, followed by permeabilization of the inner one and finally the total disintegration of both, causing leakage of the cytoplasmic contents (Hartmann et al., 2010). Similarly, when studying the bactericidal effect of zinc oxide nanoparticle against E. coli an increase of A260 was recorded indicating that the membrane was damaged releasing nucleic acid molecules (Patra et al., 2015). In another research, the membrane disruption of Staphylococcus aureus by the bacteriocin F1 produced by Lactobacillus paracasei subsp. tolerans FX-6 was demonstrated (Miao et al., 2016). Similarly, the cytoplasmic membrane damage by nisin and pediocin was reported (Kalchayanand, Dunne, Sikes, & Ray, 2004). The impact of antimicrobial peptides on the bacterial cell might depend upon the target bacterial strain, the type of peptide and its concentration. However, the reduction of cell viability in the presence of Cys2-2 bacteriocin was confirmed by the cell integrity of the target cells.

4. Conclusions

Altogether, this research indicates the efficacy of antimicrobial substances produced by native Weissella confusa Cys2-2 to inhibit both Gram-positive and Gram-negative bacteria.
The bacteriocins produced by Cys2-2 diminished the viability of *E. coli*, *Salmonella* and *Shigella* at the early exponential growth phase, exhibiting a bactericidal mode of action by disrupting the integrity of multiple target cells, which lead to rapid cell death. Nonetheless, to prove its performance *ex vitro* is required for further applications as natural preservative in different food matrices.

**Acknowledgments**

The authors wish to thank Dr. Molteni for helping with the statistical design; A. Barrigas and C. Ortega for helping with the experiments.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This research was supported by the Technical University of the North, under the Grant Number [2418, 01791]. GNT was sponsored in part by the Prometeo Project of Secretary for Higher Education, Science, Technology and Innovation (SENESCYT, Ecuador).

**References**

Abriouel, H., Lavilla Lerma, L., Casado Muñoz, M. C., Pérez Montoro, B., Kabisch, J., Pichner, R., … Benomar, N. (2015). The controversial nature of the Weissella genus: Technological and functional aspects versus whole genome analysis-based pathogenic potential for their application in food and health. *Frontiers in Microbiology*, 6, 1197.

Amari, M., Arango, L. F., Gabriel, V., Robert, H., Morel, S., Moulis, C., … Fontagné-Faucher, C. (2013). Characterization of a novel dextransucrase from Weissella confusa isolated from sourdough. *Applied Microbiology and Biotechnology*, 97, 5413–5422.

Arena, M. P., Silvain, A., Normanno, G., Grieco, F., Drider, D., Spano, G., & Fiocco, D. (2016). Use of Lactobacillus plantarum strains as a bio-control strategy against food-borne pathogenic microorganisms. *Frontiers in Microbiology*, 7, 464.

Bhatia, S., & Bharti, A. (2015). Evaluating the antimicrobial activity of nisin, lysozyme and ethylenediaminetetraacetic acid incorporated in starch based active food packaging film. *Journal of Food Science and Technology*, 52(6), 3504–3512.

Branen, J. K., & Davidson, P. M. (2004). Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *International Journal of Food Microbiology*, 90, 63–74.

Chen, C., Rui, X., Lu, Z., Li, W., & Dong, M. (2014). Enhanced shelf-life of tofu by using bacteriocinogenic Weissella hellenica D1501 as bioprotective cultures. *Food Control*, 46, 203–209.

**ORCID**

Gabriela N. Tenea [http://orcid.org/0000-0002-1256-9267](http://orcid.org/0000-0002-1256-9267)
Chopra, L., Singh, G., Kumar Jena, K., & Sahoo, D. K. (2015). Sonorenisin: A new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific Report*, 5, 13422.

Fairfax, M. R., Lephart, P. R., & Salimnia, H. (2014). Weissella confusa: Problems with identification of an opportunistic pathogen that has been found in fermented foods and proposed as a probiotic. *Frontiers in Microbiology*, 5, 254.

Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., … Franz, C. M. A. P. (2015). The genus Weissella: Taxonomy, ecology and biotechnological potential. *Frontiers in Microbiology*, 7, 155.

Galvao, M. F., Prudencio, C. V., & Vanetti, M. C. D. (2015). Stress enhanced the sensitivity of Salmonella enterica serovar typhimurium to bacteriocins. *Journal of Applied Microbiology*, 118, 1137–1143.

Garzón, K., Ortega, C., & Tenea, G. N. (2017). Characterization of bacteriocin-producing lactic acid bacteria isolated from native fruits of Ecuadorian Amazon. *Polish Journal of Microbiology*, 66(4), 473–481.

Ge, J., Sun, Y., Xin, X., Wang, Y., & Ping, W. (2016). Purification and partial characterization of a novel bacteriocin synthesized by Lactobacillus paracasei HD1-7 isolated from Chinese sauerkraut juice. *Scientific Report*, 6, 19366.

Giuliani, A., Pirri, G., & Nicoletto, S. (2007). Antimicrobial peptides: An overview of a promising class of therapeutics. *Open Life Sciences*, 2, 1–33.

Goh, H. F., & Philip, K. (2015). Purification and characterization of bacteriocin produced by Weissella confusa A3 of dairy origin. *PloS One*, 10(10), e0140434.

Hansen, L. T., Austin, J. W., & Gill, T. A. (2001). Antibacterial effect of protamine in combination with EDTA and refrigeration. *International Journal of Food Microbiology*, 66, 149–161.

Hartmann, M., Berditsh, M., Hawecker, J., Ardakani, M. F., Gerthsen, D., & Ulrich, A. S. (2010). Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy. *Antimicrobial Agents and Chemotherapy*, 54, 3132–3141.

Hector, S., Willard, K., Bauer, R., Mulako, I., Slabbert, E., Kossmann, J., & George, G. M. (2015). Diverse exopolysaccharide producing bacteria isolated from milled sugarcane: Implications for cane spoilage and sucrose yield. *PloS One*, 10(12), e0145487. doi:10.1371/journal.pone.0145487

Heng, N. C. K., Yeh, C. W., & Malik, A. (2017). Draft genome sequence of Weissella confusa MBF8-1, a glucansucrase and bacteriocin producing strain isolated from a homemade soy product. *Genome Announcement*, 5, e01497–16.

Kalchayanand, N., Dunne, P., Sikes, A., & Ray, B. (2004). Viability loss and morphology change of foodborne pathogens following exposure to hydrostatic pressures in the presence and absence of bacteriocins. *International Journal of Food Microbiology*, 91(1), 91–98.

Katina, K., Maina, N. H., Juvenon, M., Flander, L., Johansson, L., Virkki, L., … Laitila, A. (2009). In situ production and analysis of Weissella confusa dextrans in wheat sourdough. *Food Microbiology*, 26(7), 734–743.

Martin-Visscher, I. A., Yoganathan, S., Sit, S. C., Lohans, C. T., & Vederas, J. C. (2011). The activity of bacteriocins from Carnobacterium maltharomaticum UAL307 against Gram-negative bacteria in combination with EDTA treatment. *FEMS Microbiology Letters*, 317(2), 152–159.

Masuda, Y., Zendo, T., Sawa, N., Perez, R. H., Nakayama, J., & Sonomoto, K. (2012). Characterization and identification of weissellicin Y and weissellicin M, novel bacteriocins produced by Weissella hellenica QU13. *Journal of Applied Microbiology*, 112, 99–108.

Miao, J., Zhou, J., Liu, G., Chen, F., Chen, Y., Gao, X., … Cao, Y. (2016). Membrane disruption and DNA binding of Staphylococcus aureus cell induced by a novel antimicrobial peptide produced by Lactobacillus paracasei subsp. tolerans FX-6. *Food Control*, 59, 609–613.

Mokiena, M. P. (2017). Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens: A mini-review. *Molecules*, 22, 1255.

Nam, H., Ha, M., Bae, O., & Lee, Y. (2002). Effect of Weissella confusa strain PL9001 on the adherence and growth of Helicobacter pylori. *Applied and Environmental Microbiology*, 68, 4642–4645.

Ndagano, D., Lamoureux, T., Dortt, C., Vandermoten, S., & Thonart, P. (2011). Antifungal activity of 2 lactic acid bacteria of the Weissella genus isolated from food. *Journal of Food Science*, 76(6), M305–M311.

Pal, A., Ramana, K. V., & Bawa, A. S. (2010). Simplification and optimization of the Man Rogosa Sharpe (MRS) medium for enhanced production of bacteriocin by Weissella paramesenteroides DFR-8. *Journal of Food Science and Technology*, 47(3), 258–265.

Papagianni, M. (2012). Effects of dissolved oxygen and pH levels on weissellin A production Weissella paramesenteroides DX fermentation. *Bioprocess Biosystems Engineering*, 35, 1035–1041.

Papagianni, M., & Papamichael, E. M. (2011). Purification, amino acid sequence and characterization of the class Ila bacteriocin weissellin A, produced by Weissella paramesenteroides DX. *Bioresources Technology*, 102, 6730–6734.

Patra, P., Roy, S., Sarkar, S., Mitra, S., Pradhan, S., Debnath, N., & Goswami, A. (2015). Damage of lipopolysaccharides in outer cell membrane and production of ROS-mediated stress within bacteria makes nano zinc oxide a bactericidal agent. *Applied Nanoscience*, 5, 857.

Shukla, S., Shi, Q., Maina, N. H., Juvenon, M., & Goyal, A. (2014). Weissella confusa Cab3 dextransucrase: Properties and in vitro synthesis of dextran and glucooligosaccharides. *Carbohydrate Polymers*, 101, 554–564.

Sritionual, S., Yanagida, F., Lin, L. H., Hsiao, K. N., & Chen, Y. S. (2007). Weissellicin 110, a newly discovered bacteriocin from Weissella cibaria 110, isolated from Plaa-Som, a fermented fish product from Thailand. *Applied and Environmental Microbiology*, 73(7), 2247–2250.

Sturino, J. M. (2018). Literature-based safety assessment of an agriculture-and animal-associated microorganism: Weissella confusa. *Regulatory Toxicology and Pharmacology*, 95, 142–152.

Tenea, G. N., Hurtado, P., & Ortega, C. (2018). Inhibitory effect of substances produced by native Lactobacillus lactis strains of tropical fruits towards food pathogens. *Preventive Nutrition on Food Science*, 23(3), 260–268.

Yang, S. C., Lin, C. H., Sung, C. T., & Fang, J. Y. (2014). Antibacterial activities of bacteriocins: Application in food and pharmaceuticals. *Frontiers in Microbiology*, 5, 241.

Ye, K., Liu, J., Liu, M., Huang, Y., Wang, K., & Zhou, G. (2018). Effects of two Weissella viridescens strains on Listeria monocytogenes growth at different initial inoculum proportions. *CyTA - Journal of Food*, 16(1), 299–305.