MOL2NET, International Conference Series on Multidisciplinary Sciences

Antimicrobials from Native Lactic Acid Bacteria: A "Shotgun" Against Antibiotic Resistant *Staphylococcus aureus*

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Introduction

The contamination of food by microorganisms, their persistence, growth, multiplication, and/or toxin production has emerged as an important public health concern. The demand for consuming fresh and low-processed foods free of chemicals and pathogens is increasing. Despite advances in food safety, annually, more than 9 million persons developed illnesses caused by food contamination (Scallan et al., 2011). In Ecuador, the risk of diseases associated with food contaminations is increasing due to incorrect food manipulation, hygiene, and inappropriate storage conditions (Garzon et al., 2017). Although the vendors are continuously capacitated, no improvement on selling sites was made. The food is continuously sold on the street, near parks, transportation terminals, as a common habit. Along with the excessive use of chemicals for preservation, food safety is of concern. To overcome this problem, the application of natural preservation methods might be a suitable solution. Lactic acid bacteria are producing peptides or small proteins namely bacteriocins which could be the next generation of antimicrobials. Thus, their incorporation in food to prevent poisoning or spoilage has been an area of dynamic research in the last decade (Backialakshmi et al., 2015). Previously, we identified two native bacteriocinogenic strains, *Lactiplantibacillus plantarum* UTNGt2 and *L. plantarum* UTNCys5-4, producing peptides with a broad spectrum of antibacterial activity against several foodborne pathogens in vitro (Tenea and Pozo, 2019; Tenea and Guana, 2019). Moreover, the addition of those peptide extracts at the exponential phase of growth of the target bacteria (Staphylococcus aureus ATCC1026) results in a decrease of total cell viability with about 3.2-fold (log CFU/ml) order of magnitude at 6 h of incubation, indicating their bactericidal mode of action. In this study, the possible mechanism of action against *Staphylococcus aureus* was investigated through a series of cell biology analyses such as membrane permeabilization, cell integrity, and structural changes of the target cells. Altogether, the results demonstrated the effectiveness of peptides produced by native lactic acid bacteria to kill Staphylococcus and further investigation is need it to prove the effect in a food matrix.

Materials and methods

Peptide preparation

Peptide extracts of Gt2 and Cys5-4 from the producer cells of *L. plantarum* UTNGt2 (GenBank accession no. KY041688.1), and *L. plantarum* UTNCys5-4 (GenBank No. KY041686.1) were obtained as previously described (Tenea and Pozo, 2019). The data were compared with peptides produced by a commercial bacteriocinogenic strain L. plantarum ATCC8014.

Membrane permeabilization assay

To investigate the effect of each peptide extract on membrane permeabilization, the ONPG (o-nitro-phenyl-L-D-galactoside, # N1127, Sigma-Aldrich Co. LLC, Saint Louis, MO, USA) substrate was used as previously described (Tenea and Pozo, 2019). To distinguish between the cytoplasmic enzyme release and peptide uptake to the cells, β--galactosidase release was measured at different intervals of incubation from the supernatant (415nm).

Cell membrane integrity

The indicator bacterial suspension of *Staphylococcus aureus* ATCC1026 (antibiotic-resistant strain) was grown overnight in BHI broth (Brain Heart Infusion, Merck Millipore, MA, USA) media, harvested by centrifugation, and washed twice with 1 X PBS (phosphate-buffered saline, pH 7.5) as described (Tenea and Guana, 2019). In brief, the bacterial cells were treated independently with 1 X MIC of each peptide and incubated for 24 hours at 30 °C. As control one flask was maintained with no peptides added. The DNA/RNA molecules were extracted and visualized in 1 % agarose gel electrophoresis.

Results and discussion

The specific interaction between two peptides and *Staphylococcus aureus* was investigated to get comprehensive information about their possible molecular mechanism of inhibition. The pathogenic cell membrane is an important cellular structure that serves as a selective permeation barrier (Wang et al., 2017). The direct interaction of *Staphylococcus* cells with the peptide extracts in vitro results in a cytoplasmic β-galactosidase release in the cell-free medium starting with 90 min of incubation, with the highest value recorded for Cys5-4 at 120 min (Figure 1). Moreover, we showed that the

Staphylococcus cell membrane was compromised as DNA/RNA molecules were detected, suggesting that damage to the cell membrane occurred as an effect of the direct interaction between the peptides and the target (Figure 2). Transmission electronic microscopy (TEM) micrographs depicted several simultaneous secondary events such as DNA relaxation, cytoplasmic release, and membrane lysis (data not shown) after the peptide extract treatment underlying their antimicrobial actions.

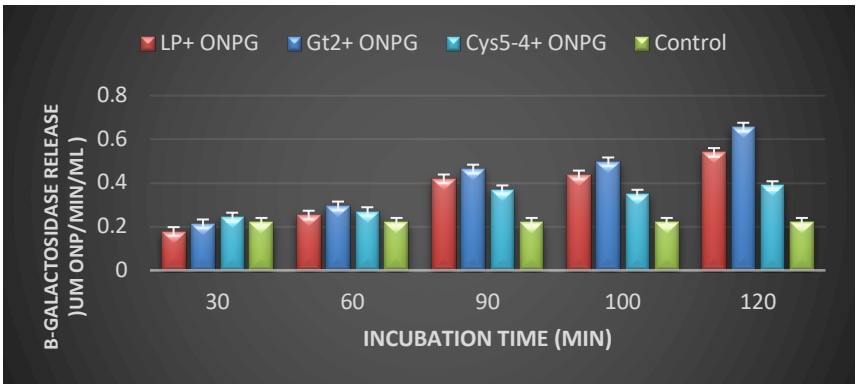

Figure 1. Permeation of *Staphylococcus aureus* ATCC1026 by the peptides during incubation. Legend: Control: cells treated with saline solution alone (no peptide added); Results are representative of three similar and independent experiments each made in triplicate.

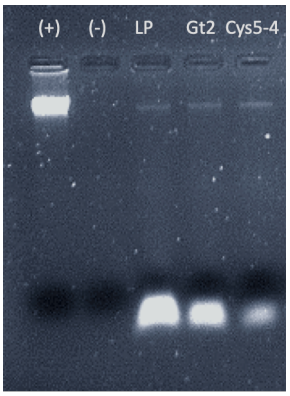

Figure 2. DNA/RNA molecules detected on gel electrophoresis as effect peptide extract treatment. Legend: (+): genomic DNA extracted from *Staphylococcus*; (-) control negative (*Staphylococcus* no peptide treated), no DNA/ RNA detected; LP, Gt2, Cys5-4: DNA/RNA molecules detected upon 1 X MIC of LP, Cys5-4 and Gt2 peptide extract treatment.

Conclusions

Lactic acid bacteria have been part of raw material and fermented foods for a century; thus, they became popular as been associated with health. Thus, the introduction of their bacteriocins as a biocontrol strategy might be a suitable solution. The membrane permeabilization may occur concomitantly with the loss of cell viability, which suggests that both Gt2 and Cys5-4 permeabilized the plasma membrane and disrupted the cell releasing aromatic molecules as the principal lethal event. Altogether, the results demonstrated the effectiveness of peptides produced by two native lactic acid bacteria to kill *Staphylococcus aureus in vitro*, and further investigation is needed to prove their efficacy on different food matrices.

References

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