TITLE: BACTERICIDAL ACTIVITY OF ENTEROCIN AGAINST VANCOMYCIN-RESISTANT *Enterococcus faecium*

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ABSTRACT:

Vancomycin-resistant *Enterococcus faecium* (VRE) has emerged as an opportunistic pathogen promoting prolonged infections and high mortality. In view of this scenario, new alternatives are being developed for the control of VRE. This study aimed to evaluate the action of enterocins on VRE isolates. The enterocin-producing isolate *E. durans* (MF2) was tested for the presence of *ent*A and *ent*B-encoding genes for enterocin, by polymerase chain reaction (PCR) method. The cell-free supernatant of isolate MF2 (CFS-MF2) was evaluated for sensitivity to proteolytic enzymes (α-chymotrypsin, protease, trypsin and proteinase K). Subsequently, enterocin obtained from CFS-MF2 was partially purified (EPP) by the precipitation method with ammonium sulfate. The minimal bactericidal concentration (MBC) of enterocin was evaluated by Microdilution in broth-MIC, and combination of enterocin with vancomycin by Checkerboard method against VRE isolates (EF29 and EF34). The viability (propidium iodide-IP staining) and cell complexity (FSC x SSC) of VRE treated with enterocin was determined by flow cytometry (FC). CFS-MF2 exhibited thermostability, with degradation by proteolytic enzymes, confirming the protein character of enterocins and exhibiting characteristics of class II bacteriocins. The MBC able of inhibiting 80% of bacterial growth (MBC80) was 0.3 µg/mL of enterocin for all VRE isolates (Anova-Tukey Test, $P < 0.05$). The fractional inhibitory concentration index (FICI), performed to assess the synergistic action between EPP and vancomycin, ranged from 0.3 to 0.4 (FICI ≤ 0.5 considered synergism). Enterocin and vancomycin in combination exhibited improved antimicrobial activity, as the MBC of enterocin was reduced by >50% when tested in combination with vancomycin, as well as reduction in the concentration of vancomycin, from 256 µg/mL to 2 µg/mL, when tested in combination with enterocin. The mean percentage of non-viable cells post-treated with the enterocin (0.3 µg/mL) was equivalent to 26%, while the quantification of FSC and SSC showed differences in cell complexity, without relevant changes in cell size. Therefore, enterocin can be considered as a promising antimicrobial compound for the control of VRE, important for the clinical context, because even at low concentrations, the compounds potentiated the antimicrobial effect against VRE isolates.

Keywords: resistance; bacteriocin; antimicrobial peptide; lactic acid bacteria.

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