

TITLE: PARTIAL CHARACTERIZATION OF MULTIPLE BACTERIOCINS PRODUCED BY A CLINICAL ISOLATE OF PENICILLIN-RESISTANT *Enterococcus faecalis*

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ABSTRACT:

Bacteriocins are antimicrobial peptides produced by bacteria. Bacteriocin production among bacteria isolated from human clinical specimens might be a beneficial asset that improves the virulence of pathogenic bacteria. The aim of this study was to characterize the bacteriocins produced by a clinical isolate of penicillin-resistant *Enterococcus faecalis*. The bacterium, designated as *E. faecalis* 20, was previously isolated from urine sample taken from a patient attended at a university hospital, in Uberaba-MG. The kinetics of bacteriocin production was evaluated along the bacterial growth curve, at 37°C, in aerobiosis; the antimicrobial activity was determined in the supernatant and pellet, using the agar well diffusion assay. The supernatant of stationary phase *E. faecalis* 20 culture was lyophilized and suspended in buffer solution. Temperature sensitivity was assessed by heating samples to 45, 60, 80 and 100°C for 30 minutes; the samples were also stored at - 20, - 80°C and room temperature for 1 week. The pH influence was evaluated after suspending the lyophilized supernatant in buffer solution with pH 4, 7 or 9. After treatments, the antimicrobial activity was determined by agar well diffusion assay. The residual antimicrobial activity was determined following the equation: $AR (\%) = (Ht/Hc) \times 100$, where AR is the residual antimicrobial activity; Ht is the halo diameter after treatment; Hc is the halo diameter of the control sample. Different bacteriocins were produced by *E. faecalis* 20, according to the bacterial growth phase: the bacteriocin active against *E. faecalis* ATCC 29212 (named Bac1) was produced during the log-phase, and production during the decelerating growth phase was observed for the bacteriocin active against *E. gallinarum* ATCC 12359 (Bac2). Both bacteriocins were stable at storage temperatures (-20, -80°C and room temperature), but Bac1 lost the biological activity when submitted to temperatures above 45°C. On the other hand, Bac2 was partially stable after heating, maintaining some biological activity even when submitted to 100°C (AR>35%). Bac1 activity gradually reduced with increasing pH (AR=64% at pH 4, 58% at pH 7 and 53% at pH 9), while Bac2 activity maintained unaltered (AR=100%). *E. faecalis* 20 produces distinct bacteriocins, varying in action spectrum, production, temperature and pH sensitivity. Additional work is needed in order to purify and determine the mechanism of action of the bacteriocins produced by *E. faecalis* 20.

Key words: *Enterococcus faecalis*, bacteriocin, antimicrobial activity, virulence

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