

Title: MOLECULAR CHARACTERIZATIONS OF THE NOVEL LANTIBIOTIC HYICIN 5580, PRODUCED BY *STAPHYLOCOCCUS HYICUS* 5580, A STRAIN ISOLATED FROM MILK OF BUFFALO WITH BOVINE MASTITIS

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Abstract:

Lantibiotics are bacterial antimicrobial peptides that contain post-translationally modified amino acids. The bacteriocin-producing strain 5580, isolated from milk of buffalo with bovine mastitis, was identified as *Staphylococcus hyicus* by 16S rRNA gene sequencing and the bacteriocin produced was named hyicin 5580. Hyicin 5580 has a broad spectrum of activity, including the foodborne pathogens *Staphylococcus aureus* and *Listeria monocytogenes*. The main goals of this work were the biochemical and molecular characterizations of hyicin 5580. The highest activity of hyicin 5580 was obtained in BHI and its production was firstly observed after 3 h (160 AU/ml) of the producer growth at 37 °C. The maximum production (10,240 AU/ml) was achieved after 14 h and the activity started decreasing after 20 h. The partially-purified hyicin 5580 (from cation exchange chromatography) exhibited a bactericidal activity against *Micrococcus luteus*. This bacteriocin was purified from the culture supernatant that was subjected to ammonium sulfate precipitation (45% saturation) and subsequently to cation exchange chromatography and HPLC. Molecular mass estimation could not be unambiguously determined by MALDI-TOF mass spectrometry. Moreover, the peptide sequence determination by MALDI-TOF/TOF spectrometry was unsuccessful. Then, genomic DNA from *S. hyicus* 5580 was sequenced by the MiSeq platform (Illumina) at the Norwegian Sequencing Centre. A total of 3,267,624 sequence reads were assembled *de novo* using the software CLC Genomics workbench, resulting in a draft genome of 2,460,692 bp, contained within 36 contigs, with a GC content of 35.7%. Genome annotation was performed using the RAST server, resulting in the delineation of 2,406 ORFs. Using BAGEL 3 and BLAST, a bacteriocin cluster was found. The gene cluster involved in hyicin 5580 biosynthesis seems to include the following genes: two *lanA* (for two different pre-peptides, probably α and β), two *lanM* (for peptide modification), *lanF* and *G* (for bacteriocin immunity), *lanP* (protease for pre-peptide cleavage) and *lanT* (for an ABC transporter). The LanA₁ amino acid sequence (molecular mass of 6,205 Da) has 39% identity to the lantibiotics lacticin 3147 A1 and mersacidin, and 32% to lichenidicin VK21 A1. The putative LanA₂ has no similarity to any protein. These results suggest that hyicin 5580 is a novel type C lantibiotic, composed of two peptides modified by LanM.

Keywords: bacteriocin, lantibiotic, *Staphylococcus hyicus*, bovine mastitis, genome sequencing

Funding Agencies: CNPq and FAPERJ