

Title: MOLECULAR CHARACTERIZATION OF HYICIN 3682, THE FIRST ANTIMICROBIAL PEPTIDE DESCRIBED IN *STAPHYLOCOCCUS HYICUS*

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Abstract:

Hyicin 3682 is the first antimicrobial peptide described in *Staphylococcus hyicus*. It is produced by strain 3682, isolated from bovine milk. The main goals of the present study were to determine the best conditions for hyicin 3682 production and activity, and to find out the genetic determinants involved in its production. The highest production of hyicin 3682 (10,240 AU/ml) was observed after 24-h growth of the producer strain in BHI medium. No bacteriocin production was observed when the strain was grown in GM17 culture medium. None of the proteolytic enzymes tested eliminated completely the bacteriocin activity. However, trypsin reduced its activity in 94%. The hyicin 3682 activity was reduced 75 and 88% in basic pH such as 9.0 and 11.0, respectively. No interference in its activity was observed in acidic pH such as 3.0 and 6.0. Heating at 121, 100 and 80°C for 15 min reduced its antimicrobial activity in 98, 94 and 75%, respectively. Its treatment at 65°C for 15 min proved to be an effective method for sterilization of preparations containing hyicin 3682. *S. hyicus* 3682 was shown to carry a single plasmid, named pRJ109, with a size of approximately 54 kb. PCR reactions using the isolated plasmid DNA as a template showed that pRJ109 carry the genes involved in hyicin 3682 production. Since pRJ109 has a very low copy number and cannot be purified in high amounts, sequencing of the entire genome of *S. hyicus* 3682 was performed in order to determine the complete nucleotide sequence of the gene cluster involved in hyicin 3682 production. *In silico* analyses of contig 28, with a lower GC content (29%), when compared with the whole genome (~36%), and carrying a *rep* gene involved in plasmid replication initiation, showed that the hyicin 3682 gene cluster was located in the same DNA strand and consisted of 8,519 bp and eight genes (*hyiA*, *hyiB*, *hyiC*, *hyiD*, *hyiP*, *hyiF*, *hyiE* and *hyiG*). The bacteriocin encoded by the structural gene *hyiA* showed 87% identity and 91% similarity to Bsa, a 22-amino acid lantibiotic related to epidermin. Regarding to epidermin, both identity and similarity were lower (68 and 79%, respectively). MALDI-TOF mass spectrometry analysis of a purified bacteriocin preparation revealed that hyicin 3682 has a molecular mass of 2,139 Da, a mass similar to that predicted (2,135 Da) by *in silico* analyses performed with the deduced product of the bacteriocin structural gene *hyiA*.

Key words: hyicin 3682, lantibiotic, *Staphylococcus hyicus*, gene cluster

Funding Agencies: CNPq, FAPERJ and CAPES