

TITLE: HYICIN 4244: MOLECULAR AND GENETIC CHARACTERIZATION

AUTHORS: Duarte, A.F.S.¹, Guimarães, A.J.², Nes, I.F.³, Bastos, M.C.F.¹

INSTITUTION: ¹IMPG – Instituto de Microbiologia Paulo de Góes - Universidade Federal do Rio de Janeiro; ²MIP - Dept. de Microbiologia e Parasitologia - Universidade Federal Fluminense; ³LMG - Lab of Microbial Gene Technology - Norwegian University of Life Science, Ås, Norway.

ABSTRACT:

The strain *Staphylococcus hyicus* 4244, isolated from milk of cows with bovine mastitis, produces the bacteriocin named hyicin 4244. This bacteriocin showed a broad spectrum of activity, inhibiting strains of different genera and species, and has potential biotechnological applications in food biopreservation and clinical treatment. Genomic DNA from *S. hyicus* 4244 was sequenced by MiSeq platform (Illumina) and a total of 3,273,442 sequence reads were assembled *de novo* using the software CLC Genomics workbench, resulting in a draft genome of 2,498,362 bp, arranged into 45 contigs and possessing a GC content of 35.6%. Genomic analysis allowed the identification of the hyicin 4244 biosynthetic gene cluster (which a genetic organization identical to the subtilisin A gene cluster): *hycS* (pre-peptide), *hycA* (radical SAM), *hycB*, *C* and *D* (immunity), *hycE* and *F* (processing) and *hycG* (putative membrane protein), transcribed in the same direction. Except for *hycC*, *hycF* and *hycG*, the remaining five genes are preceded by a putative σ^A -dependent promoter. The peptide HycS shares a high similarity to the subtilisin A precursor peptide (SboA), including three conserved C residues, C₁₂, C₁₅ and C₂₁, of the thioether donor sites. Moreover, the acceptor amino acids F₃₀, T₃₆ and F₃₉ involved in thioether linkages are also conserved in both peptides. As the numbers of amino acids found in HycS and SboA are identical and HycS is assumed to be processed at the same site as SboA, the amino acid residues involved in thioether bridge formation not only are identical but also occupy exactly the same positions in both mature bacteriocins. A palindromic sequence of 28 bp was detected downstream of *hycS*, in the intergenic region between *hycS* and *hycA*. This sequence can assume a secondary structure resembling a putative rho-dependent stem-and-loop transcriptional terminator ($\Delta G = -17.6$ kcal/mol) similar to that found in the subtilisin A gene cluster, between the *sboA* and *albA* genes. Transcription of all genes was detected by RT-PCR experiments using total RNA. Translation of the transcripts is also expected as all genes are preceded at appropriate distances by candidate RBS. Consistent with the translation of all genes is the presence of an antimicrobial peptide in the culture supernatant of *S. hyicus* 4244. Separation of the proteins, followed by an overlay of the gel with *M. luteus*, correlated the antimicrobial activity with a peptide whose *Mr* is <10 kDa. The molecular modeling analyzes predicted that the hyicin 4244 mature peptide is composed of two α -helices. Hyicin 4244 is predicted to be anionic, with a net negative charge of -2, and with a theoretical *pI* of 4.03, characteristics also shared with subtilisin A. Due to its resemblance to subtilisin A and the presence of three thioether bonds in its structure, hyicin 4244 is assumed to be a 35 amino acid circular sactibiotic, the first to be described in staphylococci.

Keywords: Genome sequencing; *Staphylococcus hyicus*; hyicin 4244; sactibiotic;