

Title: GENOME SEQUENCE ANALYSIS OF THE BACTERIOCIN-PRODUCING STRAIN *STAPHYLOCOCCUS HYICUS* 4244, ISOLATED FROM MILK OF COWS WITH BOVINE MASTITIS

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Abstract:

Bovine mastitis has the most significant impact on milk quality, since it is the most prevalent disease in dairy cattle worldwide. Coagulase-negative staphylococci have become the most common subclinical mastitis-causing agents in many countries. Some staphylococcal strains produce bacteriocins, ribosomally-synthesized peptides produced by bacteria, which have antimicrobial activity against other bacteria. *Staphylococcus hyicus* 4244, a strain isolated from milk of cows with bovine mastitis, produces hyicin 4244, a bacteriocin that has potential biotechnological applications in food biopreservation and clinical settings. Since *S. hyicus* 4244 does not seem to carry any plasmid DNA, to mine the bacteriocin gene cluster, its genomic DNA was sequenced by using the MiSeq platform (Illumina) at the Norwegian Sequencing Centre. The sequencing libraries were prepared using the Illumina Nextera XT kit. 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, contained within 45 contigs ranging from 1,211 to 282,920 bp, and with a GC content of 35.6%. Genome annotation was performed by using the RAST server, resulting in detection of 2,500 ORFs and 58 tDNAs. No evident toxin gene was found. Genes encoding teicoplanin resistance-associated membrane proteins and staphylococcal adhesins, such as clumping factor B, fibronectin binding proteins A and B, elastin binding protein S, fibrinogen-binding protein, von Willebrand factor-binding protein, autolysin Atl (implicated in biofilm formation), virulence-associated cell-wall-anchored protein SasH (LPXTG motif), SasA and F, were identified. An analysis of the presence of potential mobile genetic elements revealed two intact prophages. Moreover, using BAGEL3 and BLAST, a locus encoding a bacteriocin similar to subtilisin A was found. Hyicin 4244 amino acid sequence has 76% identity to subtilisin A from *Bacillus subtilis* 168, suggesting that hyicin 4244 is a novel variant of this circular bacteriocin. As found for subtilisin A, the gene cluster involved in hyicin 4244 biosynthesis includes the genes: *hyiS* (pre-peptide), *hyiA* (radical SAM), *hyiB*, *C* and *D* (immunity), *hyiE* and *F* (processing) and *hyiG* (putative membrane protein). According to our knowledge, it is the first time that a subtilisin A-related bacteriocin was identified in a staphylococcal strain.

Keywords: genome sequencing, bovine mastitis, *Staphylococcus hyicus*, bacteriocin, subtilisin A.

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