Title: MOLECULAR CHARACTERIZATION OF AUREOCYCLICIN 4185: THE FIRST CYCLIC BACTERIOCIN OF *STAPHYLOCOCCUS AUREUS*

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Abstract

Staphylococcus aureus 4185, a strain isolated from bovine mastitis in Brazil, produces at least two bacteriocins. One of them seems to be a cyclic peptide, a class of bacteriocin described by the first time in the genus *Staphylococcus*. This new staphylococcin was named aureocyclicin 4185 and it is encoded by plasmid pRJ101. However, it was not possible to detect aureocyclicin 4185 in the culture supernatant of the pRJ101 host strain, suggesting that this bacteriocin is produced at low levels by the cells. DNA sequencing, *in silico* and genetic analyses of pRJ101 revealed, in one DNA strand, 10 genes involved in the production of aureocyclicin 4185. This study aims to study the genes present in the gene cluster encoding this bacteriocin, to perform plasmid mobilization studies of pRJ101 and phylogenetic analysis of the Rep protein encoded by this plasmid. DNA sequencing of the genomic DNA and *in silico* analyses allowed us to conclude successfully the plasmid pRJ101 sequencing and provided interesting information about the *S. aureus* 4185 genome characteristics, as the presence of a delta-hemolysin gene encoded on the chromosome and not of a second bacteriocin. *S. aureus* 4088, a strain sensitive to *S. aureus* 4185 and non-bacteriocinogenic, was selected as a recipient of pRJ117 (pRJ101::Tn*917-lac*) in conjugation experiments to obtain a strain of *S. aureus* that produces only aureocyclicin 4185. Immunity analyses showed that *S. aureus* 4088 was resistant to the bacteriocin, highlighting the production of another antimicrobial substance by *S. aureus* 4185. Aureocyclicin 4185 was only detected in the supernatant of the *S. aureus* 4088 transconjugant when it was concentrated by ammonium sulfate precipitation (50-60%). Plasmid segregation experiments indicated that plasmid pRJ117 is stable in the *S. aureus* 4088 transconjugant and final analyses are still going on. It is also being tested the aureocyclicin 4185 production in other genetic backgrounds. Strains are being constructed by transduction of plasmid pRJ117 into *S. aureus* A53 and A70 cured of their plasmids. For evaluating the participation of the *aclH* gene, encoding an ABC transporter, in plasmid mobilization, a strain carrying both pRJ121 (pRJ101*aclH*::Tn*917-lac*) and pGO1 was constructed. Experiments aiming to investigate the plasmid mobilization are ongoing. Phylogenetic analysis of the Rep protein encoded by various plasmids indicated an evolutionary relationship between Rep_{pRJ101}, Rep_{pRJ6} and Rep_{pRJ9}.

Key-words: cyclic bacteriocin, aureocyclicin 4185, *Staphylococcus aureus,* pRJ101

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