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

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**Abstract**

*Staphylococcus aureus* 4185, a strain which was 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 (~11.7 kb). 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 by primer walking, *in silico* and genetic analyses of pRJ101 revealed, in one DNA strand, 10 genes involved in the production of aureocyclicin 4185 (*aclX*, *aclB*, *aclI*, *aclT*, *aclC*, *aclD*, *aclA*, *aclF*, *aclG* and *aclH*). However, a small region between *aclA* (the bacteriocin structural gene) and *aclF* could not be sequenced by this technique. *In silico* analyses identified in the other strand of the plasmid DNA three overlapping genes involved in plasmid mobilization (*mobC, mobA* and *mobB*), a putative *oriT* region and one gene involved in plasmid replication initiation (*rep*). The Mob proteins share a great similarity to the Mob proteins encoded by pRJ6 and pRJ9, which are *S. aureus* bacteriocinogenic plasmids. This study aims to continue the analysis of the mechanisms responsible for aureocyclicin 4185 production: (i) by studying the genes present in the gene cluster encoding this bacteriocin, (ii) by performing plasmid mobilization studies of pRJ101 and (iii) by phylogenetic analysis of the Rep protein encoded by this plasmid. DNA sequencing of the genomic DNA and *in silico* analyses revealed that the small region not previously sequenced contains 56 bp and encompasses a perfect palindromic sequence of 64 bp, capable of forming a hairpin with $\Delta G$ = -28.5 kcal/mol. Plasmid pRJ101 was shown to be mobilized by the conjugative plasmid pGO1 to other *S. aureus* strains at frequencies of $7.1 \times 10^{-8}$. A strain carrying both pRJ121 (pRJ101*aclH*::Tn*917*-lac) and pGO1 was then constructed to evaluate the involvement of the *aclH* gene, encoding an ABC transporter, in plasmid mobilization. Experiments aiming to investigate the plasmid mobilization are still on going. Phylogenetic analysis of the Rep protein sequence of 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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