Title: MOLECULAR CHARACTERIZATION OF AUREOCYCLIN 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 aureocyclin 4185 and it is encoded by plasmid pRJ101 (~11.7 kb). However, it was not possible to detect aureocyclin 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 aureocyclin 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 aureocyclin 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 ∆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 7.1x10^-8. A strain carrying both pRJ121 (pRJ101aclH::Tn917-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, aureocyclin 4185, Staphylococcus aureus, pRJ101

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