Title: MOLECULAR ANALYSIS OF THE ANTIMICROBIAL PEPTIDE AUREOCIN A53, WITH BIOTECHNOLOGICAL POTENTIAL, PRODUCED BY *STAPHYLOCOCCUS AUREUS*

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Abstract:

Aureocin A53 is an atypical class II bacteriocin produced by *Staphylococcus aureus* A53 and encoded by a 10.4-kb plasmid named pRJ9. This bacteriocin has potential in medical applications and also as a food preservative. However, optimal and rational exploitation of aureocin A53 as an antimicrobial agent requires its production in large scale. This, in turn, necessitates knowledge on all gene products required for its production. In the present work, the individual role played by *aucIA* and *aucIB* in immunity to aureocin A53 and the function of *orf7* and *orf8* in aureocin production and/or secretion were investigated. Cloning experiments with *aucIA* and *aucIB* were attempted using plasmid pT181*mcs*. The resulting recombinant plasmids were introduced into electrocompetent *S. aureus* RN4220, generating the strains MB601 (pT181*mcs*/*aucIA*) and MB602 (pT181*mcs*/*aucIB*). The immunity to aureocin A53 exhibited by these strains and the control strain *S. aureus* MB420 (strain RN4220 carrying the *aucIA*-*aucIB* genes cloned into pCC1 and ligated to pT181*mcs*) was then investigated. The growth of the strains MB601 and MB602 was completely inhibited by 160 BU of aureocin A53. However, the control strain was not inhibited by any amount of aureocin A53 tested (up to 2,560 BU), demonstrating full immunity to aureocin A53. These results suggested that the *aucIA* and *aucIB* genes, individually, do not confer immunity to aureocin A53. Probably, both genes are required to promote the immunity phenomenon. At the same time, the function of *orf7* and *orf8* in aureocin A53 externalization was investigated. Two mutants affected in *orf8*, MB38 and MB143, were obtained previously by transposon mutagenesis. These mutants exhibited a 97% reduction in the amount of aureocin A53 found in the culture supernatant, suggesting that *orf8* is important for the maintenance of the normal levels of aureocin A53 secretion. To test if aureocin A53 was being accumulated in the cytoplasm of the strains MB38 and MB143, two different cell disruption methods, including sonication and rupture by glass beads, were attempted. However, aureocin A53 could never be recovered in the supernatant, suggesting that the function encoded by *orf8* is involved in aureocin production. Moreover, RT-PCR analysis detected an amplicon corresponding to co-transcription of both *orf7* and *orf8*, confirming that these genes are transcribed as an operon. The complementation analysis of the mutant strains MB38 and MB143 are under investigation.

Keywords: aureocin A53, *Staphylococcus aureus*, staphylococcin, biotechnological potential

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