

TITLE: GENES OF AN OPERON CONTAINING THE ZINC UPTAKE REGULATOR (Zur) CONTROL VIRULENCE AND ZINC HOMEOSTASIS IN THE OPPORTUNISTIC PATHOGEN *Chromobacterium violaceum*

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ABSTRACT:

Zinc is an essential micronutrient to all forms of life. It participates in catalytical, structural and regulatory functions in both eukaryotic and prokaryotic cells. During infection, eukaryotic hosts impose zinc sequestration to delay or prevent bacterial growth, a process known as nutritional immunity. To counterattack, pathogens release compounds and express receptors that steal zinc of zinc-bound proteins from the host. In bacteria, expression of high-affinity zinc uptake systems, like the ZnuABC transporter, is under the negative control of the Zinc Uptake Regulator (Zur). In this work, we studied the role of Zur and Zur-regulated genes in zinc homeostasis and virulence of the opportunistic pathogen *Chromobacterium violaceum*. Using bioinformatics analysis, we detected a predicted Zur binding site (Zur-box) upstream of the *zur*-CV_3067-*znuCBA* cluster. Co-transcription of these genes was confirmed by reverse transcription polymerase chain reaction (RT-PCR). A whole scanning in the *C. violaceum* genome found a few additional putative Zur-boxes. We generated null-mutant strains deleted of *zur* (CV_3068), *znuCBA* (CV_3066-65-64), and a probable metallochaperone (CV_3067). Growth curves of all strains in different zinc conditions revealed a major role of ZnuCBA in zinc acquisition. Assays of metal depletion with EDTA and posterior supplementation with individual metals suggested that in addition to zinc, ZnuCBA could be involved in manganese acquisition. EDTA sensibility in agar-plates revealed that $\Delta znuCBA$ and ΔCV_3067 were more sensitive to zinc deficiency when compared to wild-type (WT) or Δzur strains. Biofilm quantification by crystal violet staining showed that the mutant strains have no difference in comparison to the WT strain. A phenotype of reduced swimming motility in semi-solid agar was only observed in $\Delta znuCBA$ strain. Virulence assays in Balb/c female mice were performed, and only the $\Delta znuCBA$ presented attenuated virulence, which was reverted in the complemented strain. Also, immunization with the $\Delta znuCBA$ strain protected mice against *C. violaceum* WT infection. In conclusion, we showed that zinc is mainly acquired by the ZnuCBA high-affinity transporter and that its absence severely compromises virulence and swimming motility in *C. violaceum*. Our data also suggest that similarly to the Zur regulon of other species, *C. violaceum* presented a compact Zur regulon.

Keywords: *Chromobacterium violaceum*, transcriptional regulator Zur, transporter ZnuCBA, zinc uptake, bacterial virulence

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