

**TITLE:** THE REGULON OF CV3905, A MarR TRANSCRIPTION FACTOR, SUGGESTS A REDOX SENSITIVE ROLE IN *Chromobacterium violaceum*.

**AUTHORS:** ALVES, J.A.; PREVIATO-MELLO, M; DA SILVA NETO, J.F.

**INSTITUTION:** FACULDADE DE MEDICINA DE RIBEIRÃO PRETO - USP (AV. BANDEIRANTES, 3900 – MONTE ALEGRE, RIBEIRÃO PRETO – SP. CEP 14049-900)

**ABSTRACT:** Transcription factors are regulatory proteins able to bind in specific regions of DNA in response to various signals to regulate gene expression. MarR family transcription factors, widely found in bacteria and archaea, control many processes, such as virulence, antibiotic resistance and oxidative stress response. *Chromobacterium violaceum* is a Gram-negative, free-living  $\beta$ -proteobacterium, able to act as a human opportunistic pathogen. The *C. violaceum* genome has fifteen predicted MarR family transcription factors, among them the protein encoded by CV3905. In this work, we identified genes regulated by this MarR family transcription factor using microarray analysis. Comparison of transcriptome profiles of wild type and  $\Delta$ CV3905 mutant strains revealed a few differentially expressed genes. However, microarray analysis comparing the  $\Delta$ CV3905 mutant with a strain overexpressing CV3905, allowed identifying about two hundred differentially expressed genes, including genes associated with nitrate assimilation and reduction pathways used for anaerobic respiration; sulfur and iron metabolism, several metabolic pathways and the *ohrR/ohrA* system. Many of these genes were previously found being regulated under cumene hydroperoxide stress in *C. violaceum*, suggesting that CV3905 could be a redox sensing transcription factor. To validate both microarrays, northern blot assay was performed for some genes, and was seen that CV3905 in fact activates *ohrA*, encoding an enzyme for organic hydroperoxide detoxification, whose expression is repressed by OhrR, a redox regulator. The positive regulation of CV3905 over *ohrA* was also seen under cumene hydroperoxide addition, where the levels of *ohrA* expression in  $\Delta$ CV3905 have not reach the same as seen in WT strain. *In vivo* assay also showed that CV3905 overexpression contributes to growth of *C. violaceum* WT and  $\Delta$ CV3905 strains in media containing tert-butyl. Additionally, we verified *in vitro* that the purified CV3905 protein containing two cysteine residues formed intermolecular dimers in non-reducing SDS-PAGE gel, after exposure to cumene hydroperoxide, hydrogen peroxide and tert-Butyl hydroperoxide. Using EMSA assays, we demonstrated a redox-dependent binding of the CV3905 protein in the promoter regions of CV3905, *ohrA* and other genes. Together, these results suggest that CV3905 is a redox-sensing transcription factor that controls expression of a large regulon required to defense against oxidative stress in *C. violaceum*.

**Keywords:** Bacterial genetics, Gene regulation, Transcription factors, *Chromobacterium violaceum*, MarR Family, Oxidative stress

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