

## The role of Rv3405c in *M. bovis* BCG Moreau intracellular lifestyle

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### ABSTRACT

*Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), an infectious disease responsible for 1.5 million deaths every year. BCG is the only available vaccine used in preventing TB. The different BCG strains used worldwide for vaccine production differ genetically and these differences can lead to variable protective efficacy. BCG Moreau, the vaccine strain used in Brazil, lost a genomic region (RD16) leading to the truncation of a transcriptional regulator, *rv3405c*. Our group demonstrated that loss of *rv3405c* affects the expression of the adjacent gene, *rv3406*. Rv3406 was characterized as an alkyl-sulfatase and sulfate metabolism seems to be important for virulence of *M. tuberculosis*. It is possible that *rv3406* constitutive expression due to *rv3405c* loss could represent a functional gain in *M. bovis* BCG Moreau. The aim of this study is to evaluate if Rv3405c plays a role in the response of bacilli to intracellular environment. THP-1-derived macrophages were infected (moi 10:1) with BCG Moreau, BCG Pasteur and with recombinant BCG Moreau expressing the intact *rv3405c* gene. CFU count was done to analyze bacilli viability during infection and showed that it is possible to recover BCG until 96h after infection. Western blot analysis of bacterial proteins recovered from infected macrophages confirmed that *rv3405c* truncation leads to Rv3406 constitutive protein expression in BCG Moreau, whereas in BCG Pasteur, bearing an intact *rv3405c*, Rv3406 expression is not detected. Electrophoretic mobility shift assays (EMSA) confirmed the capacity of recognition and binding between purified recombinant Rv3405 and the intergenic region *rv3405c-rv3406*. In addition, we selected ten genes that may be regulated by *rv3405c*, based on data from the literature and analysis of the BCG Moreau genome sequence. Binding of recombinant Rv3405c to their predicted regulatory regions is being evaluated by EMSA. Together these results may provide insights into BCG's response to the intracellular environment.

**Keywords:** *rv3405c*, EMSA, BCG Moreau

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