# EXPRESSION AND CHARACTERIZATION OF *Mycobacterium tuberculosis* PROTEINS MODIFYED POST-TRANSLATIONALLY in *Rhodococcus erythropolis*

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**Introduction.** Human tuberculosis is a major cause of death around the world, with most of the 1.5 million deaths per year attributable to the disease. Proteins released into the extracellular milieu by *M. tuberculosis*, the causative agent of disease have been the focus of research aimed at developing tuberculosis vaccines and immune-diagnostics. Development of a vaccine, recombinant or native, against tuberculosis hinges on the identification of surface or secreted antigens that induce human cell-mediated immune responses.
Recent studies have revealed that bacterial protein glycosylation and methylation are widespread post-translational modifications that are required for virulence in selected pathogenic bacteria. In the other wise, both modifications seem to have an important role in modulate the effectiveness of the host immune response (1). The glycoproteome of *M. tuberculosis* revealed the existence of considerable number of mannosylated proteins (2). In contrast, methylation has been only described in *M. tuberculosis* heparin binding hemagglutinin (Hbha). Interesting, humoral and cellular immune responses induced by Hbha and Apa depend of the level of methylation and glycosylation of the proteins (3, 4). Furthermore, both Apa and Hbha have been considered as adhesins, for their capacity to interact with Fibronectin (Apa) and Heparin and Plasminogen (Hbha). Since both modifications are lost in the recombinant proteins expressed in *Escherichia coli*, there is a need to look for surrogate bacteria that have the capacity to modify the foreign proteins. Among candidates, are some members of *Rhodococcus* genus. They are Gram-positive, GC-high content bacteria and the genome sequence of several species showed that they are close genetically related to micobacteria. Bioinformatics analysis showed that, *Rhodococcus spp* possess many orthologous of *M. tuberculosis* lipoglycoproteins with motifs that can be targets of modification (2) and also has an Hbha protein homologous to that of *M. tuberculosis* (5).
The objective of this work was to clone and express *M. tuberculosis* proteins with potentially to be post-translationally modified in R. *erythropolis* with the aimed to be used as components of a subunit vaccine.

**Methods.** The genes that codify for Hbha and for *M. tuberculosis* glycoproteins, Apa, PstS1, and LprG were chosen. The coding regions corresponding to unprocessed mycobacterial proteins were amplified from genomic DNA of *M. tuberculosis* H37Rv and were cloned in both *R. erythropolis* expression vector pNit-QC1 (Not inducible) and pTip-QC1 (Thiostrepton inducible) with His tag linked to carboxyl terminus for glycoproteins and the amino terminus for Hbha. Recombinant plasmids were electroporated in *R. erythropoli*s L88, a lysozyme sensible mutant (6). All genes were also cloned and expressed in *E. coli*. Recombinant proteins from *R. erythropolis* and *E. coli* were purified by IMAC.
Recombinant and native proteins will be resolved in SDS_PAGE and after transferred to PDVF membranes, ligand blot assays will be carried out with Con A to identified mannosylation or with and antibody raised against lysine methylated motifs. Also transferred proteins will be incubated with Fibronectin, Heparin and Plasminogen.

**Results.** Our results show that *R. erythropolis* is able to express the four mycobacterial proteins and cleave the secretion signal peptide of Apa, PstS1 and LprG. It was confirmed that the purified APA recombinant protein was recognized by Con A, indicating this observation that the protein is being mannosylated by *R. erythropolis*. Mannosylation of the other glycolipoproteins are under study as well as methylation of recombinant Hbha. Interesting, this last protein was obtained associated to lipids bodies as has been described for *R. opacus* Hbha orthologous (5). Western blot assay with the anti-methylated-lysine antibody gives a positive signal.

**Conclusions.** *R. erythropolis* could be considered as a new host for expression of post-translational modified proteins of *M. tuberculosis*.

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