

# Virulence and pathogenicity

## Approach to TlyA Cytolysin / Hemolysin structure of *Mycobacterium tuberculosis*

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TlyA protein produced by *Mycobacterium tuberculosis* has a controversial role since it has been demonstrated to be an RNA methyltransferase enzyme which confers antibiotic susceptibility to capreomycin and other studies have proved its capability to produce hemolytic activity. Prediction of secondary and tertiary structure was based on three-dimensional protein structure software (Insight II and SwissPDBviewer) and public servers for subcellular location, (SecretomeP) domains (SMART and PFAM) and topology prediction (SIGNALP and TMHMM). Possible phylogenetic relationships were explored for homologous proteins with MEGA 4.0 program. TlyA protein contained a specific ribosome binding domain (S4 residues 5-68) common for homologous protein of this family but with different sequence location. Hydrophobicity profile and non polar residues composition suggests a membrane association, although the absence of signal peptide and transmembrane helices could indicate that this protein is not secreted (via Sec) and the existence of an alternative transport mechanism is unknown. The model obtained by structural homology prediction reveals the presence of a common core, comprising a parallel  $\beta^2$ -sheet of six strands, sandwiched between two layers of helices corresponding to a RNA methyltransferase. The phylogenetic analysis for TlyA protein demonstrated that it is conserved among *Mycobacteria* species belonging to *Mycobacterium tuberculosis* complex and is not related with other proteins from intracellular bacteria. On the other hand experimental evidences from previous studies on TlyA has suggested a link between ribose modification and bacterial pathogenicity.

**Key Words:** *M. tuberculosis*, virulence, protein structure.

### Characterization of a knockout strain of *Mycobacterium bovis* IN P27 gene

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P27 lipoprotein was previously described as virulence factor in *Mycobacterium tuberculosis*. This protein is encoded by the P27 (lprG) gene and forms an operon with Rv1410 that encodes for an efflux pump, P55. OBJECTIVE: The aim of this study was

to determine the role P27 during the infection of *Mycobacterium bovis*. **METHOD:** Here, a mutant of *Mycobacterium bovis* not producing P27 was obtained by two-step mutagenesis using the counterselectable *sacB* marker in the shuttle plasmid p2nil. Complemented strains carrying a copy of each genes or the entire operon in a replicative plasmid were also obtained. The in vivo replication of strains was assessed by using an intrathacheal model of mice infection. **RESULTS:** Western blot experiments using anti-P27 polyclonal sera showed that the P27 protein was present both in the parental and in complemented strains, in which either the entire P27-P55 operon or P27 was reintroduced, but absent in the mutant strain. All of the strains showed similar growth kinetics and characteristics in culture broth. Finally, the replication of the P27 mutant was impaired in mice. **CONCLUSION:** These results indicate that P27 is also relevant for the replication of *M. bovis*.

### **The multiple methyl-branched fatty acid-containing acyltrehaloses are absent in an *mce2* mutant strain of *M. tuberculosis***

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The *mce* operons constitute four homologous regions in the *Mycobacterium tuberculosis* genome, each of which has eight ORFs. Although the function of Mce proteins is still unknown, numerous evidences relate the *mce* operons with virulence factors. Based on the identification of conserved domains in many Mce proteins, recent works strongly suggest that the *mce* genes encode for ABC transport systems. These findings, together with the fact that lipid metabolites are also regulated by the *mce3* operon regulator (unpublished data), lead us to hypothesize that the *mce* operons are likely lipid transport systems. **AIMS:** In order to demonstrate the role of the *mce2* genes in the lipid metabolism of *M. tuberculosis*, an *mce2* mutant, which carries a kanamycin cassette in the third gene of the operon, was compared to the wild type strain for their lipid contents. **METHODS:** Lipids were prepared from sub-cellular fractions of the parental strain H37Rv and of the *mce2* mutant strain. The lipidic fractions were then subjected to one- and two-dimensional thin-layer chromatography, in a variety of solvent systems, to resolve lipids of various polarities. **RESULTS:** We found that the non-mycoloylated trehalose esters, which mainly contain methyl-branched fatty acids (DAT, PAT and SL) are absent in the *mce2* mutant strain. **CONCLUSIONS:** Although these findings need further confirmation, from the results we have obtained so far, we can conclude that the *mce2* operon is related with the metabolism of this family of lipids.

**Involvement of HLP protein in *Mycobacterium leprae* adhesiveness capacity and with biofilm formation ability of *Mycobacterium smegmatis* in respiratory epithelial cells**

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The heparin-binding haemagglutinin (HBHA) and the histone-like protein (Hlp) have been frequently referred as adhesins in the interaction between *Mycobacterium tuberculosis*-epithelial cells. However, the role of these proteins in the interaction of *M. leprae* with epithelial cells has been poorly investigated. Moreover, only few studies have explored the role of these proteins in non-pathogenic mycobacterium species such as *Mycobacterium smegmatis*. The aim of the present study was to investigate whether Hlp are involved with adhesive properties of *M. smegmatis*. To this investigation wild type and mutant strain for *hlp* gene were used to interact with different substrates, appraising: hydrophobicity (adherence to *n*-hexadecane), formation of biofilm to polystyrene microtiter plates, adherence to both bronchial and alveolar epithelial cells and microcolonies formation to bronchial cells. Biofilms tests on acellular substrates suggested that Hlp affects biofilm formation. Adherence and microcolony assays on epithelial cells suggested that Hlp is not important for initial *M. smegmatis* attachment, but may promote bacterial aggregation. Finally, we investigated whether Hlp and HBHA proteins could act as adhesins in the context of *M. leprae*-epithelial cells interaction. We showed that polystyrene beads pre-treated with HBHA and Hlp recombinant proteins of *M. leprae* enhanced significantly the attachment of the beads to alveolar epithelial and bronchial cells. Altogether, our data suggest that the influence of Hlp on bacterial adhesive properties may diverse according to the species of mycobacteria. Hlp may either play a direct role as adhesion and/or, through interference with the cell wall composition, affect latter steps required for niche colonization.

**Key Words:** Histone-like protein, respiratory epithelial cells, *Mycobacterium*.

Oral Presentation

**Observations on the physiological role of *mce* operons in *M. smegmatis***

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The identification of a protein mediating mammalian cell internalization of *Mycobacterium tuberculosis* led to the recognition of a set of operons designated *mce* (mammalian cell entry) which are highly conserved through different pathogenic and non pathogenic mycobacteria (four operons in *M. tuberculosis*, six in *M. smegmatis* and three in *M. bovis*). Disruption of any of the four *mce* operons caused dramatic attenuation in *M. tuberculosis* H37Rv. Given the homology of *Mce* proteins with ABC transporters it was postulated they may take part in the uptake or efflux of substrates of unknown structure. In this study we report the construction of *M. smegmatis mce* mutants in which five or six *mce* operons were deleted and the analysis of their role(s). We analyzed growth in liquid and solid media of different composition, biofilm formation, motility pattern and modification of various substrates. We didn't observe differences in motility, growth or substrate modification; but subtle differences in morphology and biofilm formation were detected suggesting modification(s) of the cellular envelope in *mce* mutants. Disappointingly, analysis of cell envelope lipids showed no differences between the *mce* mutants and the wild-type strain. Considering that lipid composition differs between *M. tuberculosis* wild type and *mce* mutants, we suggest that the physiological function of the *mce* operons is different in non-pathogenic and pathogenic mycobacterial species. The availability of the herein reported *M. smegmatis mce* mutants and our studies currently being performed on *M. tuberculosis mce* mutants will help understanding the roles of these operons in mycobacteria.

**Key Words:** *M. tuberculosis* attenuation, *mce* operons, *M. smegmatis*, cell envelope.

### **The intergenic *narK2* / Mb1767 control region is differentially regulated during latency in *Mycobacterium bovis***

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**INTRODUCTION:** *M. tuberculosis* and *M. bovis* show high identity at the genome level but they display different phenotypic and virulence pattern to persist in the host. **OBJECTIVES:** To study the promoter activity of the intergenic control region shared by *narK2X* (nitrite/nitrate transporters) and Mb1767, genes transcribed in opposite directions. **METHODS:** promoter activity was determined upon cloning the promoters in pSM128 vector. The control region was amplified from H37Rv and *M. bovis* AF2122 genomic DNA. Transformed wild type *M. tuberculosis*, *M. bovis*, and their  $\Delta$ DosR's were exposed to NO and ethanol conditions and the promoter activity was determined using  $\beta$ -galactosidase and qRT-PCR. **RESULTS:** *M. bovis narK2* promoter showed no activity, in either strain or growing condition. In contrast, Mb1767 showed 5.7 x higher expression in *M. tuberculosis*. However, upon NO treatment, the induction in *M. bovis* was 5 x over 2.6 in *M. tuberculosis*. Rv1738 was similarly expressed in both, strains and conditions. In *M. bovis* exposed to ethanol,

Mb1767 showed a reduced induction as compared to *M. tuberculosis*. Wild type *M. bovis* and *M. tuberculosis* showed similar results on transcript abundance. Sequence alignment displayed a SNP at -17 of *M. bovis narK2*, what explains its inactivation. The *narK2*, Mb1767 and Rv1738 promoters are DosR dependent, as these are not expressed in *M. tuberculosis* nor *M. bovis ΔDosR*. CONCLUSIONS: The inactivity of *narK2* and the strong Mb1767 NO induction in *M. bovis* compared to *M. tuberculosis*, suggest the occurrence of different mechanism(s) for *M. bovis* survival.

**Key Words:** Tuberculosis, *narK2*, latency.

### Differential virulence of *Mycobacterium bovis* isolates from Argentina in murine model

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**INTRODUCTION:** Bovine tuberculosis is a significant world-wide cattle disease. The causative agent is *Mycobacterium bovis*, pathogen for humans and animals. Several authors have reported different level of virulence among *M. tuberculosis* isolates using murine models but little is known about the virulence of *M. bovis* isolates. **OBJECTIVE:** To evaluate in murine model, the virulence of selected *M. bovis* genotypes from Argentina. **MATERIAL AND METHODS:** Animal models: a) murine model by intraperitoneal infection; b) murine model of progressive pulmonary tuberculosis by intratracheal infection. Parameters studied (survival, CFU counts in spleen and lung, and spleenomegaly).

**Strains:** Five strains were selected. Two isolates from cattle and one from a wild boar possessed the prevalent spoligotype (SB0140) in Argentina (45 % of all isolates), but with MIRU patterns different. Other strain, spoligotype SB1055 was isolated from another wild boar. The fifth isolate, spoligotype SB1413, was isolated from a woman with glenohumeral monoarthritis. **RESULTS:** Reference strain showed middle virulence, producing 50 % mice survival after four months post-infection, with mild number of bacilli counts. The two strains isolated from wild boars showed higher mortality, starting to kill animals after three weeks of infection and three weeks later all the mice died, and higher CFU counts and big spleens. In contrast, strains isolated from bovines and humans were lesser virulent, permitting 100 % and 80 % survival after four months post-infection, with lower bacilli loads. **CONCLUSION:** Our results show that *M. bovis* isolates exhibited different levels of virulence, demonstrated by two murine models.

**Key Words:** *M. bovis*, virulence, genotype.