

**P1446**

**Paper Poster Session**

**Non-tuberculous mycobacteria**

**The *Mycobacterium smegmatis* MSMEG3765 (TetR-like) protein is involved in stress response**

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**Background:**

TetR-like regulators, with a conserved helix-turn-helix (HTH) DNA-binding domain and a C-terminal ligand domain, are widespread among bacteria and archaea. They regulate a wide range of cellular activities, including biosynthesis of antibiotics, multidrug resistance, efflux pumps, virulence and pathogenicity of bacteria. In the *Mycobacteria* genus they are the most abundant transcriptional regulator among the HTH regulators and the majority of them remain uncharacterized. We have identified a TetR-like regulator in *Mycobacterium smegmatis* mc2155, namely MSMEG3765, showing 74% identity with Rv1685c of *Mycobacterium tuberculosis* H37Rv.

**Material/methods:**

We performed transcriptional analysis of the MSMEG\_3765 locus by RT-PCR and expression analysis by RT-qPCR in standard and stress growth conditions. Acid-nitrosative stress conditions were obtained by exposing exponentially growing cells to 5mM NaNO<sub>2</sub>, pH 5.3, for three hours. We isolated the *M. smegmatis* Δ3765 mutant strain by a two-step homologous recombination, using the p2NIL/pGOAL system. The MSMEG\_3765 coding sequence was cloned in pet22b+, and the recombinant protein was expressed and purified by His-tag for structural studies and in vitro DNA binding analysis.

**Results:**

Transcriptional analysis shows that MSMEG\_3765 is the last of a three genes operon (MSMEG\_3762/63/65) and is down regulate under acid-nitrosative multi stress conditions. Expression studies on the wt and mutant strain (Δ3765) show that MSMEG3765 is a repressor of its own operon. Indeed, expression of MSMEG\_3762 is 60-fold up-regulated in the mutant strain. The protein was expressed in *E. coli* BL21(DE3), and the His-tag MSMEG3765 recombinant protein was found to be correctly folded by circular dichroism spectroscopy.

**Conclusions:**

The *M. smegmatis* MSMEG\_3765 gene product, annotated as TetR transcriptional regulator, shows high identity with the *M. tuberculosis* Rv1685c protein, and is conserved among other pathogenic mycobacteria, like *M. avium* and *M. abscessus*. MSMEG\_3765, regulating its own operon, is differentially expressed under acid-nitrosative stress, which the conditions found in phagosomal environments. Global expression profile of wt and mutant strain will highlight the MSMEG3765 function and the extension of its regulon.