Deciphering the role of MSMEG_5850, a probable TetR Family Transcriptional Regulator in the life cycle of Mycobacteria

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Introduction:
The presence of hostile conditions inside the host and the actively growing and dormant phase plays a key role in the regulation of gene expression in mycobacteria. Such flexibility is achieved through changes in gene expression, which is mainly mediated by transcriptional regulation. Mycobacteria is dependent on TFTRs for various metabolic processes, however, majority remains uncharacterized. In the present study, attempts have been made to decipher the role of MSMEG_5850, a probable TFTR in the life cycle of M. smegmatis by knocking out the gene and studying its regulatory networks in which it might be involved using transcriptomic analysis.

Methods:

Expression of MSMEG_5850 under normal and stress (acidic, iron, oxidative, nutritive) in M. smegmatis.

Construction of MSMEG_5850 knockout by homologous recombination

Transcriptome analysis of M. smegmatis; wild type (MSMEG_5850) and Knockout (∆MSMEG_5850) and data analysis.

Effect of knockout on growth of M. smegmatis

Results:

Fig 1: in -vitro expression analysis of MSMEG_5850 under normal and various stress conditions (acidic, oxidative, nutritive, iron). A) Semi quantitative PCR based analysis. B) qRT-PCR based analysis. Results are represented as mean ± SD of three independent experiments.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Knockout</th>
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<tbody>
<tr>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>0.6</td>
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Fig 2: A) Read alignment summary of control and Knockout (ΔMSMEG_5850) and data analysis.

B) Differentially expressed gene (p-value<0.01) C) Heat map showing the differentially expressed genes in Knockout sample D) Bar graph showing the % upregulated genes & their pathways E) Bar graph showing the % downregulated genes and their pathways

Fig 3: Effect of KO on growth of M. smegmatis

Conclusion:
- Expression pattern of MSMEG_5850 under stress conditions pointed towards its importance in intracellular survival of mycobacteria.
- Transcriptome analysis revealed the genes upregulated and downregulated are related to carbon metabolism, lipid metabolism along with other uncharacterized transcriptional factors.
- Disruption of adaptation strategy of mycobacterium and its clearance inside the host.

Reference:

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