Toxicogenomic response of *Mycobacterium bovis* to hydrogen peroxide

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**ABSTRACT**

**Background:** *Mycobacterium bovis* BCG strain Pasteur 1173P2 responds with adaptive and protective strategies against oxidative stress. Despite advances in our understanding of the responses to oxidative stress in many specific cases, the connectivity between targeted protective genes and the rest of the cell metabolism remains obscure.

**Results:** The Aim of the work was to study a pleiotropic regulator that couples iron metabolism to the oxidative stress response in the response to hydrogen peroxide stress. There were also increased levels of catalase peroxidase (KatG) and the biosynthesis operon of mycobactin. This study revealed significant upregulation of the oxidative response group of *M. bovis*, amino acid transport and metabolism, defense mechanisms, DNA replication, recombination and repair, and downregulation of cell cycle control, mitosis, and meiosis, lip transport and metabolism, and cell wall/membrane biogenesis.

**Conclusions:** This study shows that the treatment of *M. bovis* BCG with hydrogen peroxide induces iron acquisition related genes and oxidative stress response genes within one hour of treatment.

**MATERIAL AND METHOD**

- *M. bovis* exposed to hydrogen peroxide (0.5mM) for 10 and 60 min
- Affymetrix *M. bovis* BCG custom genchip analysis
- Statistical analysis of microarray data
  - Fold change in transcript level ≥ 2.0
  - Presence or marginal calls ≥ 50% replicates on both the experimental and control sets
- Quantitative real-time PCR used for the validation of the microarray data

**RESULTS AND DISCUSSION**

### Growth Inhibition of *M. bovis* BCG treated with hydrogen peroxide

![Graph showing growth inhibition](image)

Changes in the amount of ATP produced by the growth culture of *M. bovis* BCG after treatment with hydrogen peroxide were measured in 10 minute intervals for one hour. 0 mM, control (filled circle), 0.05 mM (filled square), 0.5 mM (filled triangle), 5 mM (inverted filled triangle) and 50 mM (filled diamond).

### Functional classification of genes showing statistically significant upregulation and downregulation in transcription levels after 10 and 60 minutes exposure to 0.5mM hydrogen peroxide

![Diagram showing functional classification](image)

Upregulation (mRNA level changes of 1.5 fold or more, empty square) and downregulation (filled square).

### Transcript level comparison of *Mycobacterium bovis* BCG genes between real-time PCR and microarray analyses

![Table showing transcript level comparison](image)

* The real-time PCR results are the mean of three biological replicates with three technical replicates for each gene.

**CONCLUSION**

In summary, we revealed that iron response genes were selectively upregulated during growth inhibition. This study also revealed how oxidative stress-induced genes were related and regulated in *M. bovis* BCG. Our results suggest that DNA repair proteins and catalases are among the most vital antioxidant defense systems of *M. bovis* BCG for preventing the lethal effects of reactive oxygen intermediates. A slowdown of membrane function-related genes was observed, implying that sublethal oxidative damage reduced transport through the cell membrane. We also found that growth inhibition was accompanied by the repression of cell wall/membrane biogenesis genes. It was confirmed that oxidative stress could affect iron metabolism in that many of iron-regulated genes were repressed upon exposure to hydrogen peroxide. Moreover, we showed the induction of iron uptake through the regulation of the Fur and KatG genes, induction of mycobactin biosynthesis and upregulation of biosynthesis of the polyketide backbone through putative drug-transport transmembrane ATP-binding protein ABC transporter during 10 min exposure with hydrogen peroxide. These results suggest that *M. bovis* might undergo an iron acquisition state for protection against oxidative damage upon exposure to hydrogen peroxide. In this study, hydrogen peroxide also upregulated the expression of Ple/PrpE family protein upon 10 min exposure. Further, we propose that repression of the Act-associated hemolytic activity in *M. bovis* may be affected by iron after hydrogen peroxide exposure. Subsequently, a ferric uptake regulatory gene (fur) repressed act gene expression in the presence of high amounts of iron at 10 min exposure. The repression of the glucose-inhibited division gene (gpd) of *M. bovis* at 60 min exposure may indicate a significant reduction in hemolysis and cytotoxicity which may be linked to metabolic recovery.