[C1-070]

Toxicogenomic Response of *Mycobacterium bovis* BCG to Peracetic Acid.

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Introduction

-Tuberculosis is a leading cause of death worldwide and infects thousands of Americans annually.

-*M. bovis* is part of the *M. tuberculosis* complex and is implicated in tuberculosis in humans and several animal species.

-The *M. bovis* bacillus Calmette-Guerin strain (*M. bovis* BCG) that is widely used as a human vaccine against tuberculosis was derived from *M. bovis*.

-Peracetic acid is an EPA-approved oxidative disinfectant for the eradication of pathogens including *M. tuberculosis* in the hospital and domestic environments.

-The mechanism of action of peracetic acid on any mycobacterial species from a global genomic perspective has not been reported.

Objective of Study

What is the global transcriptomic Response of *M. bovis* BCG to peracetic acid?

Microarray Technology using custom Affymetrix GeneChips was used to investigate the effects of 0.1mM Peracetic acid on *M. bovis* BCG.

Materials and Methods

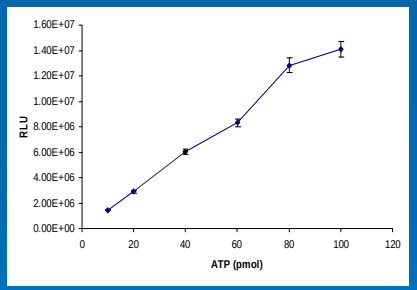
A: Growth Inhibition of *M. bovis* BCG with Peracetic acid

-ATP measurements used to monitor changes in growth after peracetic acid exposure (Using the using the Bac-titer Glo™ microbial cell viability assay and the Glomax™ luminometer).

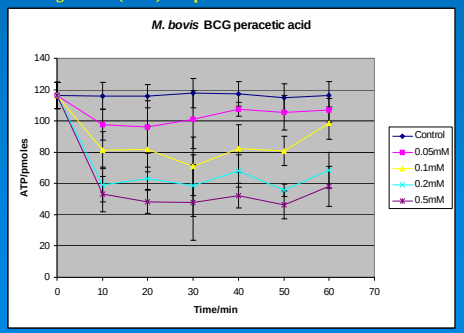
- RNA was extracted after 10 and 20 minutes of peracetic acid treatment.

-Three separate microarray experiments were performed in the absence (control) and in the presence (experimental) of 0.1mM peracetic acid .

-Microarray results were validated using quantitative real time PCR.

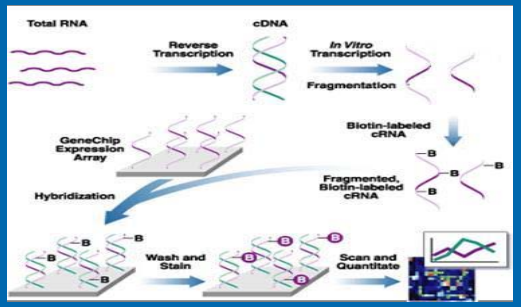


Standard curve showing the correlation between ATP measurements in relative light units (RLU) and picomoles.



M. Bovis BCG exposed to 4 concentrations of Peracetic acid and ATP Produced was measured every 10 minutes for one hour.

DNA Microarray Procedure Courtesy of Affymetrix



Statistical Analysis of Microarray Data

-p-value for 1-Way ANOVA ≤ 0.05

-Fold change in transcript level ≥ 2.0

-Presence or marginal calls ≥ 50% replicates on both the experimental and control sets for 10 and 20 min

-The array data is accessible through series number GSE 15023 at NCBI's Gene Expression Omnibus

Results and Discussion

Functional Classification of Up and Downregulated Genes

-277 genes showed statistically significant fold changes after analysis.

-These genes were functionally classified bacsed on the COG functional categories specified by the NCBI .

Filled bars: Upregulation at one or both treatment times

Empty bars: Downregulation at one or both treatment times

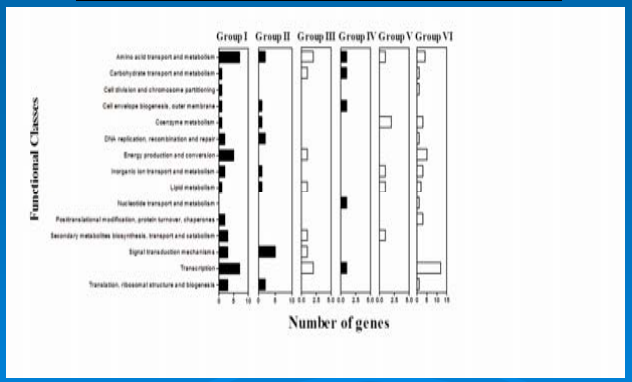


Figure 2: Classification of differentially regulated genes into six groups based on their transcription directions after 10 and 20 minutes exposure to 0.1mM peracetic acid.

Group 1: Upregulation 10 min, Upregulation 20 min

Group II: Upregulation 10 min, No change 20 min

Group III: Downregulation 10 min, No change 20 min

Group IV: No change 10 min, Upregulation 20 min

Group V: No change 10 min, Downregulation 20 min

Group VI: Downregulation 10 min, Downregulation 20 min

Group I: Genes Upregulated after both 10 and 20 minutes

-The expression of KatG, a anti-oxidative stress enzyme produced in pathogenic mycobacteria against reactive oxygen metabolites was upregulated after both treatment times.

-Upregulation of *mbt*D (BCG_2395c) which is involved in the synthesis of mycobatcin siderophores involved in iron acquisition and virulence.

-Upregulation of argininine biosynthesis genes: BCG_1691-1698 (*arg*C, *arg*I, *arg*B, *arg*D and *arg*F, *arg*G, *arg*H and *arg*R).

-Arginine biosynthesis in *M. bovis* BCG may play a role its adaptation to environmental stress

Group II: Genes Upregulated at 10 minutes Only

-Upregulation of the DevR-DevS signal transduction system after 10 minutes with return to normal transcription after 20 minutes.

- The DevR-DevS system regulates the genetic response of *M. bovis* BCG in low oxygen environments and is involved in adaptation and survival of *M. tuberculosis* within host tissues.

Group III: Genes Downregulated after 10 minutes Only

-Downregulation of the *glb*N gene involved in detoxification of reactive nitric oxide generated by activated macrophages during *M. bovis* BCG infections.

-BCG_1249 (*roc*A) plays a role in the adaptation of *Mycobacterium avium* to its niche and utilization of carbon sources.

-Downregulation of these 2 genes may contribute to the mode of action of peracetic acid in *M. bovis* BCG

Group IV: Genes Upregulated after 20 minutes Only

-After 20 minutes, cell wall associated genes were upregulated including: a putative penicillin-binding membrane protein gene, *pbp*B and BCG_2802c which encodes a putative lipoprotein.

-PbpB and lipoproteins are involved in cell shape maintenance, cell wall expansion, the formation of the cell envelope and sensing of and protection from environmental stress.

Group V: Genes Downregulated after 20 minutes only

-The DNA repair gene: *rad*A was downregulated after 20 minutes

-The *lat* gene (BCG_3319c) involved in the maintenance of latent tuberculosis infections

Group VI: Genes Downregulated after both 10 and 20 minutes

-Downregulation of DNA repair genes: *uvr*A (BCG_1676) which is critical to the nucleotide excision repair process and *nrd*F2 which is contributes to the production of deoxyribonucleotides for DNA synthesis and repair.

-Supports results in group V which indicate that peracetic acid represses DNA repair systems in *M. bovis* BCG.

Conclusions

- Regulation of iron levels and virulence factors may play an adaptive role against peracetic acid treatment in *M. bovis* BCG.

-The inhibition of DNA repair systems may contribute to the peracetic acid-induced growth inhibition.

- In addition to *kat*G which plays a major role in defense against oxidative damage, cell wall modification after 20 minutes may serve as a protective strategy against peracetic acid damage.

- TheDevR-DevS signal transduction system may play a role in the early adaptive response of *M. bovis* BCG to peracetic acid induced stress.

References

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This study has been submitted for peer review to Environmental Science and Technology.