[U-057] Toxicogenomic response of *Mycobacterium bovis* BCG to sodium hypochlorite

Hyeung-Jin Jang [1] ,Chantal Nde [2], Freshteh Toghrol [3], and William E. Bentley [2]

College of Oriental medicine, Kyunghee University, Seoul, Korea [1], Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742 [2] and Microarray Research Laboratory, Office of Pesticide Programs, U. S. Environmental Protection Agency, Fort Meade, MD 20755 [3]

INTRODUCTION

***Mycobacterium bovis* BCG (*M. bovis* BCG)**

• TB remains a potential threat to public health, although many anti-tuberculosis drugs have been developed over the past 30 years. Many countries use the *M. bovis* Bacillus Calmette-Guérin (BCG) vaccine as part of their TB control programs, especially for infants. BCG vaccines are live attenuated strains of *M. bovis*.

• In 2004, according to World Health Organization (WHO) mortality and morbidity statistics indicate that, there are 14.6 million chronic active TB cases, 8.9 million new cases, and 1.6 million deaths, mostly in developing countries.

Sodium hypochlorite (bleach) against pathogens

• Bleach is an antimicrobial agent and an active ingredient of ERA-registered disinfectants

•US Environmental Protection Agency (EPA) has endeavored to determine the mechanism of action of antimicrobials

Microarray technology (GeneChip®)

• Enables a genome-wide analysis of cellular responses to bleach

How pathogens respond to bleach?

• **Global transcription profiling by microarrays helps understand mechanisms involved in antimicrobial activity and the corresponding cellular response**

MATERIALS AND METHODS

Affymetrix *M. bovis* BCG custom GeneChip® analysis

• *M. bovis* exposed to bleach for 10 and 20 min

• 3 independent microarray experiments in the absence (control) and the presence (experimental) of bleach upon 10 and 20 min exposures

• Quantitative real-time PCR used for the validation of the microarray data

Statistical analysis of microarray data

• *p*-value for the 1-Way ANOVA ≤ 0.05

• Fold change in transcript level ≥ 2.0

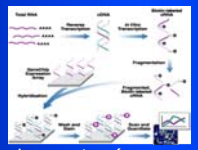


Image courtesy of Affymetrix

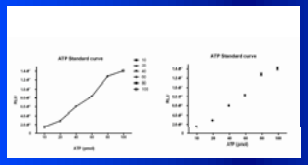
• Presence or marginal calls ≥ 50% replicates on both the experimental and control sets for 10 and 20 min

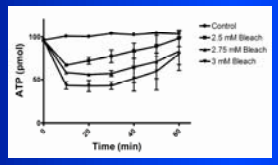
• The array data accessible through series numbers GSE13423 at NCBI's Gene Expression Omnibus

RESULTS AND DISCUSSION

1. Sodium hypochlorite-induced transcriptional changes

Growth inhibition by bleach





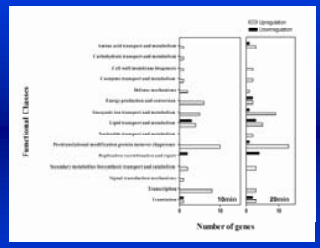
• 2.5 mM bleach triggered a growth inhibition at 10 min. After this adaptation time, cells continued to grow at a same rate as untreated cells

• To better understand how *M. bovis* initially responds to bleach and subsequently, recuperate from the damage, we employed 10 and 20 min exposure times with 2.5 mM bleach

Functional classification of differently expressed genes

• In Figure 2, the differences between the numbers of up and downregulated genes in each functional class after 10 and 20 minutes exposure to 2.5 mM of sodium hypochlorite are illustrated. Note that Figure 2 represents a total of 98 genes excluding the group of "function unknown, hypothetical protein and intergenic region" (163 genes).

• The transcriptional responses are significantly different between 10 and 20 min exposures to 2.5 mM sodium hypochlorite.



•**Classification of differently expression genes on the basis of their transcription directions**

• Group I: genes induced upon 10 and 20 min exposures (37 genes)

- Group I contains 8 genes associated with posttranslational modification, protein turnover and chaperones in *M. bovis*. Interestingly, five of these genes encode the thioredoxin, *trx*B or *trx*C. Thioredoxins are proteins that act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange.

- Decisively oxidoreductase showed the highest fold increases of 33.2 after 10 min and 25.1 after 20 min in this experiment.

• Group II: genes induced upon 10 min exposure (16 genes)

- In group II, there is heat shock protein, *hspX*, belonging to the class of "posttranslational modification, protein turnover, chaperones". In addition, another heat shock protein was upreglated in group III. It is therefore possible that heat shock proteins also play a role in the stress respose of *M. bovis* to bleach.

• Group III: genes induced upon 20 min exposure (26 genes)

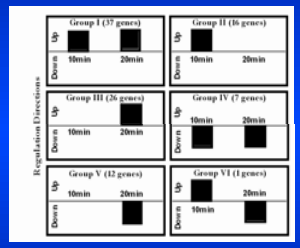
-The alkyl hydroperoxide reductase C and D (*ahp*CD) and catalase-peroxidase-peroxynitritase T (*kat*G) genes belonging to the class of inorganic ion transport and metabolism were present in group III. Oxidant defense system genes using catalase (*kat*), alkyl hydroperoxide reductase (*ahp*), and glutathione peroxidase/reductase were all upregulated.

• Group IV: : genes repressed upon 10 min and 20 min exposure (7 genes)

- Intriguingly, we observed the downregulation of BCG_0280c and BCG_3162 (*fab* G4 and *fad*E24). Mycolic acids are a key component of the mycobacterial cell wall, providing structure and forming a major permeability barrier. In *Mycobacterium tuberculosis* mycolic acids are synthesized by type I and type II fatty acid synthases. One of the enzymes of the type II system is encoded by fabG4.

• Group V: : genes repressed upon 20 min exposure (12 genes)

- BCG_0877c (*des*A1) and BCG_1154 (*des*A2) which are possibly parts of an extensively modified long-chain fatty acid complex were downregulated at 20 min. The acyl-ACP desaturases are one of the major functional classes of soluble diiron enzymes



• Fig. 3 - Groups of differentially regulated 99 genes with known functional class, which are categorized by their transcription directions upon 10 and 20 min exposures. Group I contained 37 genes upregulated upon both exposure times, while group II had 16 genes upregulated at 10 min and no significant changes upon 20 min exposure. Further, group III possessed 26 genes that were upregulated in response to 20 min exposure. Group IV contained 7 genes downregulated upon both exposure times, whereas 12 genes of group V exhibited downregulation after 10 min exposure. Finally, group VI had 1 genes that were upregulated upon 10 min and downregulated upon 20 min exposure.

CONCLUSIONS

In this study, a rapid growth culture was developed which shortened the growth time of *M. bovis* BCG from 21 days to 6 days. We also have shown that ATP quantification using luminescence is a reliable method for monitoring the growth inhibition in *M. bovis* within a short time period (0-60min). This facilitated the determination of the antimicrobial concentration and time points to be utilized for the genome-wide transcriptional analysis of *M. bovis* response to sodium hypochlorite. The data generated in this study are biologically interesting in that they may lead to improved prediction of treatment outcomes and aid in the identification of mode of action of antimicrobials in *M. bovis*.
To our knowledge, this study is the first to report a genome-wide transcriptional analysis of *M. bovis*, a very slow growing bacterium, and response to an antimicrobial in a short exposure time (1 hour).

In summary, this paper describes the first genome-wide transcriptional analysis of *M. bovis* BCG response to sodium hypochlorite. Briefly, our data based on the toxicogenomic analysis showed the following results. First, sodium hypochlorite is an oxidant and initiates a stress response and induces heat shock proteins; second, this outcome in conjunction with the extensive downregulation of the genes encoding mycolic acid biosynthesis suggests that sodium hypochlorite may inhibit biosynthesis of the mycolic acids in the outer cell wall of *M. bovis*.
Consequently, we are currently exploring whether the upregulation and/or downregulation of these genes help protect against sodium hypochlorite in *M. bovis*, and how this event is linked to the bacterial growth inhibition and metabolism in early response after10 and 20 min.

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