Appl Microbiol Biotechnol
DOI 10.1007/s00253-010-2931-6

Toxicogenomic response of *Mycobacterium bovis* BCG to peracetic acid and a comparative analysis of the *M. bovis* BCG response to three oxidative disinfectants

Chantal W. Nde · Freshteh Toghrol · Hyeung-Jin Jang · William E. Bentley

Received: 1 June 2010 / Revised: 8 September 2010 / Accepted: 1 October 2010

Abstract Tuberculosis is a leading cause of death worldwide and infects thousands of Americans annually. *Mycobacterium bovis* causes tuberculosis in humans and several animal species. Peracetic acid is an approved tuberculocide in hospital and domestic environments. This study presents for the first time the transcriptomic changes in *M. bovis* BCG after treatment with 0.1 mM peracetic acid for 10 and 20 min. This study also presents for the first time a comparison among the transcriptomic responses of *M. bovis* BCG to three oxidative disinfectants: peracetic acid, sodium hypochlorite, and hydrogen peroxide after 10 min of treatment. Results indicate that arginine biosynthesis, virulence, and oxidative stress response genes were upregulated after both peracetic acid treatment times. Three DNA repair genes were downregulated after 10 and 20 min and cell wall component genes were upregulated after 20 min. The *dev*R–*dev*S signal transduction system was upregulated after 10 min, suggesting a role in the protection against peracetic acid treatment. Results also suggest that peracetic acid and sodium hypochlorite both induce the expression of the *ctp*F gene which is upregulated in hypoxic environments. Further, this study reveals that in *M. bovis* BCG, hydrogen peroxide and peracetic acid both induce the expression of *kat*G involved in oxidative stress response and the *mbt*D and *mbt*I genes involved in iron regulation/virulence.

Keywords Microarrays · *Mycobacterium bovis* BCG · Peracetic acid · Sodium hypochlorite · Hydrogen peroxide · Transcriptomics

Introduction

Despite extensive research that has been carried out and the availability of effective chemotherapy, tuberculosis (TB) still remains a leading cause of death worldwide (Sassetti et al. 2003). Data from the Centers for Disease Control and Prevention (CDC) indicate that by the end of 2007, two billion people worldwide were infected by *Mycobacterium tuberculosis*, which is the most common cause of TB in the USA. CDC reports also indicate that although the number of TB cases is on the decrease in the USA, thousands of Americans are still infected and hundreds die annually from the disease.

M. tuberculosis and *Mycobacterium bovis* are more than 99% genetically similar (Garnier et al. 2003). *M. bovis* is part of the *M. tuberculosis* complex and is implicated in tuberculosis infections in humans and several animal species (Garnier et al. 2003; Golby et al. 2007). The tuberculosis vaccine strain *M. bovis* Bacillus Calmette-Guerin (*M. bovis* BCG) was derived from *M. bovis* (Garnier et al. 2003; Keller et al. 2008).

Electronic supplementary material The online version of this article (doi:10.1007/s00253-010-2931-6) contains supplementary material, which is available to authorized users.

C. W. Nde · W. E. Bentley
Center for Biosystems Research,
University of Maryland Biotechnology Institute,
College Park, MD 20742, USA

F. Toghrol (✉)
Microarray Research Laboratory, Biological and Economic Analysis Division, Office of Pesticide Programs,
U.S. Environmental Protection Agency,
Fort Meade, MD 20755, USA
e-mail: toghrol.freshteh@epa.gov

H.-J. Jang
College of Oriental Medicine, Kyung Hee University,
Seoul, South Korea

Published online: 28 October 2010

Oxidative disinfectants including peracetic acid, sodium hypochlorite, and hydrogen peroxide are approved by the Environmental Protection Agency as active ingredients in disinfectants used for the eradication of pathogens including *M. tuberculosis* in the hospital, domestic, and agricultural environments. Studies have reported that peracetic acid treatment leads to protein and enzyme denaturation and increased cell wall permeability of bacteria through the disruption of sulfhydryl (–SH) and disulfide bonds (S–S) (Kitis 2004; Block 2001). We have also previously shown using microarray technology that in *Staphylococcus aureus*, peracetic acid alters the expression of membrane transport genes and induces the transcription of DNA repair and replication genes (Chang et al. 2006b). To our knowledge, our recent studies of the toxicogenomic response of *M. bovis* BCG to sodium hypochlorite and hydrogen peroxide are the only studies that report the global transcriptomic response of any mycobacterial species to oxidative disinfectants (Jang et al. 2009a, b). Peracetic acid is approved for disinfection against mycobacteria, yet the mechanism of action of peracetic acid in any mycobacterial species from a global genomic perspective has not been investigated.

Several previous reports have compared microarray studies, either for analyzing the responses of one organism to different treatments or the responses of different organisms to the same treatment. However, the results of these studies are often indecisive (Small et al. 2007). A comparative analyses of the global transcriptomic effects of different antimicrobials in the same pathogenic organism will provide information on major differences and similarities between the metabolic pathways affected in that organism following treatment with these disinfectants. This information will help in the identification of commonly regulated genes in response to these antimicrobials which will facilitate the understanding of their modes of action. The current study and our previous studies (Jang et al. 2009a, b) have detailed the toxicogenomic response of *M. bovis* BCG to peracetic acid, sodium hypochlorite, and hydrogen peroxide. Using the data from these reports, we carried out a comparative analysis of the transcriptomic responses observed after 10 min of treatment of *M. bovis* BCG with the three oxidative disinfectants. We focused on data generated after 10 min of treatment in this analysis as it was a common treatment time among the three studies. A significant advantage of the comparative analysis carried out in the second section of this study is that the transcriptome data and real-time polymerase chain reaction (PCR) validation of microarray results was obtained from experiments carried out under similar experimental conditions in our laboratory.

In the first section of this study, we performed an analysis of the toxicogenomic response of the model organism, *M. bovis* BCG to 0.1 mM peracetic acid using Affymetrix *M. bovis* BCG custom arrays. Results from the first section of this study identify signature genes that are differentially regulated in mycobacteria in response to peracetic acid and improve the understanding of the genetic basis of resistance to this disinfectant. In addition, the information generated from this study sheds more light on the mechanism of action of peracetic acid in Mycobacteria. The second section of this report provides the first comparative analysis of the global gene response of *M. bovis* BCG to oxidative antimicrobials and improves the understanding of the similarities between the mechanisms of action of these disinfectants. In addition, this comparative analysis provides information that can be used for the development of more effective oxidative antimicrobials and antimicrobial mixtures.

Materials and methods

Preparation of bacterial culture and growth conditions

As previously described (Jang et al. 2009a, b), a stock culture of *M. bovis* BCG strain Pasteur 1173P2 (ATCC 35748) was inoculated into 200 ml Middlebrook 7H9 broth (Difco, Sparks, MO, USA) supplemented with 0.1% (*v*/*v*) Tween 80 (Sigma-Aldrich Co., St. Louis, MO, USA) and 10% (*v*/*v*) OADC (oleic acid, albumin, dextrose, catalase). Following incubation at 37°C with shaking at 200 rpm, the culture reached an OD_{600} of 0.3–0.4 after 5 days. One-milliliter volumes of this culture were maintained in 10% (*v*/*v*) glycerol at −80°C for subsequent use.

Measurement of cellular adenosine triphosphate

Due to the characteristic slow growth of *M. bovis* BCG, the quantity of adenosine triphosphate (ATP) produced by cells treated with peracetic acid as opposed to colony counts was used to monitor the changes in the number of viable cells. A 1-ml aliquot of the prepared *M. bovis* culture was added to the M7H9 medium and incubated at 37°C with shaking at 200 rpm to reach an OD_{600} of 0.3–0.4 after 5 days. Cells were harvested and resuspended in 200 ml of Luria–Bertani (LB) broth containing 0.1% Tween 80 and incubated for 24 h at 37°C to reach an OD_{600} of 0.3–0.6. The amount of luminescence in relative light units (RLU) produced by cells was measured using the Bac-Titer Glo™ microbial cell viability assay and the Glomax™ luminometer (Promega Co., San Luis Obispo, CA, USA). A standard curve relating luminescence (RLU) and the corresponding amount of ATP in picomoles has been previously reported (Jang et al. 2009a, b).

Peracetic acid treatment and ATP measurements

To determine a value for background luminescence prior to disinfectant treatments, cells in 1 ml of the untreated culture

in LB broth were harvested, washed in 1 ml 1× phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA, USA) and resuspended in 200 μl PBS. A 100-μl volume of the resuspended pellet of untreated cells was added to marked control wells of a 96-well plate containing 100 μl of the Bac-Titer Glo buffer–substrate mixture and luminescence was measured. LB growth cultures were dispensed into designated 50 ml tubes, and peracetic acid was added to the cultures to reach test concentrations of 0, 0.05, 0.1, 0.2, and 0.5 mM. Luminescence measurements were performed at 10-min intervals during a 1-h period for the different peracetic acid concentrations as described for the untreated culture.

RNA extraction

M. bovis BCG cells treated with peracetic acid and untreated cells were harvested and resuspended in PBS buffer. The mini-bead beater-16 (BioSpec Products Inc, Bartlesville, OK, USA) was used for breaking down the cells. Beating was carried during five 1-min periods. Microcentrifuge tubes containing the cells were stored on ice for 2 min after each beating period. RNA was extracted from untreated to peracetic acid-treated (0.1 mM) cells after 10 and 20 min using the RiboPure bacteria kit (Ambion, Inc., Austin, TX, USA). Eluted RNA was quantified using the NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). RNA quality and purity were checked using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

Complementary DNA synthesis, labeling, hybridization, staining, and scanning

Complementary DNA (cDNA) synthesis, fragmentation, labeling, hybridization, washing, and staining were performed according to instructions for the Affymetrix GeneChip arrays (Affymetrix, Inc., Santa Clara, CA, USA) as reported in our previous publications (Chang et al. 2006a, b; Jang et al. 2008; Nde et al. 2008).

Data analysis: toxicogenomic response of *M. bovis* BCG to peracetic acid

Data analysis was performed using the Affymetrix GeneChip Operating Software (GCOS), version 1.0, and GeneSpring Version 7.3 (Agilent Technologies). Parameters employed for expression analysis using GCOS include α_1=0.04, α_2=0.06, τ=0.015, and target signal was scaled to 150. Statistically significant changes in gene expression were identified by one-way ANOVA (p value≤0.05). Fold changes were calculated as the ratios between the signal averages of three untreated (control) and three peracetic acid-treated cultures. Genes with a 2-fold or more induction or repression were used in this analysis.

Data analysis: comparisons among the toxicogenomic responses of *M. bovis* BCG to sodium hypochlorite, hydrogen peroxide, and peracetic acid

The Affymetrix GCOS, version 1.0, and GeneSpring Version 7.3 (Agilent Technologies) were used for data analysis. The same parameters mentioned above were employed for expression analysis using GCOS. Using GeneSpring, three sodium hypochlorite-treated sample replicates, three hydrogen peroxide-treated replicates, and three peracetic acid-treated replicates, with exposure times of 10 min each, were normalized to three untreated (control) sample replicates. As previously described (Small et al. 2007), the lists of genes with present/marginal calls from 50% or more of the replicates were created for each sample set (three untreated control samples, three sodium hypochlorite-treated samples, three hydrogen peroxide-treated samples, and three peracetic acid-treated samples). A master list was then created by merging the four gene lists. A one-way ANOVA (p value≤0.05) was used to determine statistically significant gene expression changes within this master list. Fold changes were calculated as the ratios between the signal averages of three untreated (control) and the signal averages of each of the disinfectant-treated (sodium hypochlorite (2.5 mM; Jang et al. 2009a), hydrogen peroxide (0.5 mM; Jang et al. 2009b), and peracetic acid (0.1 mM)) cultures.

Real-time PCR analysis: toxicogenomic response of *M. bovis* BCG to peracetic acid

Quantitative real-time PCR on nine randomly selected genes was carried out in order to validate the transcript levels obtained by the microarray experiments. Primer sequences and genes used for PCR analysis are listed in Table 1. The *M. bovis* BCG 16S recombinant RNA (rRNA) housekeeping gene was used as an internal control in the PCR reactions. The iCycler iQ PCR system, the iScript cDNA synthesis kit, and the IQ SYBR Green Supermix (BioRad Laboratories, Inc., Hercules, CA, USA) were used to perform the real-time PCR reactions. For each gene, three biological replicates with three technical replicates each were employed. The conditions used for PCR reactions were 3 min at 95.0°C followed by 40 cycles of 10 s at 95.0°C, 30 s at 55.0°C, and 20 s at 72.0°C. PCR efficiencies were determined from standard curve slopes in the iCycler software v.3.1. Assessment of PCR specificity was carried out using melt-curve analysis. Single primer-specific melting temperatures were obtained from melt-curve analysis. Changes in the expression of genes relative

Table 1 Transcript level comparison of *M. bovis* BCG genes between real-time PCR and microarray analyses

Gene	mRNA level change with microarray[a]		mRNA level change with real-time PCR[b]		Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
	Fold change		Fold change			
	10 min	20 min	10 min	20 min		
BCG_1697	2.39	2.62	3.48	2.22	ATCAACGTCACCAAACGTTCGCC	TTCGGTGTAGGCGTAGATGTCCTT
BCG_1698	2.79	2.81	3.18	2.07	TAGCTCGATCATGCCGCAGAAGAA	AATCGAACACCGGCTCCTTGTCTT
BCG_2395c	2.07	2.14	2.76	2.07	ACTGGACTTGCGTAACCGACTCAA	ACGCGAAATGTCCACTTTCTGTGC
BCG_1947c	2.19	2.41	3.1	2.32	AACATCAAAGTGTCCTTCGCCGAC	GCAAAGGATTCCACGTCGGTTTGT
BCG_3156c	2.09		4.5		ATTGAACTGTGCCGCGATCTGTTG	GCCAACTCCATTCCCTTGATGTCT
BCG_3155c	2.13		3.56		ATCCTGAAGTGCAGCAACGACTCT	ACAATGGACCCACGAATTGAACGC
BCG_1249	−2.18		−2.15		TATGGCAGTGGGTGGGTACCAATA	TTCTGGCCCTGGTAATCGAATGCT
BCG_2180c		2.03		3.32	GCAATTGGAAGAGGCCAGGAAGAA	GGCATCTGGCTTGTTGTTCAGCTT
BCG_0299c	−2.57	−2.12	−2.38	−3.45	AGTATCGCATCGAGCTGAACACCA	TGCGAAAGAGAACCCGATGAACGA
16S rRNA[c]	1.00	1.00	1.00	1.00	TGC AAG TCG AAC GGA AAG GTC TCT	AAG ACA TGC ATC CCG TGG TCC TAT

[a] The microarray results are the mean of three replicates of each gene

[b] The real-time PCR results are the mean of three biological replicates with three technical replicates for each gene

[c] Internal control: 16S rRNA

to the 16S rRNA gene were used to quantify transcript level changes. Data on Table 1 indicate that our microarray results were in agreement with real-time PCR results.

Results

Growth inhibition of *M. bovis* BCG by peracetic acid

In order to determine a suitable sublethal concentration of peracetic acid that will produce strong growth inhibition, *M. bovis* BCG was exposed to four concentrations of peracetic acid (0.05, 0.1, 0.2, and 0.5 mM), and growth inhibition was monitored by the changes in the amounts of ATP in picomoles produced 10 min intervals for 1 h. In Fig. 1, the highest concentration of peracetic acid used (0.5 mM) produced a drastic growth inhibition. Therefore, a lower concentration of 0.1 mM was selected as the test concentration to observe the sublethal effects of peracetic acid on *M. bovis* BCG.

Changes in the transcriptional profiles of *M. bovis* BCG in response to peracetic acid

Three microarray replicates were used in the absence (control) and in the peracetic acid-exposed (experimental) group. Transcriptome time course effects were observed after 10 and 20 min of exposure to 0.1 mM peracetic acid. Determination of significant changes in transcription in response to peracetic acid was based on the following criteria: (1) the p value for a Mann–Whitney t test <0.05, (2) a ≥2-fold change in transcript level, and (3) a gene should have a present or marginal call (Affymetrix, Inc.) from 50% or more replicates on both experimental and control replicate sets. After a one-way ANOVA, 1,740 out of the 5,412 genes that make up the *M. bovis* BCG genome were found to be statistically significant. Further analysis of these genes revealed that a total of 277 were upregulated ≥2-fold or downregulated ≤2-fold after 10 and 20 min. All data from this study have been deposited in the National Center for Biotechnology information (NCBI) gene Expression Omnibus and can be accessed through the GEO series accession number GSE 15023.

Functional classification of upregulated and downregulated genes in *M. bovis* BCG in response to peracetic acid treatment

The 277 statistically significant genes were classified based on the COG functional categories specified by the NCBI. One hundred sixty-six genes were classified as “function unknown”, “intergenic regions”, “hypothetical”, “general function prediction only”, and “unclassified”. These genes have not been included in Figs. 2 and 3. Figure 2 illustrates

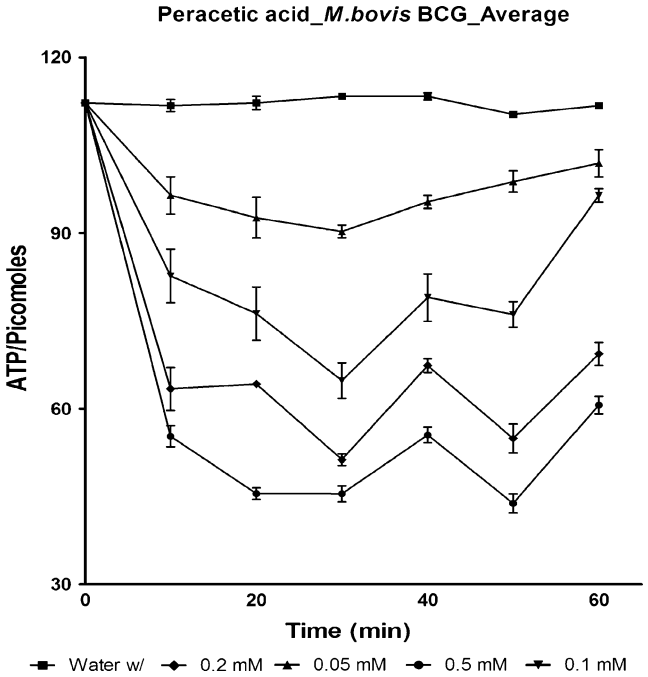

Fig. 1 Growth Inhibition of *M. bovis* BCG by peracetic acid over 60 min. ATP measurements in picomoles were monitored in 10-min intervals. The peracetic acid concentrations were as follows: control with water (*filled square*), 0.05 mM (*filled triangle*), 0.1 mM (*inverted filled triangle*), 0.2 mM (*filled diamond*), and 0.5 mM (*filled circle*). Each data point was determined from the average of three separate experiments and the *error bars* represent the standard deviations obtained

the grouping of 111 up- and downregulated genes at 10 and 20 min into different functional classes and the total number of genes in each class.

In Fig. 2, the functional classes of "coenzyme metabolism" and "inorganic ion transport and metabolism" contained more downregulated genes at 20 min compared to 10 min. The functional classes of "amino acid transport and metabolism", "DNA replication, recombination, and repair", "lipid metabolism", and "signal transduction mechanisms" contained more upregulated genes at 10 min compared to 20 min.

Grouping of functionally classified up- and downregulated genes in *M. bovis* BCG in response to peracetic acid treatment

The 111 up- and downregulated genes were placed in 6 groups based on their transcription directions. Figure 3 illustrates the six groups and the total number of genes in each group. Group I contains genes that were upregulated after 10 and 20 min. Group II is made up of genes that were upregulated after 10 min only. Group III contains genes that were downregulated only upon 10 min of peracetic acid treatment. Group IV contains genes that were upregulated after 20 min only. Group V is made up of genes that were downregulated only after 20 min. Group VI contains genes that were downregulated after both 10 and 20 min.

Changes in the transcriptional profiles of *M. bovis BCG* in response to sodium hypochlorite, hydrogen peroxide, and peracetic acid

Of the 5,412 genes represented in the *M. bovis BCG* custom array, 4,860 genes passed the present/marginal call from the four sample sets (three untreated control samples, three sodium hypochlorite-treated samples, three hydrogen peroxide-treated samples, and three peracetic acid-treated samples) to form a master list. Based on the one-way ANOVA, 2,090, 2,069, and 1,973 genes in the sodium hypochlorite, hydrogen peroxide, and peracetic acid sample sets, respectively, were statistically significant. When fold change analysis was carried out, 84 genes in the sodium hypochlorite-treated samples showed a 2-fold up- or downregulation in expression compared to the control samples. Fifteen genes in the hydrogen peroxide-treated samples showed a 2-fold up- or downregulation in expression compared to the control samples, and within the peracetic acid-treated samples, 290 genes were 2-fold up- or downregulated compared to the controls.

Venn diagram regions

A Venn diagram which shows the unions and intersections of the three disinfectants was constructed (Fig. 4). Fifty-two genes were upregulated and five genes were downregulated exclusively in response to sodium hypochlorite (region 1). Six genes were upregulated and no genes were downregulated exclusively in response to hydrogen peroxide (region 2). One hundred seventy genes were upregulated and 96 genes were downregulated exclusively in response to peracetic acid (region 3). There were six upregulated genes and no downregulated genes in common between sodium hypochlorite and hydrogen peroxide (region 4). Twenty upregulated genes and one downregulated gene were common between sodium hypochlorite and peracetic acid (region 5). There were three upregulated and no downregulated genes in common between hydrogen peroxide and peracetic acid (region 6). No genes were found in common to all the three disinfectants (region 7).

Heat map analysis of changes in the transcriptional profiles of *M. bovis* BCG in response to sodium hypochlorite, hydrogen peroxide, and peracetic acid

A heat map analysis illustrating the changes in gene expression in the control samples and experimental samples

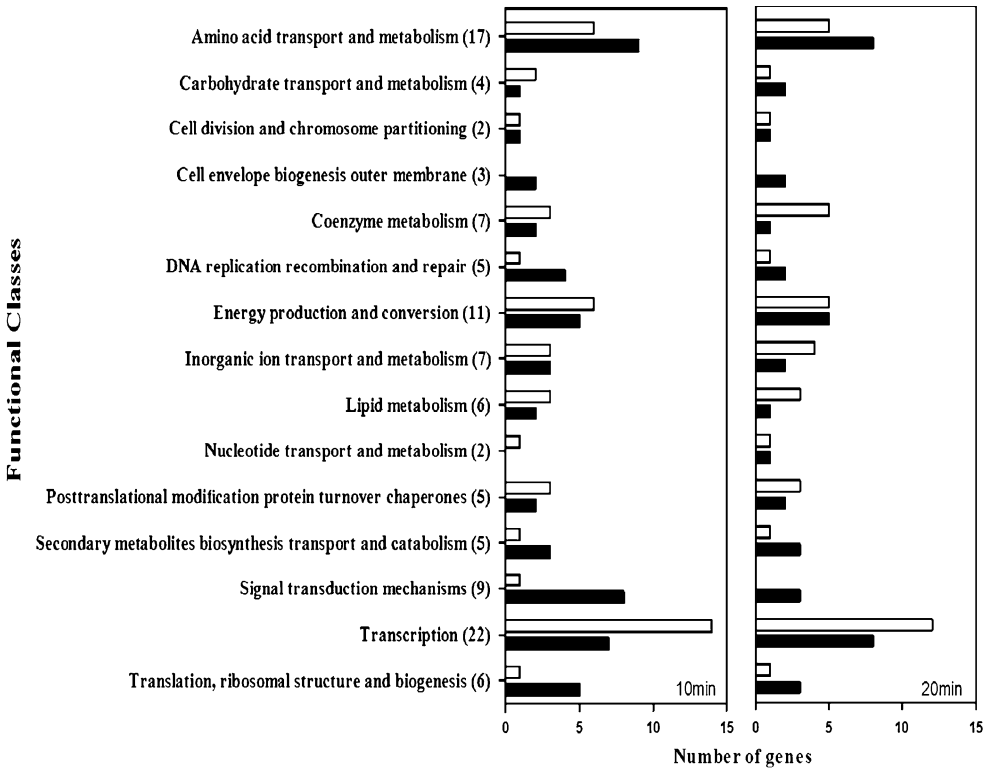

Fig. 2 Functional classification of statistically significant upregulated (*filled bars*) and downregulated (*empty bars*) genes after 10 and 20 min of exposure to 0.1 mM peracetic acid. The *numbers in parentheses* indicate the total number of genes for each functional class in both groups (a total of 111 genes)

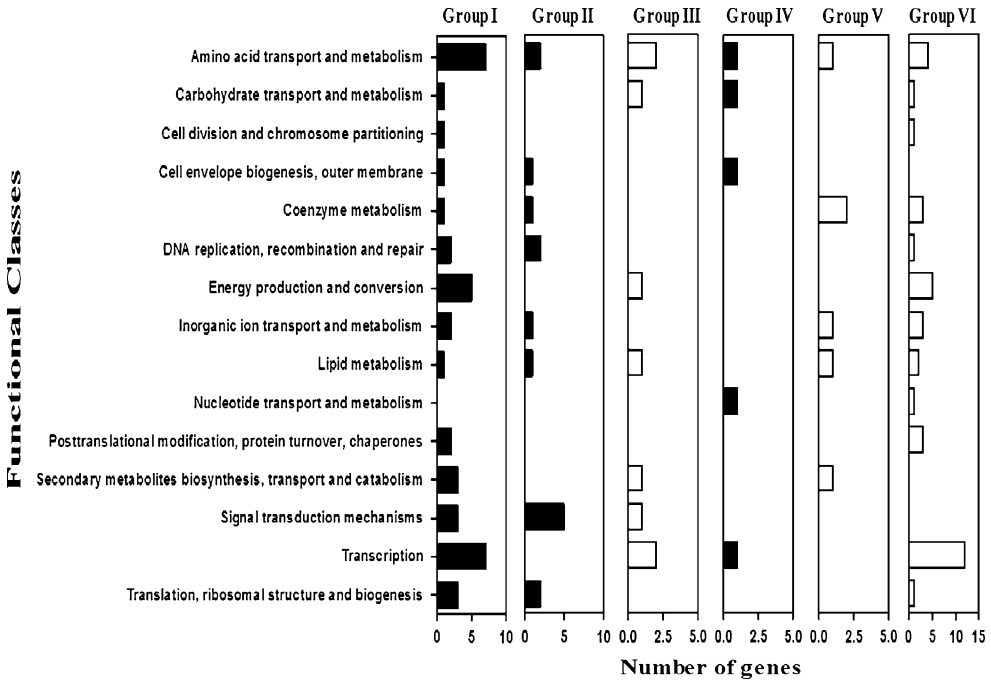

Fig. 3 Classification of significantly regulated 111 genes into 6 groups based on their transcription directions after 10 and 20 min of exposure to 0.1 mM peracetic acid. *Filled bars* indicate upregulation at either or both treatment times. *Empty bars* indicate downregulation at either one or both treatment times. Group I is made up of genes upregulated after both exposure times. Group II contains genes upregulated at 10 min, with no significant changes after 20 min of exposure. Group III consists of genes downregulated after 10 min, with no significant changes upon 20 min of treatment. Group IV is made up of genes that were upregulated in response to 20 min of treatment. Group V is made up of genes that were downregulated upon 20 min of treatment. Group VI is made up of genes that were downregulated upon both treatment times

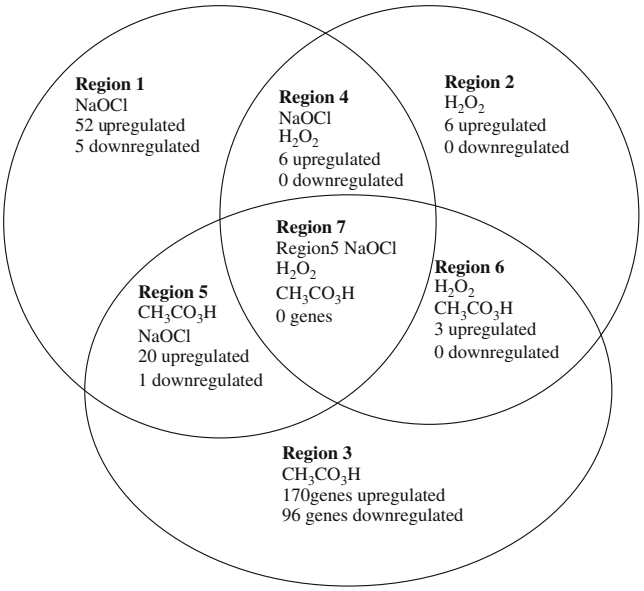

Fig. 4 Venn diagram showing intersections among the genes up- and downregulated in *M. bovis* BCG in response to sodium hypochlorite, peracetic acid, and hydrogen peroxide treatment. Designated regions are as follows: sodium hypochlorite is exclusively in region 1, hydrogen peroxide is exclusively in region 2, peracetic acid is exclusively in region 3, the intersection between sodium hypochlorite and hydrogen peroxide is in region 4, the intersection between sodium hypochlorite and peracetic acid is in region 5, the intersection between hydrogen peroxide and peracetic acid is in region 6, and the intersection of all three antimicrobials is in region 7. The genes in the regions represented in this diagram are listed in Table 3

for sodium hypochlorite, hydrogen peroxide, and peracetic acid treatment was performed. Visual inspection of the heat map indicates that sodium hypochlorite and peracetic acid treatments led to more changes in gene expression (up- and downregulation of genes) compared to hydrogen peroxide treatment (Fig. 5).

Discussion

Toxicogenomic response of *M. bovis* BCG to peracetic acid

All of the genes discussed in this report are in the Supplementary material. However, for clarity and to facilitate the reading of this report, the genes discussed below in the six groups are indicated in Table 2.

Group I: genes upregulated after 10 and 20 min of exposure to peracetic acid

This class contained eight genes relating to arginine biosynthesis namely BCG_1691–1698 (*arg*C, *arg*I, *arg*B, *arg*D and *arg*F, *arg*G and *arg*H, and *arg*R). All the genes involved in the arginine biosynthetic pathway are essential for optimal growth of both *M. tuberculosis* and *M. bovis* BCG (Sassetti et al. 2003). The upregulation of arginine biosynthesis in this study corroborates the fact that arginine is necessary for *M. bovis* BCG growth but also points to the possibility that arginine biosynthesis in *M. bovis BCG* may play a role in its adaptation to peracetic acid-induced oxidative stress.

The polyketide synthase-associated gene, *pap*A1 (BCG_3887c) which encodes a probable acyltransferase was upregulated after both treatment times. PapA1 is required for the biosynthesis of sulfolipid-1, which is a major glycolipid of the *M. tuberculosis* cell wall and is suspected to be involved in virulence (Bhatt et al. 2007). A second polyketide synthase gene *mbt*D gene (BCG_2395c) was also upregulated in this group. MbtD encodes a polyketide synthase that is involved in the biosynthesis of mycobactins which are salicylic acid-derived siderophores, important in mycobacterial iron acquisition (Barclay and Ratledge 1983; LaMarca et al. 2004; Quadri et al. 1998; Snow 1970). Iron is essential in mycobacteria as a cofactor for enzymes catalyzing redox reactions and for other cellular functions (Rodriguez and Smith 2006). In *M. tuberculosis*, mycobactins are essential for virulence and infection maintenance (Neres et al. 2008; Rodriguez and Smith 2006). Mycobactins may also function as temporary reservoirs of iron, potentially mediating the formation of reactive oxygen species (De Voss et al. 2000; Snow 1970; Vergne et al. 2000). The upregulation of these virulence-associated genes points to the possibility that the pathogenesis of *M. bovis* BCG is induced in response to peracetic acid treatment. Similar results showing the upregulation of virulence have been reported in *Pseudomonas aeruginosa* and *S. aureus* treated with different antimicrobials (Chang et al. 2006a, b; Jang et al. 2008).

The catalase-peroxidase-peroxynitritase T (*kat*G) gene was also upregulated after both treatment times. KatG is a hallmark anti-oxidative stress enzyme produced in pathogenic mycobacteria against reactive oxygen metabolites (Heym et al. 1993; Milano et al. 2001; Sherman et al. 1995). KatG is also implicated as a virulence factor of *M. tuberculosis* based on both guinea pig and mouse models (Li et al. 1998; Wilson et al. 1995). Iron regulation and oxidative stress are intricately connected (Milano et al. 2001; Zheng and Storz 2000), with iron mediating the detrimental cytotoxic effects of reactive oxygen species (Zheng and Storz 2000) and also functioning as an essential cofactor of enzymes (Ernst et al. 2005). The upregulation of both iron acquisition/virulence and oxidative stress response genes in this study suggests that these processes are all involved in the adaptive response of *M. bovis* BCG to peracetic acid treatment.

BCG_1255 (PE13) and BCG_3040c (PPE46) which belong to the PE/PPE families of genes were upregulated after both 10 and 20 min of peracetic acid exposure. The PE/PPE families of genes constitute approximately 10% of the genome of *M. tuberculosis* (Tundup et al. 2006; Voskuil

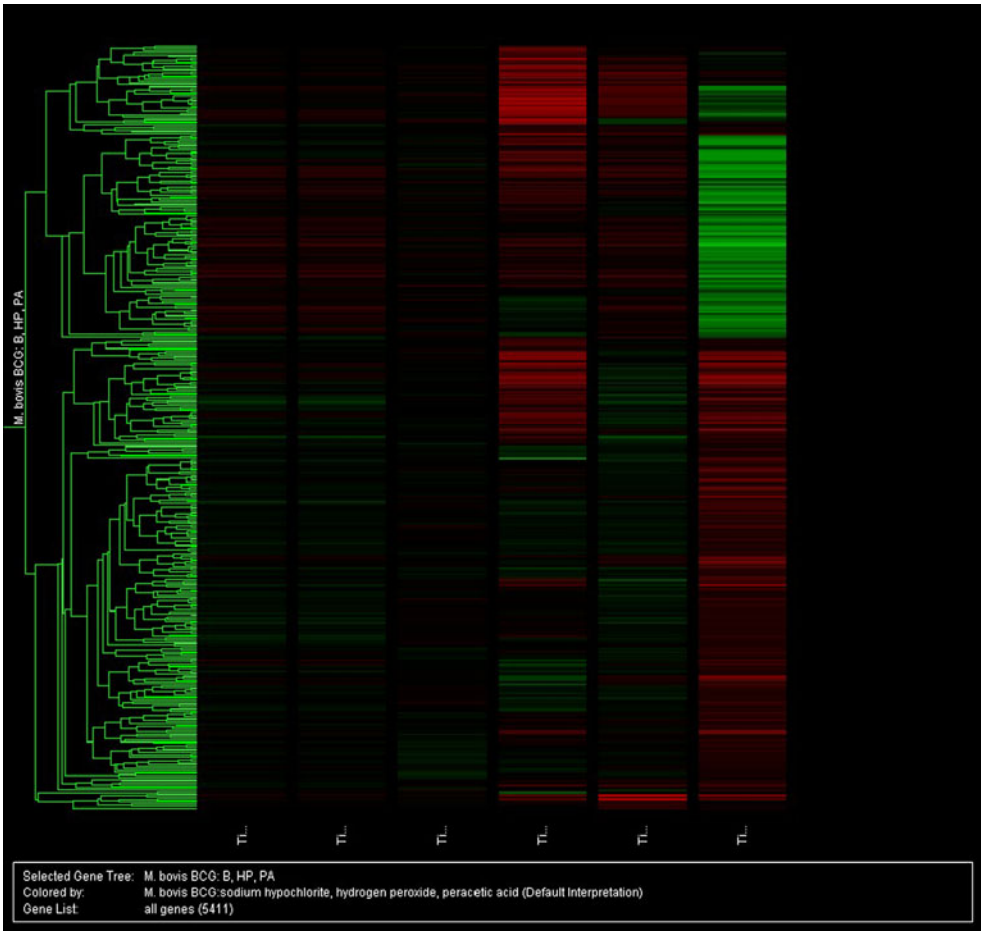

Fig. 5 A heat map illustrating the changes in gene expression in control and experimental samples of *M. bovis* BCG treated with sodium hypochlorite, hydrogen peroxide, and peracetic acid. *Lanes 1*, *2*, and *3* represent the control samples for experiments with sodium hypochlorite, hydrogen peroxide, and peracetic acid respectively as treatments. *Lanes 3*, *4*, and *5* represent the experimental samples after 10 min of exposure to 2.5 mM sodium hypochlorite, 0.5 mM hydrogen peroxide, and 0.1 mM peracetic acid, respectively. Upregulated genes are shown in *red* while downregulated genes are shown in *green*

et al. 2004) and are suggested to be cell wall proteins that could provide a diverse antigenic profile and affect immunity (Voskuil et al. 2004). Studies have shown that proteins belonging to these families may contribute to immunity if included in a tuberculosis vaccine (Chaitra et al. 2008; Tundup et al. 2008). A recent study also indicated that PPE proteins may play a role in the transport of antimicrobials across the *M. bovis* BCG outer membrane (Danilchanka et al. 2008). These results suggest that in addition to the already reported functions for the PE/PPE genes of mycobacteria, they may also play a role in the response to oxidative damage.

Group II: genes upregulated only upon 10 min of exposure to peracetic acid

Group II of Table 2 indicates the two genes of interest in this group: BCG_3156c (*dev*R) and BCG_3155c (*dev*S). The *dev*R–*dev*S genes code for a response regulator, DevR and a histidine sensor kinase, DevS, respectively, that manifest phosphorylation characteristics typical of two-component signal transduction systems (Saini et al. 2004). The DevR–DevS system regulates the genetic response of *M. tuberculosis* to hypoxia and nitric oxide exposure (Bagchi et al. 2005), both conditions which are likely to prevail during latent tuberculosis infections (Nathan and Shiloh 2000; Wayne and Sohaskey 2001). The expression of *dev*R–*dev*S is also upregulated in *M. bovis* BCG grown in low oxygen environments (Boon et al. 2001). Additionally, *dev*R has been implicated in *M. tuberculosis* virulence in a guinea pig model, suggesting that it plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within host tissues (Bagchi et al. 2005). Considering that *M. bovis* BCG in this study was grown under aerobic conditions, the upregulation of the *dev*R–*dev*S system may occur in response to the effects of oxidative stress due to the generation of reactive oxygen species from peracetic acid treatment. Therefore, the upregulation of the *dev*R–*dev*S signal transduction system after 10 min with a return to normal transcription levels after 20 min suggests that it may play a role in the early protective response of *M. bovis* BCG to peracetic acid-induced oxidative stress.

Group III: genes downregulated only upon 10 min of exposure to peracetic acid

The *glb*N gene (BCG_1594c) was downregulated after 10 min but returned to normal transcription levels after

Table 2 List of significantly up- or downregulated *M. bovis* BCG genes in response to peracetic acid treatment that are discussed in this report

Affymetrix probe ID	ORF no.	10 min[a]		20 min[a]		Description	Symbol	Functional class
		Fold change[b]	*P* value	Fold change[b]	*P* value			
Group I: upregulation (10 min)–upregulation (20 min)								
MBOV0704S00001683_at	BCG_1697	2.39	0.0018	2.62	0.0018	Putative argininosuccinate synthase argG	*arg*G	Amino acid transport and metabolism
MBOV0704S00001680_at	BCG_1694	2.49	0.000245	2.75	0.000245	Putative acetylornithine aminotransferase argD	*arg*D	Amino acid transport and metabolism
MBOV0704S00001682_at	BCG_1696	2.62	0.00107	2.58	0.00107	Putative arginine repressor argR	*arg*R	Transcription
MBOV0704S00001681_at	BCG_1695	2.65	0.000513	2.90	0.000513	Putative ornithine carbamoyltransferase, anabolic ArgF	*arg*F	Amino acid transport and metabolism
MBOV0704S00001679_at	BCG_1693	2.75	0.000529	2.87	0.000529	Putative acetylglutamate kinase argB	*arg*B	Amino acid transport and metabolism
MBOV0704S00001678_at	BCG_1692	2.77	0.000218	2.91	0.000218	Putative glutamate *N*-acetyltransferase argJ	*arg*J	Amino acid transport and metabolism
MBOV0704S00001684_at	BCG_1698	2.79	0.0112	2.81	0.0112	Putative argininosuccinate lyase argH	*arg*H	Amino acid transport and metabolism
MBOV0704S00001677_at	BCG_1691	2.33	0.00352	2.55	0.00352	Putative *N*-acetyl-gamma-glutamyl-phoshate reductase argC	*arg*C	Amino acid transport and metabolism
MBOV0704S00002377_at	BCG_2395c	2.07	0.0443	2.14	0.0443	Polyketide synthetase mbtD	*mbt*D	Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00001931_at	BCG_1947c	2.19	0.000291	2.41	0.000291	Catalase-peroxidase-peroxynitritase T katG	*kat*G	Inorganic ion transport and metabolism
MBOV0704S00003859_at	BCG_3887c	2.34	0.000352	2.48	0.000352	Putative polyketide synthase-associated protein papA1	*pap*A1	Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00001241_at	BCG_1255	2.76	0.00727	2.58	0.00727	PE family protein	PE13	
MBOV0704S00003017_at	BCG_3040c	2.36	0.0221	2.40	0.0221	PPE family protein	PPE46	
Group II: upregulation (10 min)–no change (20 min)								
MBOV0704S00003133_at	BCG_3156c	2.09	0.00667			Two-component transcriptional regulatory protein devR (probably luxR/uhpA family)	*dev*R	Signal transduction mechanisms
MBOV0704S00003132_at	BCG_3155c	2.13	0.0154			Two-component sensor histidine kinase devS	*dev*S	Signal transduction mechanisms
Group III: downregulation (10 min)–no change (20 min)								
MBOV0704S00001580_at	BCG_1594c	−2.38	0.00416			Putative hemoglobin glbN	*glb*N	General function prediction only
MBOV0704S00001880_at	BCG_1896	−2.20	0.000974			Alanine- and proline-rich secreted protein apa	*apa*	
MBOV0704S00001235_at	BCG_1249	−2.18	0.0341			Putative pyrroline-5-carboxylate dehydrogenase rocA	*roc*A	Energy production and conversion
Group IV: no change (10 min)–upregulation (20 min)								
MBOV0704S00002162_at	BCG_2180c			2.03	0.0158	Putative penicillin-binding membrane protein pbpB	pbpB	Cell envelope biogenesis, outer membrane
MBOV0704S00002781_at	BCG_2802c			2.04	0.000264	Putative lipoprotein lppU	*lpp*U	
MBOV0704S00002122_at	BCG_2140			2.09	0.0168	PPE family protein	PPE37	
Group V: no change(10 min)–downregulation (20 min)								
MBOV0704S00003622_at	BCG_3650			−2.27	4.37E−05	DNA repair protein radA	*rad*A	
MBOV0704S00003296_at	BCG_3319c			−2.07	0.0219	Putative L-lysine-epsilon aminotransferase lat	*lat*	Amino acid transport and metabolism
Group VI: downregulation (10 min)–downregulation (20 min)								
MBOV0704S00000291_at	BCG_0299c	−2.57	0.0111	−2.12	0.0111	Putative integral membrane nitrite extrusion protein	*nar*K3	Inorganic ion transport and metabolism

Table 2 (continued)

Affymetrix probe ID	ORF no.	10 min[a]		20 min[a]		Description	Symbol	Functional class
		Fold change[b]	*P* value	Fold change[b]	*P* value			
						narK3		
MBOV0704S00003084_at	BCG_3107c	−2.54	0.0035	−2.05	0.0035	Virulence-regulating transcriptional regulator virS (araC/xylS family)	*vir*S	Transcription
MBOV0704S00001993_at	BCG_2009c	−2.19	0.000331	−2.05	0.000331	Putative metal cation transporter P-type atpase G ctpG	*ctp*G	Inorganic ion transport and metabolism
MBOV0704S00001671_at	BCG_1685	−2.07	0.00683	−2.08	0.00683	PE family protein	PE17	
MBOV0704S00001662_at	BCG_1676	−2.03	0.000509	−2.01	0.000509	Excinuclease ABC, subunit a uvrA	*uvr*A	DNA replication, recombination, and repair
MBOV0704S00003049_at	BCG_3072c	−2.15	0.00128	−2.16	0.00128	Ribonucleoside-diphosphate reductase subunit beta nrdF2	*nrd*F2	Nucleotide transport and metabolism

[a] The microarray results are the mean of three replicates of each gene

[b] The fold change is a positive number when the expression level in the experiment increased compared to the control and is a negative number when the expression level in the experiment decreased compared to the control

20 min (Table 2). This gene encodes a truncated hemoglobin, designated trHbN whose sequence is identical in both *M. tuberculosis* and *M. bovis* (Wittenberg et al. 2002). Previous studies have suggested that trHbN plays a role in the detoxification of reactive nitric oxide generated by activated macrophages during physiological studies of *M. bovis* BCG and also during *M. tuberculosis* infections (Couture et al. 1999; Pawaria et al. 2008).

A second downregulated gene in this group was the *apa* gene which encodes an alanine- and proline-rich secreted protein (Table 2). The Apa molecules secreted by *M. bovis* BCG function as major immunodominant antigens (Horn et al. 1999). The addition of a poxvirus recombinant boost expressing an Apa protein of *M. tuberculosis* to a DNA vaccine led to a significant reduction of mycobacterial counts in the spleens of immunized guinea pigs comparable to the reduction obtained by the BCG vaccine (Kumar et al. 2003).

Another downregulated gene in this group was BCG_1249 (*rocA*) (Table 2). The *roc*A gene has been proposed to play a role in the adaptation of *Mycobacterium avium* subsp. *paratuberculosis* to its niche and the utilization of carbon sources within (Hughes et al. 2007).

Group IV: genes upregulated only upon 20 min of exposure to peracetic acid

In this group, BCG_2180c which encodes a putative penicillin-binding membrane protein PbpB was upregulated after 20 min of exposure to peracetic acid (Table 2). Penicillin-binding proteins are serine acyl transferases involved in the final stages of peptidoglycan synthesis and contribute to cell wall expansion, cell shape maintenance, septum formation, and cell division (Goffin and Ghuysen 2002; Popham and Young 2003). A recent study reported that the remodeling of the peptidoglycan network of *M. tuberculosis* may be involved in the adaptive response to the treatment of tuberculosis infections with a combination of antibiotics (Lavollay et al. 2008). In addition, another study reported a possible connection between peptidoglycan biosynthesis and oxidative stress defense in *Streptococcus thermophilus* (Thibessard et al. 2002). In the aforementioned study, the *pbp*2 gene (which is reported to be implicated in peptidoglycan biosynthesis during the process of cell elongation) is shown to be involved in the response to hydrogen peroxide-induced oxidative stress.

A second upregulated gene in this group was BCG_2802c which encodes a putative lipoprotein. Mycobacterial lipoproteins are usually cell surface-associated and are important for the formation of the cell envelope and sensing of and protection from environmental stress, and they play a role in host pathogen reactions (Rezwan et al. 2007). In addition, lipoprotein metabolism has been

established as a major virulence determinant for tuberculosis (Sander et al. 2004).

The upregulation of these cell wall-associated genes in response to peracetic acid treatment may be indicative of cell wall modification as a protective strategy against peracetic acid treatment. In addition, lipoprotein-associated virulence further supports the results in group I which indicate that virulence mechanisms in *M. bovis* BCG may contribute to the adaptive response to peracetic acid exposure.

Another gene of the PPE family BCG_2140 (PPE37) was upregulated only upon 20 min of exposure to peracetic acid (Table 2). Two genes belonging to the PE/PPE family of genes were upregulated after both treatment times (see group I). Currently, the functions of all PPE proteins are not known. These results support the fact that these genes elicit diversified metabolic functions.

Group V: genes downregulated only upon 20 min of exposure to peracetic acid

The *rad*A gene (BCG_3650) which is involved in DNA repair was downregulated after 20 min (Table 2). The transcription of the *rad*A gene was upregulated in *M. tuberculosis* grown in a simulated phagosomal environment (Schnappinger et al. 2003). The expression of *rad*A in *M. tuberculosis* also increased following induced DNA damage (Rand et al. 2003). These results suggest that peracetic acid on *M. bovis BCG* may include the inhibition of some DNA repair genes.

A second downregulated gene in this group was the *lat* gene (BCG_3319c) which encodes a putative L-lysine-epsilon aminotransferase. Expression of the LAT protein was upregulated approximately 40-fold during the latent phase of *M. tuberculosis*, suggesting that it plays a significant role for survival (Tripathi and Ramachandran 2006). As such, the downregulation of the *lat*A gene may contribute to the peracetic acid-induced growth inhibition observed in this study.

Group VI: genes downregulated after 10 and 20 min of exposure to peracetic acid

The *nar*K3 gene (BCG_0299c), which codes for a putative integral membrane nitrite extrusion protein, was downregulated after both treatment times (Table 2). The *Escherichia coli nar*K gene is implicated in nitrate uptake or nitrite excretion (DeMoss and Hsu 1991; Noji et al. 1989). The *M. tuberculosis nar*K1 through *nar*K3 and *nar*U are homologous to the *E. coli nar*K and *nar*U (Cole et al. 1998). In *M. tuberculosis*, nitrite production is upregulated during anaerobic conditions, and the transcription of the nitrite transport gene, *nar*K2, is also upregulated (Sohaskey and Wayne 2003). However, in *M. bovis*, only low levels of nitrite were produced, and this was not induced by hypoxia (Sohaskey and Wayne 2003). The downregulation of *nar*K3 gene in this study suggests that when *M. bovis* BCG is subjected to oxidative stress, anaerobic metabolism where nitrate is used as a terminal electron acceptor and is converted to nitrite is not favored.

Another downregulated gene in this group was BCG_3107c, *vir*S. The *vir*S gene encodes a transcriptional regulator which belongs to the AraC family and is involved in the regulation of pathogenesis of *M. tuberculosis* (Gupta et al. 1999; Gupta and Tyagi 1993). In a recent study, the virulence regulator *vir*S was upregulated during the reactivation phase of tuberculosis and was suggested to be one of the master regulators in the reactivation of tuberculosis (Talaat et al. 2007). The downregulation of *vir*S after both 10 and 20 min contrasts the results obtained in group I which support the upregulation of virulence in response to peracetic acid treatment. This suggests that the virulence genes of *M. bovis* BCG may elicit different metabolic roles, thus are differentially affected by peracetic acid treatment.

A third downregulated gene in this group was *ctp*G, which encodes a putative metal cation-transporting P-type ATPase. In a previous study, *ctp*G appeared to be induced by low iron and it was theorized that *ctp*G may transport iron (De Voss et al. 2000). The downregulation of *ctp*G in this study, therefore, supports the results in group 1 that indicate that peracetic acid treatment may elicit the regulation of intracellular iron levels to ensure growth and survival but also to combat the effect of oxidant-induced damage.

BCG_1685 (PE 17) which belongs to the PE/PPE family of genes was downregulated after both 10 and 20 min of exposure to peracetic acid. This further showed that the PE/PPE families of genes elicit diversified metabolic functions.

The *uvr*A gene (BCG_1676) was downregulated approximately 2-fold after both treatment times. UvrA is critical to the nucleotide excision repair (NER) process which is used by cells to repair a wide range of DNA lesions (Croteau et al. 2006). During the NER process, UvrA initially recognizes distortions caused by damage in DNA and then transfers the damaged DNA to UvrB which makes a more detailed evaluation of the nature of damage (Croteau et al. 2006, 2008; Zou et al. 1998). The downregulation of the *uvr*A gene after both treatment times suggests that peracetic acid may affect DNA damage/repair systems in *M. bovis* BCG. The downregulation of *nrd*F2 gene (BCG_3072c) further supports this theory. The *nrd*F2 encodes the ribonucleoside-diphosphate reductase subunit beta. Ribonucleotide reductases are critical to all living cells because they provide deoxyribonucleotides for DNA synthesis and repair (Mowa et al. 2009). In addition, both

Table 3 List of genes represented in the Venn diagram by region

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class
		Fold change	*P* value			
Region 1: genes upregulated by sodium hypochlorite only: 52 genes						
AFFX-BioB-M_at		4.02	0.0132	*E. coli*/GEN=bioB/DB_XREF=gb:J04423.1/ NOTE=SIF corresponding to nucleotides 2482–-2739 of gb:J04423.1/DEF=*E. coli* 7,8-diamino-pelargonic acid (bioA), biotin synthetase (bioB), 7-keto-8-amino-pelargonic acid synthetase (bioF), bioC protein, and dethiobiotin synthetase (bioD), complete cds		
MBOV0704S00000016_s_at	BCG_0019	5.37	0.00175	Putative thioredoxin reductase trxB2 (TRXR)	*trx*B2_2	Posttranslational modification, protein turnover, chaperones
MBOV0704S00000017_s_at	BCG_0020	5.28	0.00356	Thioredoxin trxC (TRX) (MPT46)	*trx*C_2	Posttranslational modification, protein turnover, chaperones
MBOV0704S00000171_at	BCG_0178	3.17	0.000664	Hypothetical protein		
MBOV0704S00000356_at	BCG_0364	2.65	0.00123	Putative transcriptional regulatory protein (possibly arsR family)		Inorganic ion transport and metabolism
MBOV0704S00000358_at	BCG_0366c	2.25	0.00637	Putative cytochrome P450 135A1 cyp135A1		Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00000362_at	BCG_0370	8.96	0.000245	Putative dehydrogenase/reductase		General function prediction only
MBOV0704S00000414_at	BCG_0422c	2.59	0.0033	Putative endopeptidase ATP-binding protein (chain b) clpB	*clp*B	Posttranslational modification, protein turnover, chaperones
MBOV0704S00000604_at	BCG_0614	2.12	0.00479	Hypothetical protein		General function prediction only
MBOV0704S00001035_at	BCG_1046c	2.63	0.0106	Hypothetical serine-rich protein		General function prediction only
MBOV0704S00001095_at	BCG_1107	15.21	0.000248	Putative transcriptional repressor protein		Transcription
MBOV0704S00001096_at	BCG_1108	22.89	0.000118	Putative oxidoreductase		General function prediction only
MBOV0704S00001118_at	BCG_1130	2.15	0.00191	Putative transmembrane protein		Function unknown
MBOV0704S00001119_at	BCG_1131	3.03	0.00062	Hypothetical protein		Function unknown
MBOV0704S00001267_at	BCG_1281	2.16	0.0357	Alternative RNA polymerase sigma factor sigE		Function unknown
MBOV0704S00001268_at	BCG_1282	2.02	0.00106	Hypothetical protein		Function unknown
MBOV0704S00001518_at	BCG_1532	7.23	0.000208	Putative thioredoxin trxB1	*trx*B1	Posttranslational modification, protein turnover, chaperones
MBOV0704S00001519_at	BCG_1533	2.44	0.00245	Putative enoyl-CoA hydratase echA12	*ech*A12	Lipid metabolism
MBOV0704S00001566_at	BCG_1580c	3.62	0.000928	Putative polyketide synthase-associated protein papA4	*pap*A4	
MBOV0704S00001697_at	BCG_1711c	3.49	0.00592	Hypothetical protein		Function unknown

						and metabolism	
MBOV0704S00001763_at	BCG_1777	2.26	0.00102	Hypothetical protein		Function unknown	
MBOV0704S00001895_at	BCG_1911	4.15	0.00167	Hypothetical protein		Function unknown	
MBOV0704S00002008_at	BCG_2024c	2.01	0.00493	Putative ferredoxin fdxA	*fdx*A	Energy production and conversion	
MBOV0704S00002034_at	BCG_2050c	2.28	0.0011	Heat shock protein hspX	*hsp*X	Posttranslational modification, protein turnover, chaperones	
MBOV0704S00002053_at	BCG_2069	2.31	0.0204	Hypothetical protein		Function unknown	
MBOV0704S00002055_at	BCG_2071c	2.06	0.00624	Hypothetical protein		Function unknown	
MBOV0704S00002056_at	BCG_2072c	2.09	0.00187	Putative transmembrane protein			
MBOV0704S00002122_at	BCG_2140	2.62	0.00144	PPE family protein		Cell motility and secretion	
MBOV0704S00002394_at	BCG_2412c	2.29	0.00359	Putative sulfate-transport ATP-binding protein ABC transporter cysA1	*cys*A1	Inorganic ion transport and metabolism	
MBOV0704S00002395_at	BCG_2413c	2.40	0.00119	Putative sulfate-transport integral membrane protein ABC transporter cysW	*cys*W	Inorganic ion transport and metabolism	
MBOV0704S00002397_at	BCG_2415c	2.17	0.00074	Putative sulfate-binding lipoprotein subI	*sub*I	Inorganic ion transport and metabolism	
MBOV0704S00002623_at	BCG_2644c	5.09	0.00016	Putative transmembrane protein			
MBOV0704S00002686_at	BCG_2707c	2.40	0.000641	Hypothetical protein		Function unknown	
MBOV0704S00002691_at	BCG_2712c	2.39	0.000585	Hypothetical protein		Function unknown	
MBOV0704S00002702_at	BCG_2723	2.38	0.0248	RNA polymerase sigma factor sigB		Transcription	
MBOV0704S00002739_at	BCG_2760c	2.09	0.0119	Conserved 35 kDa alanine-rich protein		Transcription	Signal transduction mechanisms
MBOV0704S00002740_at	BCG_2761c	2.23	0.0022	Putative transcriptional regulatory protein			
MBOV0704S00003054_at	BCG_3077c	2.67	0.00906	Putative glutaredoxin electron transport component of nrdEF (glutaredoxin-like protein) nrdH		Posttranslational modification, protein turnover, chaperones	
MBOV0704S00003175_at	BCG_3198	5.37	0.000665	Putative short-chain dehydrogenase/reductase		General function prediction only	
MBOV0704S00003209_at	BCG_3232c	3.78	0.00142	Putative molybdenum cofactor biosynthesis protein moeB1	*moe*B1	Coenzyme metabolism	
MBOV0704S00003255_at	BCG_3278c	2.32	0.0366	Putative transcriptional regulatory protein (probably tetR family)		Transcription	
MBOV0704S00003256_at	BCG_3279c	2.67	0.0203	Putative rubredoxin rubB	*rub*B	Energy production and conversion	
MBOV0704S00003257_at	BCG_3280c	2.18	0.0157	Putative rubredoxin rubA	*rub*A	Energy production and conversion	
MBOV0704S00003941_s_at	BCG_3971	5.08	0.00128	Putative thioredoxin reductase trxB2	*trx*B2_2	Posttranslational modification, protein turnover, chaperones	
MBOV0704S00003942_s_at	BCG_3972	4.84	0.0067	Thioredoxin trxC	*trx*C_2	Posttranslational modification, protein turnover, chaperones	
MBOV0704S00003964_at		3.21	0.00477	Intergenic region 21218–21326			
MBOV0704S00004348_at		4.81	7.24E−05	Intergenic region 1204273–1204430			

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class	
		Fold change	*P* value				
MBOV0704S00004420_at		3.02	0.0346	Intergenic region 1394907–1395063			
MBOV0704S00004638_at		2.02	0.00359	Intergenic region 2125784–2126298			
MBOV0704S00004919_at		2.07	0.0144	Intergenic region 2977118–2977249			
MBOV0704S00005341_at		3.51	0.0177	Intergenic region 4366073–4366181			
Region 1: genes downregulated by sodium hypochlorite only: 5 genes							
MBOV0704S00000528_at	BCG_0536	−2.00	0.05	Putative transcriptional regulatory protein (probably gntR family)		Transcription	
MBOV0704S00000864_at	BCG_0876c	−2.05	0.0135	Putative transcriptional regulatory protein		Translation, ribosomal structure, and biogenesis	
MBOV0704S00001594_at	BCG_1608	−2.64	0.00483	Putative membrane protein mmpS6			
MBOV0704S00002000_at	BCG_2016c	−2.16	0.00833	Putative integral membrane protein		Amino acid transport and metabolism	
MBOV0704S00004336_at		−2.10	0.0244	Intergenic region 1174953–1175048			
Region 2: genes upregulated by hydrogen peroxide only: 6 genes							
MBOV0704S00001930_at	BCG_1946c	3.82	6.75E−05	Hypothetical protein		Function unknown	
MBOV0704S00001932_at	BCG_1948c	4.88	6.78E−05	Ferric uptake regulation protein furA	*fur*A	Inorganic ion transport and metabolism	
MBOV0704S00002378_at	BCG_2396c	4.46	4.22E−05	Polyketide synthetase mbtC	*mbt*C	Secondary metabolites biosynthesis, transport, and catabolism	
MBOV0704S00002379_at	BCG_2397c	3.65	0.000857	Phenyloxazoline synthase mbtB	*mbt*B	Secondary metabolites biosynthesis, transport, and catabolism	
MBOV0704S00002985_at	BCG_3008c	2.40	0.000441	Putative 3-isopropylmalate dehydratase small subunit leuD	*leu*D	Amino acid transport and metabolism	
MBOV0704S00002986_at	BCG_3009c	3.39	0.00332	Putative 3-isopropylmalate dehydratase large subunit leuC	*leu*C	Amino acid transport and metabolism	
Region 3: genes upregulated by peracetic acid only: 170 genes							
MBOV0704S00000025_s_at	BCG_0028c	2.20	0.000886	Putative hemolysin		Function unknown	
MBOV0704S00000058_at	BCG_0062	2.09	0.00327	Putative remnant of a transposase			
MBOV0704S00000058_x_at	BCG_0062	2.43	0.0174	Putative remnant of a transposase			
MBOV0704S00000080_at	BCG_0084	2.08	0.000417	Putative 30s ribosomal protein s6 rpsF	*rps*F	General function prediction only	
MBOV0704S00000110_at	BCG_0113	2.64	0.0151	Hypothetical protein		Function unknown	
MBOV0704S00000111_at	BCG_0114	3.60	0.000512	Putative transcriptional regulatory protein		Transcription	
MBOV0704S00000112_at	BCG_0115	4.10	0.00135	Putative oxidoreductase		Energy production and conversion	
MBOV0704S00000113_at	BCG_0116	4.60	0.000108	Putative oxidoreductase		Energy production and	Inorganic ion

						conversion	transport and metabolism
MBOV0704S00000114_at	BCG_0117	2.52	0.0179	Putative formate hydrogenlyase hycD (FHL)	*hyc*D	Energy production and conversion	
MBOV0704S00000117_at	BCG_0120	3.36	0.0269	Putative formate hydrogenase hycQ (FHL)	*hyc*Q	Energy production and conversion	
MBOV0704S00000125_at	BCG_0128c	3.33	0.0144	Hypothetical protein		Function unknown	
MBOV0704S00000144_at	BCG_0147	2.11	0.0104	Putative dehydratase		Amino acid transport and metabolism	
MBOV0704S00000261_at	BCG_0269	3.12	0.00162	Putative transcriptional regulatory protein (probably tetR/acrR family)		Transcription	
MBOV0704S00000319_at	BCG_0327	2.24	0.00159	Hypothetical protein TB9.8		Function unknown	
MBOV0704S00000381_at	BCG_0389	2.46	0.00349	Putative chaperone protein dnaK	*dna*K	Posttranslational modification, protein turnover, chaperones	
MBOV0704S00000382_at	BCG_0390	2.76	0.00229	Putative grpE protein (hsp-70 cofactor)	*grp*E	Posttranslational modification, protein turnover, chaperones	
MBOV0704S00000606_at	BCG_0616c	2.25	0.00378	Hypothetical protein		Function unknown	
MBOV0704S00000609_at	BCG_0619c	2.06	0.00836	Hypothetical protein		Function unknown	
MBOV0704S00000703_at	BCG_0714	2.24	0.0167	Hypothetical protein		Function unknown	
MBOV0704S00000735_at	BCG_0746	2.02	0.00833	Putative dehydrogenase		Amino acid transport and metabolism	
MBOV0704S00000756_at	BCG_0767	2.05	0.00463	Putative 30S ribosomal protein S14 rpsN1	*rps*N1	Translation, ribosomal structure, and biogenesis	
MBOV0704S00000757_at	BCG_0768	2.18	0.0031	Putative 30S ribosomal protein S8 rpsH	*rps*H	Translation, ribosomal structure, and biogenesis	
MBOV0704S00000762_at	BCG_0773	2.10	0.00479	Putative 50S ribosomal protein L15 rplO	*rpl*O	Translation, ribosomal structure and biogenesis	
MBOV0704S00001017_at	BCG_1028c	3.91	0.00265	Putative acetyl-/propionyl-coa carboxylase subunit beta accD2	*acc*D2	Lipid metabolism	
MBOV0704S00001018_at	BCG_1029c	2.63	0.000285	Putative acyl-CoA dehydrogenase fadE13	*fad*E13	General function prediction only	Function unknown
MBOV0704S00001019_at	BCG_1030c	2.98	7.95E−05	Hypothetical protein		Function unknown	
MBOV0704S00001093_s_at	BCG_1105	2.62	0.0365	Putative IS1081 transposase		DNA replication, recombination, and repair	
MBOV0704S00001237_at	BCG_1251	2.39	0.0117	Putative alternative RNA polymerase sigma factor sigI′	*sig*I	Transcription	General function prediction only
MBOV0704S00001241_at	BCG_1255	2.76	0.00878	PE family protein			
MBOV0704S00001297_at	BCG_1311c	3.24	0.0002	Hypothetical protein		Function unknown	
MBOV0704S00001312_at	BCG_1326c	2.29	0.000766	Putative transcriptional regulatory protein embR		Signal transduction mechanisms	
MBOV0704S00001316_at	BCG_1330c	2.03	0.0295	Hypothetical secreted protein		Function unknown	

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class
		Fold change	*P* value			
MBOV0704S00001406_at	BCG_1420	2.87	0.0108	Putative transcriptional regulatory protein		General function prediction only
MBOV0704S00001421_at	BCG_1435c	2.05	0.00325	Hypothetical protein		Function unknown
MBOV0704S00001422_at	BCG_1436	2.03	0.0212	Hypothetical protein		Function unknown
MBOV0704S00001590_at	BCG_1604	2.72	0.023	Putative fumarate reductase [flavoprotein subunit] frdA	*frd*A	Energy production and conversion
MBOV0704S00001663_at	BCG_1677c	2.21	0.0181	Hypothetical protein		Function unknown
MBOV0704S00001677_at	BCG_1691	2.33	1.97E−05	Putative *N*-acetyl-gamma-glutamyl-phoshate reductase argC	*arg*C	Amino acid transport and metabolism
MBOV0704S00001678_at	BCG_1692	2.77	3.66 E−05	Putative glutamate *N*-acetyltransferase argJ	*arg*J	Amino acid transport and metabolism
MBOV0704S00001679_at	BCG_1693	2.75	0.000283	Putative acetylglutamate kinase argB	*arg*B	Amino acid transport and metabolism
MBOV0704S00001680_at	BCG_1694	2.49	4.31E−06	Putative acetylornithine aminotransferase argD	*arg*D	Amino acid transport and metabolism
MBOV0704S00001681_at	BCG_1695	2.64	0.000105	Putative ornithine carbamoyltransferase, anabolic ArgF	*arg*F	Amino acid transport and metabolism
MBOV0704S00001682_at	BCG_1696	2.62	9.47E−05	Putative arginine repressor argR	*arg*R	Transcription
MBOV0704S00001683_at	BCG_1697	2.39	0.000598	Putative argininosuccinate synthase argG		
MBOV0704S00001684_at	BCG_1698	2.79	0.00182	Putative argininosuccinate lyase argH	*arg*H	Amino acid transport and metabolism
MBOV0704S00001741_at	BCG_1755	2.12	0.00433	Hypothetical protein		Function unknown
MBOV0704S00001832_at	BCG_1847c	2.36	0.0102	Hypothetical protein		Function unknown
MBOV0704S00001969_at	BCG_1986	2.01	0.00596	Hypothetical protein		Function unknown
MBOV0704S00002004_at	BCG_2020c	4.06	0.00156	Hypothetical protein		Function unknown
MBOV0704S00002005_at	BCG_2021c	2.62	0.000634	Hypothetical protein		Function unknown
MBOV0704S00002006_at	BCG_2022c	2.42	0.000251	Hypothetical protein		Function unknown
MBOV0704S00002031_at	BCG_2047c	2.80	0.00472	Hypothetical protein		Function unknown
MBOV0704S00002032_at	BCG_2048c	3.78	0.023	Putative phosphofructokinase pfkB	*pfk*B	Carbohydrate transport and metabolism
MBOV0704S00002061_at	BCG_2077c	4.07	0.0159	Ribosomal protein L28		Translation, ribosomal structure, and biogenesis
MBOV0704S00002062_at	BCG_2078	2.09	0.00453	Hypothetical protein		Function unknown
MBOV0704S00002266_at	BCG_2284c	2.11	0.000275	Hypothetical protein		Function unknown
MBOV0704S00002280_at	BCG_2298	3.23	0.000126	Hypothetical protein		Function unknown
MBOV0704S00002320_at	BCG_2338	3.52	0.0212	Putative sugar-transport integral membrane protein ABC Transporter uspB		Carbohydrate transport and metabolism
MBOV0704S00002544_at	BCG_2565	2.60	0.0134	Putative lipoprotein lppA	l*pp*A	
MBOV0704S00002629_at	BCG_2650	2.13	0.00343	Hypothetical protein TB31.7		Function unknown
MBOV0704S00002630_at	BCG_2651c	4.06	0.00755	Hypothetical protein		Function unknown

MBOV0704S00002658_at	BCG_2679	9.18	0.00461	Putative transposase for insertion sequence element IS1081		DNA replication, recombination, and repair	
MBOV0704S00002658_s_at	BCG_2679	2.51	0.0313	Putative transposase for insertion sequence element IS1081		DNA replication, recombination, and repair	
MBOV0704S00002737_at	BCG_2758c	2.06	0.0453	Hypothetical arginine-rich protein			
MBOV0704S00002757_at	BCG_2778c	2.10	0.000216	Hypothetical protein			
MBOV0704S00002759_at	BCG_2780c	2.07	0.000123	Putative dihydrofolate reductase dfrA	*dfr*A	Coenzyme metabolism	
MBOV0704S00002784_at	BCG_2805	2.18	0.00344	Hypothetical alanine-rich protein		Cell division and chromosome partitioning	
MBOV0704S00002988_at	BCG_3011c	2.04	0.0102	Hypothetical protein		Function unknown	
MBOV0704S00002997_at	BCG_3020c	2.91	0.0393	Hypothetical protein		Function unknown	
MBOV0704S00003017_at	BCG_3040c	2.36	0.0314	PPE family protein		Cell motility and secretion	
MBOV0704S00003021_at	BCG_3044c	3.60	0.0427	PPE family protein		Cell motility and secretion	
MBOV0704S00003112_at	BCG_3135	2.86	0.0182	Putative pterin-4-alpha-carbinolamine dehydratase moaB1		Coenzyme metabolism	
MBOV0704S00003117_s_at	BCG_3140	2.68	0.0181	Putative transposase		DNA replication, recombination, and repair	
MBOV0704S00003122_at	BCG_3145	4.50	0.0115	Putative transcriptional regulatory protein		Signal transduction mechanisms	
MBOV0704S00003132_at	BCG_3155c	2.13	0.000929	Two-component sensor histidine kinase devS	*dev*S	Signal transduction mechanisms	
MBOV0704S00003133_at	BCG_3156c	2.09	0.00397	Two-component transcriptional regulatory protein devR (probably luxR/uhpA family)	*dev*R	Signal transduction mechanisms	Transcription
MBOV0704S00003134_at	BCG_3157c	2.08	0.0181	Hypothetical protein		Function unknown	
MBOV0704S00003180_at	BCG_3203	2.39	0.0369	Hypothetical protein		Function unknown	
MBOV0704S00003300_s_at	BCG_3323c	2.32	0.0127	Hypothetical protein		Function unknown	
MBOV0704S00003371_at	BCG_3396c	2.47	0.0056	Hypothetical protein		Function unknown	
MBOV0704S00003378_at	BCG_3403c	2.65	0.0156	Hypothetical proline-rich protein		Function unknown	
MBOV0704S00003397_at	BCG_3425c	3.12	6.13E−07	Hypothetical protein		Function unknown	
MBOV0704S00003424_at	BCG_3452c	2.21	0.000551	Putative polyprenyl synthetase idsB	*ids*B	Coenzyme metabolism	
MBOV0704S00003444_at	BCG_3472c	2.80	0.00725	Hypothetical protein		Function unknown	
MBOV0704S00003484_at	BCG_3512c	2.20	0.00455	Hypothetical alanine- and valine-rich protein			
MBOV0704S00003492_at	BCG_3520c	2.36	0.000802	Putative tRNA pseudouridine synthase A truA	*tru*A	Translation, ribosomal structure, and biogenesis	
MBOV0704S00003494_at	BCG_3522c	2.67	7.35E−05	Putative DNA-directed RNA polymerase (alpha chain) rpoA	*rpo*A	Transcription	
MBOV0704S00003495_at	BCG_3523c	2.10	0.00116	Putative 30S ribosomal protein S4 rpsD	*rps*D	Translation, ribosomal structure, and biogenesis	
MBOV0704S00003668_at	BCG_3696	5.04	0.0371	Putative transposase		DNA replication,	

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class
		Fold change	*P* value			
						recombination, and repair
MBOV0704S00003760_at	BCG_3787	6.72	0.0221	Putative oxidoreductase		Function unknown
MBOV0704S00003859_at	BCG_3887c	2.34	3.11E−06	Putative polyketide synthase-associated protein papA1	*pap*A1	Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00003950_s_at	BCG_3980c	2.08	0.0015	Putative hemolysin		Function unknown
MBOV0704S00003968_at		2.01	0.00286	Intergenic region 31192–31718		
MBOV0704S00003975_at		2.03	0.0303	Intergenic region 43225–43379		
MBOV0704S00003981_at		7.14	0.0144	Intergenic region 57091–57242		
MBOV0704S00004006_at		2.96	0.00329	Intergenic region 119222–119315		
MBOV0704S00004068_at		3.16	0.00756	Intergenic region 330684–330896		
MBOV0704S00004077_at		7.23	0.00699	Intergenic region 367247–367495		
MBOV0704S00004078_at		3.52	0.0364	Intergenic region 370001–370290		
MBOV0704S00004100_at		2.10	0.0353	Intergenic region 450536–450761		
MBOV0704S00004129_at		3.39	0.0454	Intergenic region 572726–572956		
MBOV0704S00004140_at		4.99	8.61E−05	Intergenic region 594243–594378		
MBOV0704S00004171_at		3.36	0.00012	Intergenic region 695709–695931		
MBOV0704S00004172_at		2.22	0.000724	Intergenic region 696274–696740		
MBOV0704S00004178_at		2.08	0.00394	Intergenic region 709958–710110		
MBOV0704S00004205_at		2.16	0.00716	Intergenic region 798730–799092		
MBOV0704S00004290_at		2.73	0.0408	Intergenic region 1026753–1026980		
MBOV0704S00004310_at		3.71	0.00858	Intergenic region 1088008–1088122		
MBOV0704S00004322_at		2.84	0.00166	Intergenic region 1120652–1120849		
MBOV0704S00004372_at		2.01	0.000653	Intergenic region 1253271–1253374		
MBOV0704S00004388_at		2.00	0.00736	Intergenic region 1300425–1300566		
MBOV0704S00004412_at		2.32	0.00223	Intergenic region 1374889–1374978		
MBOV0704S00004418_at		2.50	0.00298	Intergenic region 1393082–1393222		
MBOV0704S00004440_at		3.99	0.00596	Intergenic region 1452539–1452787		
MBOV0704S00004446_at		4.97	0.0283	Intergenic region 1472793–1472905		
MBOV0704S00004458_at		2.10	0.00196	Intergenic region 1503528–1503647		
MBOV0704S00004473_at		5.70	0.00127	Intergenic region 1564159–1564296		
MBOV0704S00004498_at		5.40	0.0355	intergenic region 1644493–1644598		
MBOV0704S00004500_at		3.03	0.0105	Intergenic region 1647501–1648098		
MBOV0704S00004502_at		2.10	0.0461	Intergenic region 1659650–1660050		
MBOV0704S00004503_at		20.89	0.000859	Intergenic region 1663249–1663399		

MBOV0704S00004532_at	2.08	0.00731	Intergenic region 1769644–1770012
MBOV0704S00004557_at	2.47	0.00154	intergenic region 1867500–1867592
MBOV0704S00004558_at	4.13	0.0421	Intergenic region 1870650–1870842
MBOV0704S00004559_at	2.23	6.92E−05	Intergenic region 1879319–1879426
MBOV0704S00004582_at	2.02	0.00554	intergenic region 1966948–1967386
MBOV0704S00004606_at	2.15	0.00553	Intergenic region 2040353–2040454
MBOV0704S00004632_at	3.07	0.000399	Intergenic region 2104002–2104141
MBOV0704S00004662_at	6.76	0.00862	Intergenic region 2208963–2209860
MBOV0704S00004668_at	3.53	4.99E−05	Intergenic region 2218613–2218813
MBOV0704S00004683_at	2.05	0.0191	Intergenic region 2263777–2263891
MBOV0704S00004684_at	5.00	0.00357	Intergenic region 2264735–2264945
MBOV0704S00004704_at	2.53	0.031	Intergenic region 2360982–2361107
MBOV0704S00004718_at	4.80	0.0167	Intergenic region 2403152–2403253
MBOV0704S00004719_at	3.69	0.021	Intergenic region 2404727–2404935
MBOV0704S00004752_at	2.08	0.000654	Intergenic region 2495194–2495431
MBOV0704S00004755_at	2.06	0.00793	Intergenic region 2508435–2508563
MBOV0704S00004768_at	2.53	0.00704	Intergenic region 2542127–2542420
MBOV0704S00004784_at	6.31	0.000285	Intergenic region 2580557–2580789
MBOV0704S00004800_at	2.23	0.0363	Intergenic region 2640860–2640957
MBOV0704S00004803_at	5.81	0.00584	intergenic region 2648165–2648269
MBOV0704S00004836_at	2.42	0.00615	Intergenic region 2749776–2750383
MBOV0704S00004862_at	2.35	0.0269	Intergenic region 2834817–2835226
MBOV0704S00004873_at	2.21	0.000936	Intergenic region 2868989–2869267
MBOV0704S00004891_at	2.57	7.12E−05	Intergenic region 2919866–2920211
MBOV0704S00004893_at	2.31	0.00658	Intergenic region 2923635–2923778
MBOV0704S00004897_at	2.10	0.00241	Intergenic region 2929362–2929535
MBOV0704S00004923_at	2.21	0.0226	Intergenic region 2985223–2985529
MBOV0704S00004942_at	2.38	0.0209	Intergenic region 3055611–3055811
MBOV0704S00004993_at	2.24	0.0304	Intergenic region 3262707–3262828
MBOV0704S00004997_at	4.35	0.00663	Intergenic region 3267835–3267934
MBOV0704S00005016_at	2.55	0.00214	Intergenic region 3319020–3319195
MBOV0704S00005021_at	2.46	0.00532	Intergenic region 3332847–3333141
MBOV0704S00005049_at	11.34	0.00683	Intergenic region 3393460–3393557
MBOV0704S00005051_at	2.57	0.00585	intergenic region 3411759–3412696
MBOV0704S00005062_at	2.76	0.0331	Intergenic region 3440775–3441216
MBOV0704S00005064_at	2.33	0.0146	Intergenic region 3443362–3443517
MBOV0704S00005065_at	2.74	0.0136	Intergenic region 3445892–3446369
MBOV0704S00005081_at	10.75	0.0258	Intergenic region 3502522–3502808
MBOV0704S00005101_at	7.30	0.00142	Intergenic region 3546718–3546949
MBOV0704S00005125_at	5.82	0.00345	Intergenic region 3645870–3646101

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class
		Fold change	*P* value			
MBOV0704S00005160_at		2.38	0.0089	Intergenic region 3787671–3787859		
MBOV0704S00005172_at		3.91	0.00525	Intergenic region 3831716–3831947		
MBOV0704S00005207_at		9.10	0.000377	Intergenic region 3964338–3964448		
MBOV0704S00005211_at		2.01	0.0387	Intergenic region 3976524–3976871		
MBOV0704S00005223_at		6.73	0.0118	Intergenic region 4020234–4020336		
MBOV0704S00005261_at		2.18	0.00666	Intergenic region 4110228–4110318		
MBOV0704S00005274_at		3.07	0.0161	Intergenic region 4146183–4146522		
MBOV0704S00005281_at		2.34	0.0496	Intergenic region 4168909–4169125		
MBOV0704S00005310_at		3.28	0.0358	Intergenic region 4272008–4272213		
Region 3: genes downregulated by peracetic acid only: 96 genes						
AFFX-LysX-3_at		−2.53	0.0392	*B. subtilis*/GEN=lys/DB_XREF=gb:X17013.1/ NOTE=SIF corresponding to nucleotides 1061–1343 of gb:X17013.1, not 100% identical/ DEF=*B. subtilis* lys gene for diaminopimelate decarboxylase (EC 4.1.1.20).		
AFFX-LysX-5_at		−2.39	0.0103	*B. subtilis*/GEN=lys/DB_XREF=gb:X17013.1/ NOTE=SIF corresponding to nucleotides 387–658 of gb:X17013.1, not 100% identical/DEF=*B. subtilis* lys gene for diaminopimelate decarboxylase (EC 4.1.1.20).		
AFFX-LysX-M_at		−2.59	0.00795	*B. subtilis*/GEN=lys/DB_XREF=gb:X17013.1/ NOTE=SIF corresponding to nucleotides 720–990 of gb:X17013.1, not 100% identical/DEF=*B. subtilis* lys gene for diaminopimelate decarboxylase (EC 4.1.1.20).		
AFFX-PheX-5_at		−2.26	0.0349	*B. subtilis*/GEN=pheA/DB_XREF=gb:M24537.1/ NOTE=SIF corresponding to nucleotides 2028–2375 of gb:M24537.1, not 100% identical/ DEF=*Bacillus subtilis* sporulation protein (spoOB), GTP-binding protein (obg), phenylalanine biosynthesis associated protein (pheB), and monofunctional prephenate dehydratase (pheA) genes, complete cds.		
AFFX-r2-Bs-thr-3_s_at		−2.60	0.0441	*B. subtilis*/GEN=thrB/DB_XREF=gb:X04603.1/ NOTE=SIF corresponding to nucleotides 1735–2143 of gb:X04603.1/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine synthase		
AFFX-r2-Bs-thr-M_s_at		−2.28	0.0319	*B. subtilis*/GEN=thrC, thrB/DB_XREF=gb: X04603.1/NOTE=SIF corresponding to nucleotides 1045–1556 of gb:X04603.1/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine synthase		

AFFX-ThrX-3_at		−2.43	0.0243	*B. subtilis*/GEN=thrB/DB_XREF=gb:X04603.1/ NOTE=SIF corresponding to nucleotides 1689–2151 of gb:X04603.1/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine synthase (EC 2.7.1.39 and EC 4.2.99.2, respectively).			
AFFX-ThrX-5_at		−2.23	0.0332	*B. subtilis*/GEN=thrC/DB_XREF=gb:X04603.1/ NOTE=SIF corresponding to nucleotides 288–932 of gb:X04603.1/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine synthase (EC 2.7.1.39 and EC 4.2.99.2, respectively).			
MBOV0704S00000013_s_at	BCG_0013	−2.20	0.000339	Putative para-aminobenzoate synthase component II trpG	*trpG*_1	Amino acid transport and metabolism	
MBOV0704S00000040_s_at	BCG_0043	−2.28	0.000461	Putative anthranilate synthase component II trpG	*trp*G_2	Amino acid transport and metabolism	Coenzyme metabolism
MBOV0704S00000074_at	BCG_0078c	−2.15	0.0176	Hypothetical protein		Function unknown	
MBOV0704S00000092_at	BCG_0095A	−2.51	0.00329	Hypothetical protein, antitoxin			
MBOV0704S00000164_at	BCG_0171c	−2.19	0.00012	Putative transcriptional regulatory protein			
MBOV0704S00000170_at	BCG_0177c	−2.18	0.000506	Hypothetical protein		Function unknown	
MBOV0704S00000183_at	BCG_0190c	−2.09	0.00162	Putative acyl-CoA dehydrogenase fadE2			
MBOV0704S00000225_at	BCG_0233	−2.10	0.000396	Putative transcriptional regulatory protein			
MBOV0704S00000289_at	BCG_0297c	−2.22	0.00688	Hypothetical protein		Function unknown	
MBOV0704S00000290_at	BCG_0298c	−2.28	0.00557	Putative transcriptional regulatory protein		Transcription	Coenzyme metabolism
MBOV0704S00000291_at	BCG_0299c	−2.57	0.0189	Putative integral membrane nitrite extrusion protein narK3		Inorganic ion transport and metabolism	
MBOV0704S00000371_at	BCG_0379	−2.11	0.00723	Hypothetical protein		Function unknown	
MBOV0704S00000397_at	BCG_0405c	−2.16	0.00391	Hypothetical protein		Function unknown	
MBOV0704S00000522_at	BCG_0530	−2.01	0.000873	Putative phosphoglycerate mutase 1 gpm1	*gpm*1	Carbohydrate transport and metabolism	
MBOV0704S00000557_at	BCG_0565	−2.11	6.19E−05	Putative gaba permease gabP (4-amino butyrate transport carrier)	*gab*P	Amino acid transport and metabolism	
MBOV0704S00000603_at	BCG_0613	−2.23	0.00302	Putative cytochrome p450 135b1 cyp135B1	*cyp*135B1	Secondary metabolites biosynthesis, transport, and catabolism	
MBOV0704S00000616_at	BCG_0626	−2.45	0.00177	Hypothetical protein		Function unknown	
MBOV0704S00000622_at	BCG_0632	−2.24	0.000448	Hypothetical integral membrane protein yrbE2A	*yrb*E2A	Secondary metabolites biosynthesis, transport, and catabolism	
MBOV0704S00000685_at	BCG_0696c	−2.37	0.0496	Hypothetical protein		Function unknown	
MBOV0704S00000777_at	BCG_0788	−2.07	0.000296	Hypothetical protein		Function unknown	
MBOV0704S00000797_at	BCG_0808c	−2.46	0.0277	Hypothetical protein		Function unknown	
MBOV0704S00000853_at	BCG_0864	−2.18	0.00142	Putative amino acid aminotransferase		General function prediction only	

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class	
		Fold change	*P* value				
MBOV0704S00001050_at	BCG_1061c	−2.58	0.00175	Hypothetical protein		Function unknown	
MBOV0704S00001080_at	BCG_1091c	−2.20	0.0407	Two-component transcriptional regulator trcR	*trc*R	Signal transduction mechanisms	
MBOV0704S00001151_at	BCG_1163c	−2.11	0.00168	Hypothetical protein		Function unknown	
MBOV0704S00001152_at	BCG_1164	−2.17	0.000196	Putative para-nitrobenzyl esterase		Lipid metabolism	
MBOV0704S00001158_at	BCG_1170	−2.41	0.0115	Putative lytB-related protein lytB2	l*yt*B2		
MBOV0704S00001178_at	BCG_1190c	−2.27	0.0409	Putative transcriptional regulator protein		Transcription	General function prediction only
MBOV0704S00001179_at	BCG_1191	−4.67	0.0283	Hypothetical protein		Function unknown	
MBOV0704S00001199_at	BCG_1212c	−2.29	0.0022	Putative transcriptional regulatory protein		Transcription	
MBOV0704S00001235_at	BCG_1249	−2.18	0.0392	Putative pyrroline-5-carboxylate dehydrogenase rocA	*roc*A	Energy production and conversion	
MBOV0704S00001489_at	BCG_1503	−2.16	2.92E−09	Putative biotin sulfoxide reductase bisC	*bis*C	Energy production and conversion	
MBOV0704S00001507_at	BCG_1521	−2.04	0.000985	Putative transcriptional regulatory protein		Transcription	
MBOV0704S00001534_at	BCG_1548c	−2.19	0.00776	Hypothetical protein		Function unknown	
MBOV0704S00001580_at	BCG_1594c	−2.38	0.00507	Putative hemoglobin glbN	*glb*N	General function prediction only	
MBOV0704S00001608_at	BCG_1622	−2.25	0.00766	Putative 8-amino-7-oxononanoate synthase bioF1	*bio*F1	Coenzyme metabolism	
MBOV0704S00001662_at	BCG_1676	−2.03	0.00062	Excinuclease ABC, subunit a uvrA	*uvr*A	DNA replication, recombination, and repair	
MBOV0704S00001671_at	BCG_1685	−2.07	0.0174	PE family protein			
MBOV0704S00001823_at	BCG_1837c	−2.25	0.00258	Hypothetical protein		Function unknown	
MBOV0704S00001827_at	BCG_1841	−2.02	0.0133	PPE family protein			
MBOV0704S00001866_at	BCG_1882c	−2.11	0.00717	Putative transcriptional regulatory protein		Transcription	
MBOV0704S00001874_at	BCG_1890c	−2.00	0.00687	Putative NADH dehydrogenase ndh	*ndh*	Energy production and conversion	
MBOV0704S00001880_at	BCG_1896	−2.20	0.000548	Alanine- and proline-rich secreted protein apa	*apa*		
MBOV0704S00001883_at	BCG_1899c	−2.06	0.0183	Putative integral membrane protein		General function prediction only	
MBOV0704S00001897_at	BCG_1913	−2.02	0.00299	Putative integral membrane protein		Amino acid transport and metabolism	
MBOV0704S00001977_at	BCG_1994	−2.79	0.00078	Hypothetical protein		Function unknown	
MBOV0704S00001993_at	BCG_2009c	−2.19	0.00637	Putative metal cation transporter P-type atpase G ctpG	*ctp*G	Inorganic ion transport and metabolism	
MBOV0704S00001994_at	BCG_2010c	−2.34	0.00687	Hypothetical protein		Function unknown	
MBOV0704S00001995_at	BCG_2011c	−2.32	0.00646	Putative transcriptional regulatory protein		Transcription	

MBOV0704S00002071_at	BCG_2088	−2.24	0.00997	Putative RNA polymerase sigma factor, ECF subfamily, SigC	*sig*C	Transcription
MBOV0704S00002128_at	BCG_2146c	−2.09	1.96E−05	Putative oxidoreductase		Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00002253_at	BCG_2271	−3.41	0.00463	Putative secreted unknown protein		
MBOV0704S00002325_at	BCG_2343c	−2.26	0.00771	Putative ornithine aminotransferase (N-terminus part) rocD1	*roc*D1	Amino acid transport and metabolism
MBOV0704S00002326_at	BCG_2344c	−2.65	0.00115	Hypothetical protein		Function unknown
MBOV0704S00002350_at	BCG_2368	−2.11	0.000125	Putative transmembrane protein		
MBOV0704S00002371_at	BCG_2389	−2.18	0.0193	Hypothetical protein		Function unknown
MBOV0704S00002384_at	BCG_2402c	−2.12	0.00798	Putative oxygen-independent coproporphyrinogen III oxidase hemN	*hem*N	Coenzyme metabolism
MBOV0704S00002399_at	BCG_2417c	−2.07	0.00174	Hypothetical protein		Function unknown
MBOV0704S00002502_at	BCG_2523c	−2.06	0.00978	Putative succinyl-CoA:3-KETOACID-COENZYME A TRANSFERASE subunit beta scoB	*sco*B	Lipid metabolism
MBOV0704S00002646_at	BCG_2667c	−2.34	0.00158	Putative transcriptional regulatory protein (probably arsR family)		Transcription
MBOV0704S00002664_at	BCG_2685	−2.11	0.00182	Putative secreted protease		General function prediction only
MBOV0704S00002668_at	BCG_2689c	−2.00	0.00443	Hypothetical protein		Function unknown
MBOV0704S00002674_at	BCG_2695c	−2.25	0.00156	Putative 1-deoxy-D-xylulose 5-phosphate synthase dxs1	*dxs*1	Translation, ribosomal structure, and biogenesis
MBOV0704S00002705_at	BCG_2726	−2.00	0.000285	Putative soluble pyridine nucleotide transhydrogenase sthA	*sth*A	Energy production and conversion
MBOV0704S00002743_at	BCG_2764c	−2.38	0.0187	Putative cell division transmembrane protein ftsK	*fts*K	Cell division and chromosome partitioning
MBOV0704S00002773_at	BCG_2794c	−2.04	0.00317	Hypothetical protein		Function unknown
MBOV0704S00002797_at	BCG_2818	−2.22	0.00895	Putative hydrolase		General function prediction only
MBOV0704S00002820_at	BCG_2843c	−2.53	0.0276	Hypothetical protein		Function unknown
MBOV0704S00002980_at	BCG_3003c	−2.04	0.0127	Putative glycerol-3-phosphate dehydrogenase [NAD(P)+] gpdA2	*gpd*A2	Energy production and conversion
MBOV0704S00003005_at	BCG_3028	−2.06	0.00203	Putative lipoprotein lppZ	*lpp*Z	Carbohydrate transport and metabolism
MBOV0704S00003047_at	BCG_3070c	−2.20	0.000119	Hypothetical protein		Function unknown
MBOV0704S00003049_at	BCG_3072c	−2.16	0.0315	Ribonucleoside-diphosphate reductase subunit beta nrdF2	*nrd*F2	Nucleotide transport and metabolism
MBOV0704S00003084_at	BCG_3107c	−2.54	0.0018	Virulence-regulating transcriptional regulator virS (araC/xylS family)	*vir*S	Transcription
MBOV0704S00003214_at	BCG_3237c	−2.25	0.00561	Hypothetical protein		Function unknown
MBOV0704S00003233_s_at	BCG_3256c	−2.07	6.77E−05	Hypothetical protein		Function unknown
MBOV0704S00003260_at	BCG_3283	−2.22	0.0138	Hypothetical protein		Function unknown
MBOV0704S00003275_at	BCG_3298	−2.73	0.0187	Hypothetical protein		Function unknown

Table 3 (continued)

Affymetrix probe ID	ORF no.	10 min		Description	Symbol	Functional class
		Fold change	*P* value			
MBOV0704S00003276_at	BCG_3299	−2.19	0.00587	Putative metal cation-transporting P-type atpase C ctpC	*ctp*C	Inorganic ion transport and metabolism
MBOV0704S00003398_at	BCG_3426	−2.16	0.000104	Hypothetical protein		Function unknown
MBOV0704S00003573_at	BCG_3601	−2.01	0.00519	Putative dehydrogenase		Secondary metabolites biosynthesis, transport, and catabolism
MBOV0704S00003623_at	BCG_3651	−2.14	0.00739	Hypothetical protein		Function unknown
MBOV0704S00003698_at	BCG_3726c	−2.42	0.000289	Putative protease		
MBOV0704S00003775_at	BCG_3803	−2.11	0.00201	Transcriptional regulatory protein (probably arsR family)		Transcription
MBOV0704S00003784_at	BCG_3812c	−2.15	0.00568	Hypothetical protein		Function unknown
MBOV0704S00004157_at		−2.14	0.00379	Intergenic region 643702–643835		
MBOV0704S00004401_at		−2.12	0.01	Intergenic region 1332561–1332651		
MBOV0704S00004664_at		−2.06	0.0281	Intergenic region 2212293–2212535		
MBOV0704S00005076_at		−2.72	0.00405	Intergenic region 3495938–3496073		
Region 4: genes upregulated by both sodium hypochlorite and hydrogen peroxide: 6 genes						
MBOV0704S00000169_at	BCG_0176	9.37	0.000285	Hypothetical protein		Function unknown
MBOV0704S00002466_at	BCG_2486c	13.11	0.00113	Hypothetical protein		Function unknown
MBOV0704S00003056_at	BCG_3079c	7.26	0.00435	Hypothetical protein		Function unknown
MBOV0704S00003500_at	BCG_3528	9.92	0.000262	Hypothetical protein		Function unknown
MBOV0704S00003874_at	BCG_3902	3.69	0.000511	Hypothetical protein		Function unknown
MBOV0704S00004610_at		3.26	7.56E−05	Intergenic region 2047845–2048024		
Region 5: genes upregulated by both sodium hypochlorite and peracetic acid: 20 genes						
AFFX-PheX-M_at		2.30	0.00538	*B. subtilis*/GEN=pheA/DB_XREF=gb:M24537.1/ NOTE=SIF corresponding to nucleotides 2437–2828 of gb:M24537.1/DEF=*Bacillus subtilis* sporulation protein (spoOB), GTP-binding protein (obg), phenylalanine biosynthesis associated protein (pheB), and monofunctional prephenate dehydratase (pheA) genes, complete cds.		
AFFX-r2-Bs-lys-5_at		2.44	0.00666	*B. subtilis*/GEN=lys/DB_XREF=gb:X17013.1/ NOTE=SIF corresponding to nucleotides 411–659 of gb:X17013.1, not 100% identical/DEF=*B. subtilis* lys gene for diaminopimelate decarboxylase (EC 4.1.1.20).		
AFFX-r2-Bs-thr-5_s_at		2.04	0.025	*B. subtilis*/GEN=thrC/DB_XREF=gb:X04603.1/ NOTE=SIF corresponding to nucleotides 290–888 of gb:X04603.1/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine		

				synthase.			
AFFX-ThrX-M_at		2.20	0.016	*B. subtilis*/GEN=thrC, thrB/DB_XREF=gb: X04603.1/NOTE=SIF corresponding to nucleotides 995–1562 of gb:X04603.1, not 100% identical/DEF=*B. subtilis* thrB and thrC genes for homoserine kinase and threonine synthase (EC 2.7.1.39 and EC 4.2.99.2, respectively).			
MBOV0704S00000607_at	BCG_0617c	2.35	0.000328	Hypothetical protein		Function unknown	
MBOV0704S00001758_at	BCG_1772c	2.62	0.000164	Putative transmembrane protein			
MBOV0704S00001998_at	BCG_2014	2.14	2.65E−05	Putative metal cation transporter P-type atpase A ctpF	*ctp*F	Inorganic ion transport and metabolism	
MBOV0704S00002033_at	BCG_2049c	2.08	0.00222	Hypothetical protein		Function unknown	
MBOV0704S00002035_at	BCG_2051	2.47	0.000231	Hypothetical protein Acg		Energy production and conversion	
MBOV0704S00002631_at	BCG_2652c	2.48	0.0037	Putative transmembrane alanine- and leucine-rich protein		General function prediction only	
MBOV0704S00002632_at	BCG_2653c	3.38	7.35E−05	Hypothetical protein		Function unknown	
MBOV0704S00002633_at	BCG_2654c	2.38	0.0004	Hypothetical protein		Function unknown	
MBOV0704S00002634_at	BCG_2655	2.24	2.23E−05	Hypothetical protein		Function unknown	
MBOV0704S00003020_at	BCG_3043c	2.02	0.00997	PE family protein			
MBOV0704S00003055_at	BCG_3078c	3.19	0.00189	Hypothetical protein		Function unknown	
MBOV0704S00003126_at	BCG_3149	2.61	0.0149	Hypothetical protein		Function unknown	
MBOV0704S00003130_at	BCG_3153c	2.31	0.000584	Hypothetical protein		Function unknown	
MBOV0704S00003131_at	BCG_3154	2.30	0.0014	Hypothetical protein		Function unknown	
MBOV0704S00003176_at	BCG_3199	2.97	0.00387	Putative amidase		Translation, ribosomal structure, and biogenesis	
MBOV0704S00004478_at		2.06	0.00744	Intergenic region 1572691–1572991			
Region 5: genes downregulated by both sodium hypochlorite and peracetic acid: 1 gene							
MBOV0704S00001742_at	BCG_1756	−2.24	0.00723	Hypothetical protein		Function unknown	
Region 6: genes upregulated by both hydrogen peroxide and peracetic acid: 3 genes							
MBOV0704S00001931_at	BCG_1947c	5.52	1.29E−05	Catalase-peroxidase-peroxynitritase T katG	*kat*G	Inorganic ion transport and metabolism	
MBOV0704S00002377_at	BCG_2395c	3.22	0.00279	Polyketide synthetase mbtD	*mbt*D	Metabolites biosynthesis, transport, and catabolism	
MBOV0704S00002382_at	BCG_2400c	2.11	0.00387	Putative isochorismate synthase mbtI	*mbt*I	Amino acid transport and metabolism	Coenzyme metabolism

The microarray results are the mean of three replicates of each gene. The fold change is a positive number when the expression level in the experiment increased compared to the control and is a negative number when the expression level in the experiment decreased compared to the control

*uvr*A and *nrd*F2 are directly regulated by the RecA-NDp promoter. In contrast to these results, *uvr*A has been shown to be induced in *M. tuberculosis* treated with mitomycin which is a DNA damaging agent (Brooks et al. 2001).

The discussion from this point on focuses on the comparison of the *M. bovis* BCG response to the oxidative disinfectants, peracetic acid, hydrogen peroxide, and sodium hypochlorite and provides conclusions to the two sections of the study.

Discussion: comparisons among the toxicogenomic responses of *M. bovis* BCG to sodium hypochlorite, hydrogen peroxide, and peracetic acid

Table 3 contains the genes found in the seven regions of the Venn diagram (Fig. 4). However, in this discussion, we have focused on the genes in the intersecting regions among the three disinfectants (regions 4, 5, and 6) since our prior publications (Jang et al. 2009a, b) and part of the current study have described in detail the toxicogenomic responses of *M. bovis* BCG to sodium hypochlorite, hydrogen peroxide, and peracetic acid.

Region 4: genes up- and downregulated in common between sodium hypochlorite and hydrogen peroxide

Five of the upregulated genes in this region were hypothetical proteins and the sixth gene was an intergenic region with no known function.

Region 5: genes up- and downregulated in common between sodium hypochlorite and peracetic acid

The *ctp*F gene which encodes a putative metal cation transporter P-type atpase A was the only gene with a known function in this region. Interestingly, in another study, the *ctp*F gene was upregulated in *M. tuberculosis* in response to exposure to reactive nitrogen intermediates (Ohno et al. 2003). Further, the *ctp*F gene was upregulated in *M. tuberculosis* in response to growth in a hypoxic environment (Sherman et al. 2001).

Region 6: genes up- and downregulated in common between peracetic acid and hydrogen peroxide

The three upregulated genes in this region were the *kat*G gene which encodes a catalase-peroxidase-peroxynitritase T enzyme and the *mbt*D and *mbtI* genes which encode polyketide synthases involved in the biosynthesis of mycobactins. As earlier mentioned, *kat*G is an anti-oxidative stress enzyme produced in mycobacteria to counteract the effects of reactive oxygen intermediates. Mycobactins are salicylic acid-derived siderophores, important in mycobacterial iron acquisition/virulence. These results further emphasize the intricate connection between iron regulation and oxidative stress response in *M. bovis* BCG exposed to both disinfectants.

Region 7: no genes were upregulated in common between sodium hypochlorite, hydrogen peroxide, and peracetic acid

In conclusion, the first section of this report suggests that the regulation of arginine levels and virulence factors may play an adaptive role against peracetic acid treatment in *M. bovis* BCG. The results from this section also suggest that, in addition to the upregulation of *kat*G which plays a major role in defense against oxidative damage, cell wall modification due to upregulation of genes coding for cell wall components after 20 min may also function as a protective strategy against peracetic acid damage. The downregulation of DNA repair genes after both 10 and 20 min indicates that the inhibition of DNA repair may contribute to the mechanism of action of peracetic acid. Further, the upregulation of the *dev*R–*dev*S signal transduction system, which is a regulator of the genetic response of *M. tuberculosis* in oxygen-deficient environments after 10 min, indicates that this system plays a role in the early adaptive response of *M. bovis* BCG to peracetic acid-induced oxidative stress. In summary, the complex interplay of the changes in these metabolic processes may contribute to both the inhibitory effect of peracetic acid on *M. bovis* BCG and the resistance strategies utilized by *M. bovis* BCG against the effect of peracetic acid treatment. This is the first report of the genome-wide response of *M. bovis* BCG to peracetic acid treatment and therefore advances the understanding of the mode of peracetic acid in *M. bovis* BCG. The second section of this study reports that iron acquisition/virulence is affected in *M. bovis* BCG in response to hydrogen peroxide and peracetic acid treatment. This comparative analysis also determined that the *ctp*F gene was upregulated in response to both peracetic acid and sodium hypochlorite treatment. This comparative analysis helps in the identification of commonly activated genes between these oxidative antimicrobials, which further improves the understanding of their modes of action in mycobacteria. The information generated in this study will benefit other researchers studying the transcriptomic response of mycobacteria to peracetic acid specifically and to oxidative biocides in general.

Acknowledgment This research is supported by the United States Environmental Protection Agency Grant number T-83284001-4.

Although the research described in this paper has been funded wholly by the United States Environmental Protection Agency, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the EPA nor does the mention of trade names or commercial products constitute endorsement of recommendation of use.

References

Bagchi G, Chauhan S, Sharma D, Tyagi JS (2005) Transcription and autoregulation of the Rv3134c–*dev*R–*dev*S operon of *Mycobacterium tuberculosis*. Microbiol 151:4045–4053

Barclay R, Ratledge C (1983) Iron-binding compounds of *Mycobacterium avium*, *M. intracellulare*, *M. scrofulaceum*, and mycobactin-dependent *M. paratuberculosis* and *M. avium*. J Bacteriol 153:1138–1146

Bhatt K, Gurcha SS, Bhatt A, Besra GS, Jacobs WR Jr (2007) Two polyketide-synthase-associated acyltransferases are required for sulfolipid biosynthesis in *Mycobacterium tuberculosis*. Microbiol 153:513–520

Block SS (2001) Disinfection, sterilization and preservation. Lippincott Williams & Wilkins, Baltimore

Boon C, Li R, Qi R, Dick T (2001) Proteins of *Mycobacterium bovis* BCG induced in the Wayne dormancy model. J Bacteriol 183:2672–2676

Brooks PC, Movahedzadeh F, Davis EO (2001) Identification of some DNA damage-inducible genes of *Mycobacterium tuberculosis*: apparent lack of correlation with LexA binding. J Bacteriol 183:4459–4467

Chaitra MG, Shaila MS, Nayak R (2008) Characterization of T-cell immunogenicity of two PE/PPE proteins of *Mycobacterium tuberculosis*. J Med Microbiol 57:1079–1086

Chang W, Small DA, Toghrol F, Bentley WE (2006a) Global transcriptome analysis of *Staphylococcus aureus* response to hydrogen peroxide. J Bacteriol 188:1648–1659

Chang W, Toghrol F, Bentley WE (2006b) Toxicogenomic response of *Staphylococcus aureus* to peracetic acid. Environ Sci Technol 40:5124–5131

Cole ST, Brosch R et al (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393:537–544

Couture M, Yeh SR, Wittenberg BA, Wittenberg JB, Ouellet Y, Rousseau DL, Guertin M (1999) A cooperative oxygen-binding hemoglobin from *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 96:11223–11228

Croteau DL, DellaVecchia MJ, Wang H, Bienstock RJ, Melton MA, Van Houten B (2006) The C-terminal zinc finger of UvrA does not bind DNA directly but regulates damage-specific DNA binding. J Biol Chem 281:26370–26381

Croteau DL, DellaVecchia MJ, Perera L, Van Houten B (2008) Cooperative damage recognition by UvrA and UvrB: identification of UvrA residues that mediate DNA binding. DNA Repair Amst 7:392–404

Danilchanka O, Mailaender C, Niederweis M (2008) Identification of a novel multidrug efflux pump of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 52:2503–2511

De Voss JJ, Rutter K, Schroeder BG, Su H, Zhu Y, Barry CE 3rd (2000) The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. Proc Natl Acad Sci USA 97:1252–1257

DeMoss JA, Hsu PY (1991) NarK enhances nitrate uptake and nitrite excretion in *Escherichia coli*. J Bacteriol 173:3303–3310

Ernst FD, Homuth G, Stoof J, Mader U, Waidner B, Kuipers EJ, Kist M, Kusters JG, Bereswill S, van Vliet AH (2005) Iron-responsive regulation of the *Helicobacter pylori* iron-cofactored superoxide dismutase SodB is mediated by Fur. J Bacteriol 187:3687–3692

Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, Duthoy S, Grondin S, Lacroix C, Monsempe C, Simon S, Harris B, Atkin R, Doggett J, Mayes R, Keating L, Wheeler PR, Parkhill J, Barrell BG, Cole ST, Gordon SV, Hewinson RG (2003) The complete genome sequence of *Mycobacterium bovis*. Proc Natl Acad Sci USA 100:7877–7882

Goffin C, Ghuysen JM (2002) Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent. Microbiol Mol Biol Rev 66:702–738, table of contents

Golby P, Hatch KA, Bacon J, Cooney R, Riley P, Allnutt J, Hinds J, Nunez J, Marsh PD, Hewinson RG, Gordon SV (2007) Comparative transcriptomics reveals key gene expression differences between the human and bovine pathogens of the *Mycobacterium tuberculosis* complex. Microbiology 153:3323–3336

Gupta S, Tyagi AK (1993) Sequence of a newly identified *Mycobacterium tuberculosis* gene encoding a protein with sequence homology to virulence-regulating proteins. Gene 126:157–158

Gupta S, Jain S, Tyagi AK (1999) Analysis, expression and prevalence of the *Mycobacterium tuberculosis* homolog of bacterial virulence regulating proteins. FEMS Microbiol Lett 172:137–143

Heym B, Zhang Y, Poulet S, Young D, Cole ST (1993) Characterization of the *kat*G gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. J Bacteriol 175:4255–4259

Horn C, Namane A, Pescher P, Riviere M, Romain F, Puzo G, Barzu O, Marchal G (1999) Decreased capacity of recombinant 45/47-kDa molecules (Apa) of *Mycobacterium tuberculosis* to stimulate T lymphocyte responses related to changes in their mannosylation pattern. J Biol Chem 274:32023–32030

Hughes V, Smith S, Garcia-Sanchez A, Sales J, Stevenson K (2007) Proteomic comparison of *Mycobacterium avium* subspecies paratuberculosis grown in vitro and isolated from clinical cases of ovine paratuberculosis. Microbiol 153:196–205

Jang HJ, Chang MW, Toghrol F, Bentley WE (2008) Microarray analysis of toxicogenomic effects of triclosan on *Staphylococcus aureus*. Appl Microbiol Biotechnol 78:695–707

Jang HJ, Nde C, Toghrol F, Bentley WE (2009a) Global transcriptome analysis of the *Mycobacterium bovi*s BCG response to sodium hypochlorite. Appl Microbiol Biotechnol 85:127–140

Jang HJ, Nde C, Toghrol F, Bentley WE (2009b) Microarray Analysis of *Mycobacterium bovis* BCG revealed induction of iron acquisition related genes in response to hydrogen peroxide. Environ Sci Technol 43:9465–9472

Keller PM, Bottger EC, Sander P (2008) Tuberculosis vaccine strain *Mycobacterium bovis* BCG Russia is a natural *rec*A mutant. BMC Microbiol 8:120

Kitis M (2004) Disinfection of wastewater with peracetic acid: a review. Environ Int 30:47–55

Kumar P, Amara RR, Challu VK, Chadda VK, Satchidanandam V (2003) The Apa protein of *Mycobacterium tuberculosis* stimulates gamma interferon-secreting CD4+ and CD8+ T cells from purified protein derivative-positive individuals and affords protection in a guinea pig model. Infect Immun 71:1929–1937

LaMarca BB, Zhu W, Arceneaux JE, Byers BR, Lundrigan MD (2004) Participation of *fad* and *mbt* genes in synthesis of mycobactin in *Mycobacterium smegmatis*. J Bacteriol 186:374–382

Lavollay M, Arthur M, Fourgeaud M, Dubost L, Marie A, Veziris N, Blanot D, Gutmann L, Mainardi JL (2008) The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by L, D-transpeptidation. J Bacteriol 190:4360–4366

Li Z, Kelley C, Collins F, Rouse D, Morris S (1998) Expression of *kat*G in *Mycobacterium tuberculosis* is associated with its growth and persistence in mice and guinea pigs. J Infect Dis 177:1030–1035

Milano A, Forti F, Sala C, Riccardi G, Ghisotti D (2001) Transcriptional regulation of *fur*A and *kat*G upon oxidative stress in *Mycobacterium smegmatis*. J Bacteriol 183:6801–6806

Mowa MB, Warner DF, Kaplan G, Kana BD, Mizrahi V (2009) Function and regulation of class I ribonucleotide reductase-encoding genes in mycobacteria. J Bacteriol 191:985–995

Nathan C, Shiloh MU (2000) Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA 97:8841–8848

Nde CW, Jang HJ, Toghrol F, Bentley WE (2008) Toxicogenomic response of *Pseudomonas aeruginosa* to ortho-phenylphenol. BMC Genomics 9:473

Neres J, Labello NP, Somu RV, Boshoff HI, Wilson DJ, Vannada J, Chen L, Barry CE 3rd, Bennett EM, Aldrich CC (2008) Inhibition of siderophore biosynthesis in *Mycobacterium tuberculosis* with nucleoside bisubstrate analogues: structure–activity relationships of the nucleobase domain of 5′-*O*-[*N*-(salicyl) sulfamoyl]adenosine. J Med Chem 51:5349–5370

Noji S, Nohno T, Saito T, Taniguchi S (1989) The *nar*K gene product participates in nitrate transport induced in *Escherichia coli* nitrate-respiring cells. FEBS Lett 252:139–143

Ohno H, Zhu G, Mohan VP, Chu D, Kohno S, Jacobs WR Jr, Chan J (2003) The effects of reactive nitrogen intermediates on gene expression in *Mycobacterium tuberculosis*. Cell Microbiol 5:637–648

Pawaria S, Lama A, Raje M, Dikshit KL (2008) Responses of *Mycobacterium tuberculosis* hemoglobin promoters to in vitro and in vivo growth conditions. Appl Environ Microbiol 74:3512–3522

Popham DL, Young KD (2003) Role of penicillin-binding proteins in bacterial cell morphogenesis. Curr Opin Microbiol 6:594–599

Quadri LE, Sello J, Keating TA, Weinreb PH, Walsh CT (1998) Identification of a *Mycobacterium tuberculosis* gene cluster encoding the biosynthetic enzymes for assembly of the virulence-conferring siderophore mycobactin. Chem Biol 5:631–645

Rand L, Hinds J, Springer B, Sander P, Buxton RS, Davis EO (2003) The majority of inducible DNA repair genes in *Mycobacterium tuberculosis* are induced independently of RecA. Mol Microbiol 50:1031–1042

Rezwan M, Grau T, Tschumi A, Sander P (2007) Lipoprotein synthesis in mycobacteria. Microbiology 153:652–658

Rodriguez GM, Smith I (2006) Identification of an ABC transporter required for iron acquisition and virulence in *Mycobacterium tuberculosis*. J Bacteriol 188:424–430

Saini DK, Malhotra V, Dey D, Pant N, Das TK, Tyagi JS (2004) DevR–DevS is a bona fide two-component system of *Mycobacterium tuberculosis* that is hypoxia-responsive in the absence of the DNA-binding domain of DevR. Microbiology 150:865–875

Sander P, Rezwan M, Walker B, Rampini SK, Kroppenstedt RM, Ehlers S, Keller C, Keeble JR, Hagemeier M, Colston MJ, Springer B, Bottger EC (2004) Lipoprotein processing is required for virulence of *Mycobacterium tuberculosis*. Mol Microbiol 52:1543–1552

Sassetti CM, Boyd DH, Rubin EJ (2003) Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48:77–84

Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK (2003) Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. J Exp Med 198:693–704

Sherman DR, Sabo PJ, Hickey MJ, Arain TM, Mahairas GG, Yuan Y, Barry CE 3rd, Stover CK (1995) Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. Proc Natl Acad Sci USA 92:6625–6629

Sherman DR, Voskuil M, Schnappinger D, Liao R, Harrell MI, Schoolnik GK (2001) Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding α-crystallin. Proc Natl Acad Sci USA 98:7534–7539

Small DA, Chang W, Toghrol F, Bentley WE (2007) Comparative global transcription analysis of sodium hypochlorite, peracetic acid and hydrogen peroxide on *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 76:1093–1105

Snow GA (1970) Mycobactins: iron-chelating growth factors from mycobacteria. Bacteriol Rev 34:99–125

Sohaskey CD, Wayne LG (2003) Role of *nar*K2X and *nar*GHJI in hypoxic upregulation of nitrate reduction by *Mycobacterium tuberculosis*. J Bacteriol 185:7247–7256

Talaat AM, Ward SK, Wu CW, Rondon E, Tavano C, Bannantine JP, Lyons R, Johnston SA (2007) Mycobacterial bacilli are metabolically active during chronic tuberculosis in murine lungs: insights from genome-wide transcriptional profiling. J Bacteriol 189:4265–4274

Thibessard A, Frenandez A, Gitnz B, Leblond-Bourget N, Decaris B (2002) Effects of roda and pbp2b disruption on cell morphology and oxidative stress response of *Streptococcus thermophilus* CNRZ368. J Bacteriol 184:2821–2826

Tripathi SM, Ramachandran R (2006) Overexpression, purification and crystallization of lysine epsilon-aminotransferase (Rv3290c) from *Mycobacterium tuberculosis* H37Rv. Acta Crystallogr F Struct Biol Cryst Commun 62:572

Tundup S, Akhter Y, Thiagarajan D, Hasnain SE (2006) Clusters of PE and PPE genes of *Mycobacterium tuberculosis* are organized in operons: evidence that PE Rv2431c is co-transcribed with PPE Rv2430c and their gene products interact with each other. FEBS Lett 580:1285–1293

Tundup S, Pathak N, Ramanadham M, Mukhopadhyay S, Murthy KJ, Ehtesham NZ, Hasnain SE (2008) The co-operonic PE25/PPE41 protein complex of *Mycobacterium tuberculosis* elicits increased humoral and cell mediated immune response. PLoS ONE 3: e3586

Vergne AF, Walz AJ, Miller MJ (2000) Iron chelators from mycobacteria (1954–1999) and potential therapeutic applications. Nat Prod Rep 17:99–116

Voskuil MI, Schnappinger D, Rutherford R, Liu Y, Schoolnik GK (2004) Regulation of the *Mycobacterium tuberculosis* PE/PPE genes. Tuberc Edinb 84:256–262

Wayne LG, Sohaskey CD (2001) Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu Rev Microbiol 55:139–163

Wilson TM, de Lisle GW, Collins DM (1995) Effect of *inh*A and *kat*G on isoniazid resistance and virulence of *Mycobacterium bovis*. Mol Microbiol 15:1009–1015

Wittenberg JB, Bolognesi M, Wittenberg BA, Guertin M (2002) Truncated hemoglobins: a new family of hemoglobins widely distributed in bacteria, unicellular eukaryotes, and plants. J Biol Chem 277:871–874

Zheng M, Storz G (2000) Redox sensing by prokaryotic transcription factors. Biochem Pharmacol 59:1–6

Zou Y, Bassett H, Walker R, Bishop A, Amin S, Geacintov NE, Van Houten B (1998) Hydrophobic forces dominate the thermodynamic characteristics of UvrA–DNA damage interactions. J Mol Biol 281:107–119