



Toxicogenomic response of *Mycobacterium bovis* to hydrogen peroxide



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ABSTRACT

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

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

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

Mycobacterium bovis BCG genes that showed statistically significant mRNA level changes upon either 10 or 60 min exposure to hydrogen peroxide

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

Gene Annotation	10min		60min		Description	Functional group
	P-value	Fold	P-value	Fold		
Group I: Upregulation (10min) - Upregulation (60 min) 7 genes						
BCC_1947G	0.00124	5.5	0.00124	2.7	Catalase-peroxidase, peroxidase 7 katG	Inorganic ion transport and metabolism
BCC_1948G	0.00111	4.9	0.00111	2.3	Ferric uptake regulation protein furA	Inorganic ion transport and metabolism
BCC_2396G	0.00381	4.5	0.00381	1.9	Polyketide synthase mbtC	Secondary metabolites biosynthesis, transport and catabolism
BCC_2397C	0.00139	3.6	0.00139	2.0	l-phenylalanine synthase mbtB	Secondary metabolites biosynthesis, transport and catabolism
BCC_3009G	0.00589	3.4	0.00589	1.5	putative 3-isopropylmalate dehydratase large subunit leuC	Amino acid transport and metabolism
BCC_1712G	0.0124	1.9	0.0124	1.6	putative transcriptional regulatory protein	Inorganic ion transport and metabolism
BCC_0020	0.028	1.6	0.028	1.5	Thioredoxin hxc (TRX) (MPT4)	Posttranslational modification, protein turnover, chaperones
Group II: Upregulation (10min) - No change (60 min) 11 genes						
BCC_2395G	0.00811	3.2			Polyketide synthase mbtD	Secondary metabolites biosynthesis, transport and catabolism
BCC_3008G	0.0278	2.4			putative 3-isopropylmalate dehydratase small subunit leuD	Amino acid transport and metabolism
BCC_2405G	0.00508	2.1			putative isochlorogenic acid synthase mbtI	Amino acid transport and metabolism
BCC_2394G	0.0132	2.0			Peptide synthetase mbtE	Secondary metabolites biosynthesis, transport and catabolism
BCC_1590G	0.0235	1.9			putative polyketide synthase associated protein mbtA	Secondary metabolites biosynthesis, transport and catabolism
BCC_2140	0.0156	1.9			PPE family protein	
BCC_0328	0.0246	1.7			PPE family protein	
BCC_1411	0.0273	1.7			inhibitor drug-transport transmembrane ATP-binding protein ABC transporter	Defense mechanism
BCC_1410	0.00425	1.7			putative drug-transport transmembrane ATP-binding protein ABC transporter	Defense mechanism
BCC_0862G	0.00822	1.5			putative 3-hydroxyacyl-CoA dehydrogenase rnaB	Lipid transport and metabolism
BCC_1408	0.0207	1.5			putative acyl carrier protein	Lipid transport and metabolism
Group III: No change (10 min) - Upregulation (60min) 3 genes						
BCC_3227G			0.0205	1.8	putative ATP-dependent DNA helicase	DNA replication, recombination and repair
BCC_1377G			0.00117	1.6	putative methylated DNA-protein-cysteine Methyltransferase ogf	DNA replication, recombination and repair
BCC_3228G			0.00607	1.6	putative ATP-dependent DNA helicase	DNA replication, recombination and repair
Group IV: No change (10 min) - Downregulation (60min) 14 genes						
BCC_0029G			0.011	-1.5	putative chromosome partitioning protein parB	Transcription
BCC_0024G			0.0196	-1.8	putative chromosome partitioning protein parA	Cell cycle control, mitosis and meiosis
BCC_0025G			0.015	-1.5	putative glucose-inhibited division protein B GID	Cell wall/membrane biogenesis
BCC_0119			0.0254	-1.5	putative hydrogenase hsdG	Energy production and conversion
BCC_0567			0.0169	-1.7	putative transmembrane protein	
BCC_1457G			0.0222	-1.6	PE-PGRS family protein	
BCC_1747			0.0334	-1.6	putative inhibition inhibitor protein	Cell cycle control, mitosis and meiosis
BCC_1751			0.00439	-1.6	putative cytidylate kinase omk	Nucleotide transport and metabolism
BCC_1754			0.021	-1.6	putative 3-hydroxybutyryl-CoA dehydrogenase hsdD2	Lipid transport and metabolism
BCC_2969G			0.0419	-1.5	putative fatty acid-CoA ligase hsdD2	Lipid transport and metabolism
BCC_3075G			0.026	-1.6	putative chromosome partitioning protein parB	Transcription
BCC_3076G			0.0283	-1.7	putative chromosome partitioning protein parA	Cell cycle control, mitosis and meiosis
BCC_3077G			0.0134	-1.5	putative glucose-inhibited division protein B gid	Cell wall/membrane biogenesis
BCC_3078G			0.0072	-1.5	hypothetical protein similar to pig protein	General function prediction only

Gene	mRNA level change with microarray		mRNA level change with real-time PCR		Sense primer sequence	Antisense primer sequence
	Fold change		Fold change			
	10min	60min	10min	60min		
BCC_1947G	5.5	2.7	18.0 (± 0.1)	5.5 (± 0.3)	5'- AAC ATC AAA GTG TCC TTC GCC GAC -3'	5'- GCA AAG GAT TCC ACC TCG GTT TGT -3'
BCC_1948G	4.9	2.3	10.3 (± 0.1)	2.9 (± 0.2)	5'- TCG GAC CAT AAC GGG TCG CTO TT -3'	5'- GAT GTG ATC GCG AAG TGT CGG ATA -3'
BCC_2396G	4.5	1.9	7.1 (± 0.1)	5.3 (± 0.2)	5'- CTT TCA CAC GCG GGT TCA AGG TAT -3'	5'- TCG TCG TTG GAG AAC TCG ACC AAA -3'
BCC_2397C	3.6	2.0	7.5 (± 0.1)	4.8 (± 0.3)	5'- ATC TCG CGA CTT TCC CAT CAG TT -3'	5'- GTC AAC GGA AGT TCG AAT ACC GGA -3'
BCC_3009G	3.4	1.5	7.1 (± 0.1)	2.5 (± 0.4)	5'- AAA GCA TTG GCG TAC ATG GCC TT -3'	5'- TTC AAT GCG ACC GTT GGT ACA GGA -3'
BCC_0020	1.6	1.5	4.5 (± 0.1)	3.3 (± 0.1)	5'- AAA GTT ACC GAC GCA TCC TTG GCG -3'	5'- TAG CAT CTT GCA AGG TCG AGA GCA -3'
BCC_2395G	3.2	-	11.7 (± 0.1)	-	5'- AGG ACT ACC ACC TGG TAG GAA ACA -3'	5'- TCG GCA TTA CCG GAG TAA AGA ACT -3'
BCC_3008G	2.4	-	3.0 (± 0.6)	-	5'- AAG CCT TTC ACA CCC ACT CTC GTA -3'	5'- TCA GAA AGA CCG CGG GAA TGA TGT -3'
BCC_2405G	2.1	-	5.9 (± 0.4)	-	5'- TGG CTC GTG ATG ACC TGG AAT GAA -3'	5'- TAA TCT CCT CAA GCG AAG ACC GGA -3'
BCC_2394G	2.0	-	2.6 (± 0.2)	-	5'- FAT TGG AGG GAC GCA TGT CGC ATA -3'	5'- TTC GGA ATC CCG GAA TCT TGA CCT -3'
BCC_1410	1.7	-	5.0 (± 0.2)	-	5'- GGA TCC GGT GAA TTT GTT GCC GTT -3'	5'- AAT TCG GTT TCG TCG AGT GTC ACT -3'
16S rRNA*	1.0	1.0	1.0	1.0	5'- TGC AAG TCG AAC GGA AAG GTC TCT -3'	5'- AAG ACA TGC ATC CCG TCG TCC TAT -3'

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

MATERIAL AND METHOD

• *M. bovis* exposed to hydrogen peroxide (0.5mM) for 10 and 60min

• Affymetrix *M. bovis* BCG custom genechip analysis

• Statistical analysis of microarray data

- p-value for the t-test ≤ 0.05

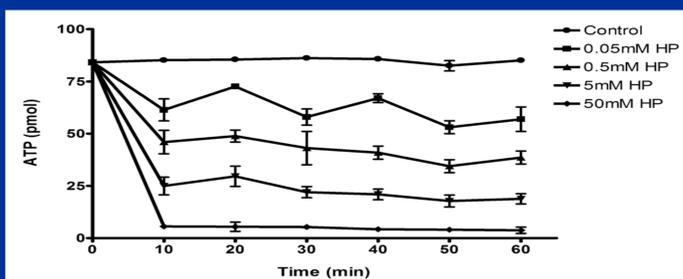
- Fold change in transcript level ≥ 2.0

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

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

RESULTS AND DISCUSSION

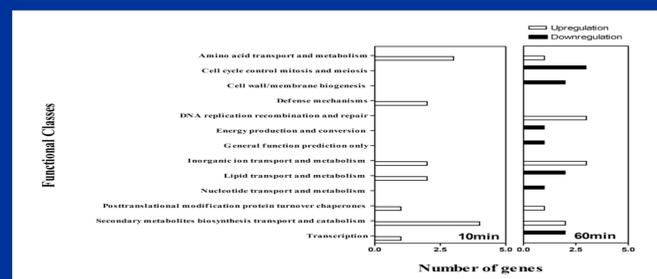
Growth Inhibition of *M. bovis* BCG treated with hydrogen peroxide



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

* The genes were grouped based on their regulation directions upon 10 and 60 min exposures.

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



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