Quantitative proteomics reveals novel insights into isoniazid susceptibility in mycobacteria mediated by a universal stress protein

Xinling Hu[1,#], Xiaojing Li[1,#], Lige Huang[1], John Chan[2], Yuling Chen[3], Haiteng Deng[3], Kaixia Mi[1,4*]

[1]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, CAS, Beijing 100101, China
[2] Departments of Medicine and Microbiology & Immunology, Albert Einstein College of Medicine, NY 10461, USA
[3] School of Life Sciences, Tsinghua University, Beijing 100084, China
[4] Beijing Key Laboratory of Microbial Drug Resistance and Resistome, Beijing 100101, China

[#] These authors contributed equally to this study

[*]To whom correspondence should be addressed: Institute of Microbiology, No.1 Beichen West Road, Chaoyang District, Beijing 100101, China.
Tel: 86-010-64806082;
E-mail: mik@im.ac.cn;
Fax: 8610-64807468

Figure S1: Level of KatG activation of INH.

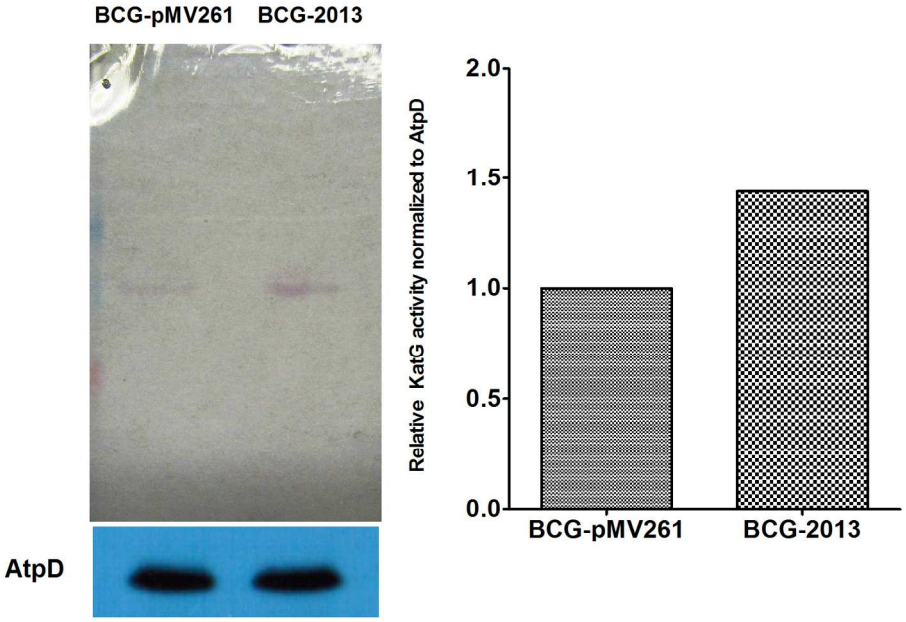
Figure S2: MS and MS/MS spectra for the peptide IVEHSYLVAQAHQIVEQAHK from BCG_2013.

Table S1: Oligonucleotide primers used in this study.

Table S2: Proteins up-regulated in BCG-2013 compared with BCG-pMV261.

Table S3: Proteins down-regulated in BCG-2013 compared with BCGpMV261.

Table S4: Functional Annotation Clustering of the differentially proteins expressed between BCG-Rv1996 and BCG-pMV261.



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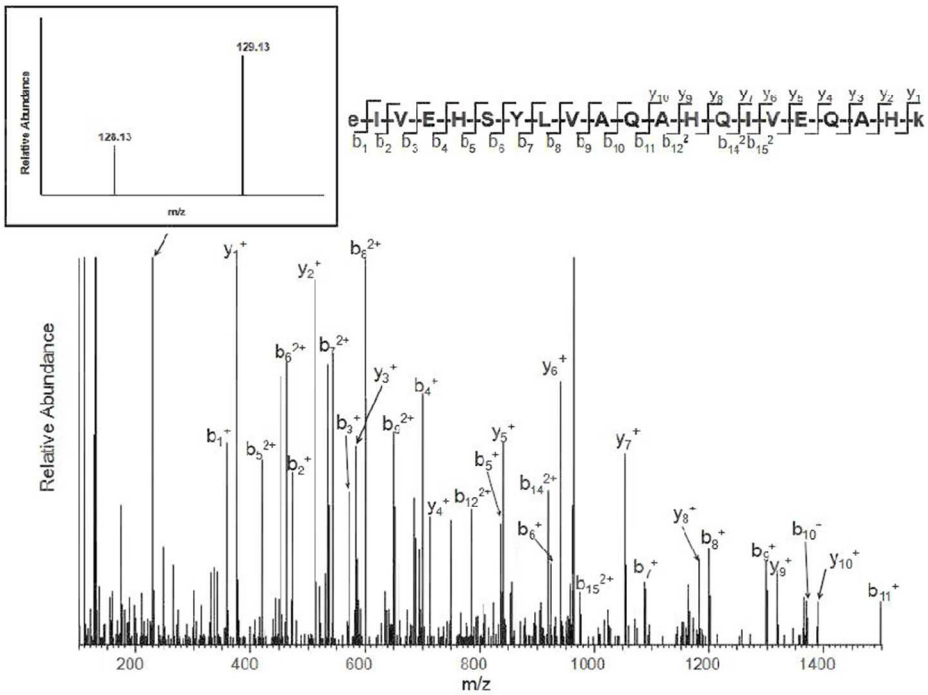

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Table S1. Oligonucleotide primers used in this study*

Name	Sequence (5'-3')	Purpose
pMV261F	CATCCCCGATCCGGAGGAATC	To confirm pMV261-*BCG2013*
pMV261R	GTCTTTCGACTGAGCCTTTC	To confirm pMV261-*BCG2013*
BCG2013F	GCTGAATTCATGTCAGCCCAACAAACGAAC	To clone pMV261-*BCG2013*
BCG2013R	GTTAAGCTTCAGATCGCACAGGCCCTCAG	To clone pMV261-*BCG2013*
A1KM41-qF	TGATGACCGAGCTTAGCGAG	q-RT-PCR
A1KM41-qR	TTGGCTTCCAGCAGATGGTT	q-RT-PCR
A1KK40-qF	CACCAAATCGTCGAACAGGC	q-RT-PCR
A1KK40-qR	CTTGAGCTGACCGATCCCAG	q-RT-PCR
A1KJX3-qF	GATTGTTTTCGCCGGCAACT	q-RT-PCR
A1KJX3-qR	TGGCTTCCTTGCCCCAATAG	q-RT-PCR
A1KIH7-qF	CGATGAGAGACGGAGGAATCG	q-RT-PCR
A1KIH7-qR	TGCCCAAGACTTCATCGCTC	q-RT-PCR
A1KF72-qF	CGCCGAGATCGTCAAGGATG	q-RT-PCR
A1KF72-qR	GTAGCGGATCTCACGCAGG	q-RT-PCR
A1KGB9-qF	AGATCAAAAACGGCCAACGC	q-RT-PCR
A1KGB9-qR	CGCGGAAGCAGAAACTTCAC	q-RT-PCR
A1KH68-qF	CGGCCTTTCACGAGAGGTAT	q-RT-PCR
A1KH68-qR	TGTAGTTGACCTGCTGAGCG	q-RT-PCR
A1KN45-qF	CTGGGTTCTACCTGCCGATG	q-RT-PCR
A1KN45-qR	GAGCTCGAACAGTAGCTCGT	q-RT-PCR
A1KHB7-qF	GAGTCGATGGCCATGAGGTT	q-RT-PCR
A1KHB7-qR	CGGATTGGCGATCAGCATCT	q-RT-PCR
A1KJ77-qF	AGCTCACCGACTCGTTTGTC	q-RT-PCR
A1KJ77-qR	AAAATCTTGTTCGCCTCGCC	q-RT-PCR
A1KQB1-qF	GTGTGGTCGAACCTGGTGAT	q-RT-PCR
A1KQB1-qR	CTCCAGTTTCTCCGGTGAGC	q-RT-PCR
A1KPL9-qF	CGTGTCGATCCAGCATTTCG	q-RT-PCR
A1KPL9-qR	TCGACGACGTAGACACACTG	q-RT-PCR
A1KF36-qF	CACACCTGGTCACCCTCATC	q-RT-PCR
A1KF36-qR	GTTGCCAGAATTCGATGGCG	q-RT-PCR

* Restriction enzyme sites are underlined.

Table S2. Up-regulated proteins in BCG-2013 compared with BCG-pMV261*

Accession	Protein description	Score	Coverage	Peptides matched	Variability（%）	Ratio±SD
A1KFR2	60 kDa chaperonin 1	5553	81	35	0.9-25.5	1.5± 0.1
A1KK40	Universal stress protein BCG_2013	2401	80	16	2.0-24.6	3.0± 0.6
A1KFH2	Chaperone protein DnaK	1222	75	38	0.4-27.7	1.6± 0.1
A1KJX3	Catalase-peroxidase KatG	409	49	25	0.3-21.2	1.9± 0.2

A1KFK5	Probable endopeptidase ATP binding protein (Chain b) ClpB	227	45	15	0.1-12.8	1.6± 0.1
A1KPT2	Probable ATP-dependent clp proteasE ATP-binding subunit ClpC	196	50	14	0.6-23	1.6± 0.2
A1KFU9	Heparin-binding hemagglutinin	141	46	7	0.3-25.6	1.5± 0.1
A1KMS6	Phenolpthiocerol synthesis type-I polyketide synthase PpsA	119	12	5	2.0-21.4	1.7± 0.2
A1KGD2	50S ribosomal protein L7/L12	116	48	6	1.3-21	1.7± 0.2
A1KFH3	Protein GrpE	104	60	7	0.1-25.7	1.6± 0.2
A1KF91	Probable fatty-acid-CoA ligase FadD2	81	26	6	0.3-17.1	1.6± 0.1
A1KGI1	30S ribosomal protein S10	81	76	5	0.4-25.7	1.6± 0.1
A1KIH7	Putative integration host factor MihF	78	30	7	4.1-22.4	1.7± 0.1
A1KKJ3	Cell division protein FtsZ	69	36	4	0.3-27	1.6± 0.2
A1KKT7	Meromycolate extension acyl carrier protein AcpM	59	38	4	2.3-26.8	1.6± 0.1
A1KIY8	Putative uncharacterized protein BCG_1610	54	41	4	0.3-13.4	1.6± 0.1
A1KMR0	Probable signal recognition particle protein Ffh	51	20	4	1.1-29.3	1.6± 0.3
A1KFG4	Isoniazid inductible gene protein IniA	51	19	3	0.5-12.2	1.6± 0.2
A1KHF1	Putative polyketide synthase Pks16	47	17	3	1.8-12.1	1.6± 0.1
A1KFH4	Chaperone protein DnaJ 1	45	32	2	4.9-21.7	1.6± 0.1
A1KEM1	30S ribosomal protein S6	42	50	4	3.2-25.9	1.8± 0.1
A1KF72	Heat shock protein Acr2	40	57	7	15-28.2	3.3± 0.3
A1KLD7	50S ribosomal protein L27	39	62	3	0.4-18.1	1.8± 0.5
A1KL63	Chaperone protein DnaJ 2	34	14	4	2.3-24.9	1.6± 0.1
A1KFK0	Possible protein transport protein SecE2	33	58	2	12.2-18.3	1.5± 0.1
A1KNP2	Protein translocase subunit SecA 1	30	9	2	9.5-23.9	1.9± 0.2
A1KI52	Putative uncharacterized protein BCG_1324	26	31	2	0.9-16.1	1.7± 0.1
A1KMC8	30S ribosomal protein S15	26	20	2	1.8-6.1	1.7± 0.1
A1KFQ7	Putative conserved atpase	25	14	2	4.7-28.7	1.6± 0.1
A1KMU3	Putative uncharacterized protein BCG_2970c	24	33	2	5.3-10	1.5± 0.1
A1KKE0	Sec-independent protein translocase protein TatA	22	47	2	4.4-7.4	1.5± 0.1
A1KGJ8	30S ribosomal protein S14 type Z	21	21	1	6.1	1.8± 0.1
A1KEN4	Putative uncharacterized protein BCG_0096	21	39	3	3.2-29.5	1.5± 0.1

A1KG43	UPF0234 protein BCG_0611c	20	24	2	0.7-12.4	1.7± 0.2
A1KMX6	Glycerol-3-phosphate dehydrogenase [NAD(P)+] 2	20	16	2	2.6-29.5	1.7± 0.5
A1KEM3	30S ribosomal protein S18	19	49	5	0-24.1	1.8± 0.2
A1KQ07	Putative uncharacterized protein BCG_3736c	18	23	2	4.0-5.4	1.5± 0.2
A1KGN5	Probable methylmalonate-semialdehyde dehydrogenase MmsA	16	20	2	3.5-21.6	1.6± 0.2
A1KIS9	Putative uncharacterized protein BCG_1551	16	16	1	4.3	1.6
A1KKR9	Putative uncharacterized protein BCG_2243	16	10	2	6.9-27.1	2.0± 0.3
A1KII9	Probable lipase LipH	16	6	2	0.6-9.3	1.6± 0.1
A1KG39	Probable polyprenyl-diphosphate synthase GrcC1	16	23	2	3.7-19.8	1.6± 0.1
A1KQK7	Putative uncharacterized protein BCG_3935c	15	30	2	5.5-9.6	1.7± 0.1
A1KNK8	Putative uncharacterized protein TB9.4	12	59	2	3.1-15.8	1.7± 0.2
A1KHB5	50S ribosomal protein L32	12	16	2	8.0-21.2	1.8± 0.1
A1KMI4	Probable N utilization substance protein A NusA	12	10	2	5.6-20.2	1.8± 0.2
A1KGK3	50S ribosomal protein L30	10	31	2	10.5-21.5	1.5± 0.1
A1KK96	Putative uncharacterized protein BCG_2069	10	9	1	4.3	1.8
A1KMI8	Proline--tRNA ligase	10	10	1	13.3	1.7± 0.3
A1KJA5	Tyrosine--tRNA ligase	10	3	1	22.7	1.5

Table S3. Down-regulated proteins in BCG-2013 compared with BCG-pMV261*

Accession	Protein description	Score	Coverage	Peptides matched	Variability（%）	Ratio± SD
A1KI28	MCE-family protein Mce1B	224	43	10	2.0-23.3	0.6± 0.2
A1KMY1	Putative uncharacterized protein BCG_0253	213	66	6	8.0-29.7	0.7± 0.1
A1KFI4	Probable fructose-bisphosphate aldolase Fba	184	67	11	0.4-19.4	0.7± 0.1
A1KMY0	Probable transcriptional regulatory protein (Possibly ArsR-family)	128	31	4	0.4-17.5	0.7± 0.1
A1KMY2	Transcription antitermination protein NusG	99	27	7	1.1-16.4	0.7± 0.1
A1KF36	Periplasmic phosphate-binding lipoprotein PstS1	95	26	7	5.4-25.2	0.7± 0.2

A1KGB9	Response regulator MprA	89	56	7	2.3-16.6	0.6± 0.1
A1KQD8	Putative uncharacterized protein BCG_1078	87	30	4	0.5-15	0.6± 0.1
A1KLV3	Possible GTP-binding translation elongation factor TypA	73	46	6	3.5-20.9	0.7± 0.2
A1KN73	Malate dehydrogenase	64	28	5	2.7-20.6	0.6± 0.1
A1KG53	ATP synthase epsilon chain	49	15	4	9.1-25.1	0.7± 0.1
A1KHF9	Putative uncharacterized protein BCG_1489c	42	34	3	4.3-17.5	0.6± 0.1
A1KQJ8	Possible chalcone synthase Pks10	37	23	2	5.0-18.4	0.6± 0.1
A1KH68	Probable transcriptional regulatory protein BCG_2628c	28	12	2	0.3-25.7	0.5± 0.1
A1KN45	Probable DNA-binding protein HU homolog HupB	22	13	3	3.9-11.8	0.7± 0.1
A1KHB7	3-isopropylmalate dehydratase small subunit	20	26	2	0.6-7.1	0.5± 0.1
A1KQB1	3-isopropylmalate dehydratase large subunit	19	17	2	2.0-7.1	0.7± 0.1
A1KNJ7	Ribonucleoside-diphosphate reductase (Beta chain) NrdF2	18	20	1	17.6	0.7± 0.2
A1KI99	Putative uncharacterized protein BCG_3100c	17	28	1	2.4	0.7± 0.1
A1KJ77	NADH pyrophosphatase	17	10	2	2.2-17.9	0.6± 0.1
A1KQ94	4-hydroxy-2-oxovalerate aldolase	15	16	1	12.5	0.7± 0.1
A1KQD7	19 kDa lipoprotein antigen LpqH	12	16	1	28.5	0.7± 0.1
A1KEZ0	Putative oxidoreductase	12	13	1	12.1	0.6± 0.1
A1KPL9	Secreted MPT51/MPB51 antigen protein FbpD	11	14	2	6.0-22.2	0.5± 0.1
A1KIL7	Antigen 85-A	10	9	1	7.4	0.7± 0.1
A1KHV6	Hypothetical alanine rich protein	10	9	1	23.7	0.6± 0.2

* The "Peptides Matched" was assigned when the criteria were as followed: 1) quantitation was performed for proteins with at least two unique identified peptides. 2) quantitative precision was expressed as protein ratio variability. When the protein ratio variability was smaller than 30%, the ratios were accepted as the quantitative ratios of proteins.

Table S4 Functional Annotation Clustering of the differentially proteins expressed between BCG-Rv1996 and BCG-pMV261

Annotation Cluster	Count	p-value
Translation	10	2.00E-05
Stress response	7	2.90E-09
protein transport	3	2.50E-02

Cellular carbohydrate catabolic process	4	5.30E-02
metal ion binding	10	2.40E-01
cell membrane	4	4.50E-01
carboxylic acid biosynthetic process	4	3.50E-01
GTP-binding	3	3.20E-02
ATPase	3	2.90E-02
NAD-binding domain	4	4.40E-01