Table S1: Summary of the S-mycothiolated proteins in *Corynebacterium glutamicum, Corynebacterium diphtheriae* and *Mycobacterium smegmatis*

Accession	Protein	Function	Conservation/function of Cys-SSM	Peptide-SSM Sequence
Corynebacterium glutamicum [1]				
Metabolism of carbohydrates and amino acids				
Q6M5P4	MalP	Maltodextrin phosphorylase	Cys180 not cons.	(R)ASNQLVV$\mathbf{C}_{180}$(+484)FDDm(+16)K(T)
Q8NRB3	MetE	Methionine synthase	**Cys713 Zn-binding active site**	(R)QLWVNPD$\mathbf{C}_{713}$(+484)GLK(T)
P08499	Hom	Homoserine dehydrogenase	Cys239 not cons.	(R)VTADDVY$\mathbf{C}_{239(}$+484)EGISNISAADIEAAQQAGHTIK(L)
Q8NLE6	Ino-1	Myo-inositol-1-phosphate synthase	Cys79 not cons.	(K)VGIDLADATEASQN$\mathbf{C}_{79}$(+484)TIK(I)
P19537	Fba	Fructose-bisphosphate aldolase	Cys332 not cons.	(R)IIES$\mathbf{C}_{332}$(+484)QDLK(S)
P77844	Pta	Phosphate acetyltransferase	Cys367 not cons.	(R)RLNPEL$\mathbf{C}_{367}$(+484)VDGPLQFDAAVDPGVAR(K)
Q6M8P0	XylB	Sugar (pentulose and hexulose) kinase	Cys338 not cons.	(R)GVLAGLN$\mathbf{C}_{338}$(+484)ATTR(E)
Metabolism of coenzymes and nucleotides				
Q8NM99	GuaB1	Inosine-5'-monophosphate dehydrogenase	**Cys302 active site, Thioimidate intermediate**	(K)VGVGPGAm$\mathbf{C}_{302}$(+484)TTR(M)
Q8NSR5	GuaB2	Inosine-5'-monophosphate dehydrogenase	**Cys317 active site, Thioimidate intermediate**	(K)VGIGPGSI$\mathbf{C}_{317}$(+484)TTR(V)
Q8NMI5	PurL	Phosphoribosylformylglycinamidine synthase 2	Cys716 not cons.	(K)LG$\mathbf{C}_{716}$(+484)TNDSAVIAVK(G)
Q8NQH1	TheD(ThiD1)	Thiamine biosynthesis multifunctional protein	**Cys451 HMP substrate binding**	(R)VNTTNSHGTG$\mathbf{C}_{451}$(+484)SLSASLATK(I)
Q8NQN6	PheT	Phenylalanyl-tRNA synthetase beta subunit	**Cys89 in tRNA binding domain**	(R)H$\mathbf{C}_{89}$(+484)HVNVGDANGTGELQSIVcGAR(N)
Q8NRI1	NadC	Nicotinate-nucleotide pyrophosphorylase	Cys114 not cons.	(R)TSGIATLTS$\mathbf{C}_{114}$(+484)YVAEVK(G)
Protein biosynthesis and quality control				
Q8NT01	RpsC	30S ribosomal protein S3	**Cys153 cons.**	(K)VV$\mathbf{C}_{153}$(+484)SGR(L)
Q8NST6	RplM	50S ribosomal protein L13	Cys50 not cons.	(K)GKPLYAPNVD$\mathbf{C}_{50}$(+484)GDHVIVINADK(V)
Q8NSV6	RpsM	30S ribosomal protein S13	Cys86 not cons.	(R)KIEIG$\mathbf{C}_{86}$(+484)YQGIR(H)
Q8NLF9	RpsF	30S ribosomal protein S6	**Cys67 cons.**	(K)$\mathbf{C}_{67}$(+484)ESATVLELDR(V)
P42439	Tuf	Elongation factor Tu	**Cys277 cons.**	(K)LLDSAEAGDN$\mathbf{C}_{277}$(+484)GLLLR(G)
Enzymes involved in detoxification				
Q8NRG3	Tpx	Thiol peroxidase	**Cys60 peroxidatic**	(K)LVLNIFPSVDTGV$\mathbf{C}_{60}$(+484)ATSVR(K)
			Cys94 resolving	(R)F$\mathbf{C}_{94}$(+484)SAEGIENVTPVSAFR(S)
Q8NMI2	Mpx	Mycothiol peroxidase	**Cys36 peroxidatic**	(K)$\mathbf{C}_{36}$(+484)GLTPQYEGLQK(L)
Q8NNH5	HmuO	Heme oxygenase	Cys165 not cons.	(R)EYGVSEEALSFY$\mathbf{C}_{165}$(+484)FEDLGK(L)
Q8NLL5	MsrA	Peptide methionine sulfoxide reductase	**Cys91 cons.**	(R)EV$\mathbf{C}_{91}$(+484)SGR(T)
Proteins with other/unknown functions				
I0LKH6	WA5_1783	Uncharacterized protein (WA5_1783)	Cys322 not cons.	(R)QVF$\mathbf{C}_{322}$(+484)LSSSGR(E)
Corynebacterium diphtheriae [2]				
Metabolism of carbohydrates and amino acids				
Q6NHC1	Ndh (DIP1217)	NADH dehydrogenase	Cys159 not cons.	(R)AEm$\mathbf{C}_{159}$(+484)EDPKER(E)
Q6NG08	DIP1726	4-alpha-glucanotransferase	Cys45 not cons.	(R)SLGV$\mathbf{C}_{45}$(+484)FGNEDEPATDHEPLTGPMPSEDQIR(Y)
Q6NH35	Gap (DIP1310)	Glyceraldehyde 3-phosphate dehydrogenase	**Cys153 cons. ; NAD binding domain**	(K)HNIISNAS$\mathbf{C}_{153}$(+484)TTNCLAPMAK(V)
Q6NEN5	GlpD (DIP2237)	Putative glycerol-3-phosphate dehydrogenase	Cys10 not cons.	(K)SH$\mathbf{C}_{10}$(+484)TFNPDYYQDVWQR(F)
Q6NFU0	DIP1796	Putative ribose/galactose isomerase	Cys143 not cons.	(R)RIDIL$\mathbf{C}_{143}$(+484)EYER(T)
Q6NIW1	Rbsk (DIP0655)	Ribokinase	Cys171 not cons.	(R)GTVVVNLAPVIDVDRD$\mathbf{C}_{171}$(+484)LLR(A)
Q6NHU4	ThrA (DIP1036)	Homoserine dehydrogenase	Cys243 not cons.	(R)VTYADVY$\mathbf{C}_{243}$(+484)EGISK(I)
Q6NJA1	DIP0511	4-hydroxy-tetrahydrodipicolinate synthase	Cys141 not cons.	(R)AVAAATSLPVIAYDIPV$\mathbf{C}_{141}$(+484)VHTK(L)
Q6NGP4	DapA (DIP1464)	4-hydroxy-tetrahydrodipicolinate synthase	Cys161 not cons.	(R)SVVPIAPDTL$\mathbf{C}_{161}$(+484)R(L)
Q6NI06	DIP0974	Putative aminotransferase	Cys138 not cons.	(R)$\mathbf{C}_{138}$(+484)DAPHELPNDDIDLVFINSPSNPTGR(V)
Q6NG84	GlnA1 (DIP1644)	Glutamine synthetase	Cys220 not cons.	(R)QHPE$\mathbf{C}_{220}$(+484)GTGSQQEINYR(F)
Q6NHM7	LeuB (DIP1105)	3-isopropylmalate dehydrogenase	Cys130 not cons.	(R)EGTEGLY$\mathbf{C}_{130}$(+484)GNGGTLR(E)
Metabolism of coenzymes and nucleotides				
Q6NG96	DIP1631	Uncharacterized protein	**Cys43 cons.**	(R)IAVQPGG$\mathbf{C}_{43}$(+484)SGLR(Y)
Q6NJ33	GuaB (DIP0580)	Inosine-5'-monophosphate dehydrogenase	**Cys317 cons. ; active site**	(K)VGIGPGSI$\mathbf{C}_{317}$(+484)TTR(V)
Q6NF38	PurA (DIP2063)	Adenylosuccinate synthetase	Cys423 not cons.	(R)DQTIV$\mathbf{C}_{423}$(+484)HDVMEA(-)
Protein biosynthesis and quality control				
P60453	RplC (DIP0473)	50S ribosomal protein L3	Cys154 not cons.	(R)VGGIGA$\mathbf{C}_{154}$(+484)ATPGR(V)
Q6NJ66	RpsM (DIP0546)	30S ribosomal protein S13	**Cys86 cons.**	(K)IEIG$\mathbf{C}_{86}$(+484)YQGLR(H)
Q6NI81	Pth (DIP0897)	Peptidyl-tRNA hydrolase	Cys49 not cons.	(K)ASGAVIEVGG$\mathbf{C}_{49}$(+484)R(V)
Q6NGV2	DIP1398	Uncharacterized RNA methyltransferase	**Cys376 cons ; nucleophile**	(R)AIAQSGPQAAIHIG$\mathbf{C}_{376}$(+484)DPATFAR(D)
Enzymes involved in detoxification				
Q6NGT3	DirA (DIP1420)	Iron repressible polypeptide	**Cys61 cons. ; active site**	(K)DFTFV$\mathbf{C}_{61}$(+484)PTEIAAFGK(L)
Proteins with other/unknown functions				
Q6NI65	DIP0913	Uncharacterized protein	Cys22 not cons.	(K)ERPTAGPQLYPVT$\mathbf{C}_{22}$(+484)EAVVSAIR(A)
Q6NHV4	DIP1026	Conserved hypothetical ATP-binding protein	Cys75 not cons.	(R)I$\mathbf{C}_{75}$(+484)LEADLGPVR(F)
Q6NHN0	DIP1102	Putative uncharacterized protein	Cys441 not cons.	(R)LLSA$\mathbf{C}_{441}$(+484)PESGLYK(G)
Q6NH90	DIP1250	M18 family aminopeptidase	**Cys401 cons.**	(K)AGSSHQVFVGNNSVP$\mathbf{C}_{401}$(+484)GSTIGPITATR(L)
Q6NH59	DIP1287	UPF0210 protein DIP1287	Cys324 not cons.	(K)GGMMA$\mathbf{C}_{324}$(+484)SR(V)
Q6NG33	GlmS (DIP1700)	Glutamine--fructose-6-phosphate aminotransferase	Cys74 not cons.	(K)VQALEQELETSPMPQS$\mathbf{C}_{74}$(+484)LGIGHTR(W)
Mycobacterium smegmatis [3]				
Metabolism of carbohydrates and amino acids				
A0QYH2	MSMEG_3657	Putative fumarate reductase/succinate dehydrogenase	Cys225 not cons.	(K)HFGETG$\mathbf{C}_{225}$(+484)VK(A)
A0R729	GlpK3 (MSMEG_6759)	Glycerol kinase	Cys294 not cons.	(K)NGLLTTV$\mathbf{C}_{294}$(+484)YR(L)
			Cys389 not cons.	(R)ATLESI$\mathbf{C}_{389}$(+484)YQSR(D)
A0QWW4	TpiA (MSMEG_3086)	Triosephosphate isomerase	Cys192 not cons.	(R)VAGAADAQEV$\mathbf{C}_{192}$(+484)K(A)
A0QPG1	MSMEG_0386	NAD dependent epimerase/dehydratase family protein	Cys194 not cons.	(R)WDPVGPNGEHAVPLPS$\mathbf{C}_{194}$(+484)ASR(T)
A0QS69	MSMEG_1371	Phosphomannose isomerase-like protein	Cys116 not cons.	(R)HLD$\mathbf{C}_{116}$(+484)VYGK(T)
A0QQX6	AceA (MSMEG_0911)	Isocitrate lyase	Cys268 not cons.	(K)NGLEP$\mathbf{C}_{268}$(+484)IAR(A)
A0R421	CitA (MSMEG_5676)	Citrate (Si) synthase	**Cys143 cons.**	(R)TIDE$\mathbf{C}_{143}$(+484)PTVTAR(F)
A0R2B1	Kgd (MSMEG_5049)	Multifunctional 2-oxoglutarate metabolism enzyme	**Cys695 cons.**	(R)SSEY$\mathbf{C}_{695}$(+484)TDVAK(M)
Q9X5M1	LysA (MSMEG_4958)	Diaminopimelate decarboxylase	Cys442 not cons.	(R)YNLL$\mathbf{C}_{442}$(+484)RPAVVAVADGQAR(L)
A0QSR5	MSMEG_1574	Glutamate decarboxylase	Cys344 not cons.	(R)AGYTQVMQ$\mathbf{C}_{344}$(+484)LSQTAR(W)
A0QZX1	HisG (MSMEG_4180)	ATP phosphoribosyltransferase	Cys179 not cons.	(R)TLGLHNLVAFGDPL$\mathbf{C}_{179}$(+484)ESEAVLIER(D)
A0QUX8	IlvC (MSMEG_2374)	Ketol-acid reductoisomerase	**Cys150 cons.**	(K)GVP$\mathbf{C}_{150}$(+484)LIAIDQDPKGEGQALALSYAAAIGGAR(A)
Metabolism of coenzymes and nucleotides				
A0QZ17	CobN (MSMEG_3864)	Cobaltochelatase, CobN subunit	Cys787 not cons.	(R)LAGTE$\mathbf{C}_{787}$(+484)EIDQVLR(A)
A0QQX8	UmaA (MSMEG_0913)	Methoxy mycolic acid synthase 1	**Cys76 cons. ; S-AdoMet binding**	(K)LDLKPGMTLLDVG$\mathbf{C}_{76}$(484)GWGGALER(A)
A0R7G6	Ino1 (MSMEG_6904)	Inositol-3-phosphate synthase	**Cys18 cons.**	(R)VAIVGVGN$\mathbf{C}_{18}$(+484)ASSLVQGVQYYR(N)
A0R061	YfhF2 (MSMEG_4272)	HesB/YadR/YfhF family protein	**Cys47 cons.**	(R)IAVQPGG$\mathbf{C}_{47}$(+484)AGLR(Y)
A0R1P5	MSMEG_4827	Aminoglycoside phosphotransferase	Cys44 not cons.	(R)FLSGGTQNVIYEL$\mathbf{C}_{44}$(+484)R(G)
A0QQL0	ThiG (MSMEG_0793)	Thiazole synthase	**Cys75 cons.**	(R)LGIAALPNTAG$\mathbf{C}_{75}$(+484)R(G)
A0QUZ6	MSMEG_2394	Cys/Met metabolism pyridoxal-phosphate-dependent enzyme	Cys212 not cons.	(K)SLSGHSDLIAGYVAG$\mathbf{C}_{212}$(+484)QPELAER(I)
A0QS64	Mkl (MSMEG_1366)	ABC transporter, ATP-binding protein	Cys71 not cons.	(R)GSIVIDGTDILQ$\mathbf{C}_{71}$(+484)SAK(E)
P0CH00	NrdE2 (MSMEG_2299)	Ribonucleoside-diphosphate reductase subunit alpha 2	**Cys396 cons. ; active site**	(K)ITHSNL$\mathbf{C}_{396}$(+484)SEILQVSTPSEFNDDLSYAK(V)
A0QSU3	GuaB (MSMEG_1602)	Inosine-5'-monophosphate dehydrogenase	**Cys325 cons. ; active site**	(K)VGVGPGSI$\mathbf{C}_{325}$(+484)TTR(V)
A0QYE8	GuaB2 (MSMEG_3634)	IMP dehydrogenase family protein	**Cys302 cons. ; thioimidate**	(K)VGVGPGAm$\mathbf{C}_{302}$(+484)TTR(M)
A0R4I0	PurC (MSMEG_5841)	Phosphoribosylaminoimidazole-succinocarboxamide synthase	Cys125 not cons.	(K)TGTV$\mathbf{C}_{125}$(+484)GIALPPGLGEASKFDEPLFTPATK(A)
A0QVQ5	Pnp (MSMEG_2656)	Polyribonucleotide nucleotidyltransferase	Cys248 not cons.	(K)AL$\mathbf{C}_{248}$(+484)AAQQELADR(A)

Protein biosynthesis and quality control

A0QSD1	RplC (MSMEG_1436)	50S ribosomal protein L3	**Cys154 cons.**	(R)RPGSIGG**C$_{154}$**(+484)ATPGR(V)
A0QSL5	RpsM (MSMEG_1521)	30S ribosomal protein S13	**Cys86 cons.**	(R)KIEIG**C$_{86}$**(+484)YQGLR(H)
A0R7F7	RpsR2 (MSMEG_6895)	30S ribosomal protein S18 2	**Cys57 cons.**	(R)VTGN**C$_{57}$**(+484)VQHQR(D)
A0QUW9	GatC (MSMEG_2364)	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	Cys71 not cons.	(K)DVNVTRPDTVQP**C$_{71}$**(+484)LTQDEALAAAPK(A)
A0QYT3	PheT (MSMEG_3777)	Phenylalanine--tRNA ligase beta subunit	**Cys89 cons.**	(K)VDVGDQNVDGAPRDIV**C$_{89}$**(+484)GAR(N)
A0QQQ5	Lon1 (MSMEG_0839)	ATP-dependent protease La (LON) domain subfamily protein	Cys72 not cons.	(R)IID**C$_{72}$**(+484)QNLGANR(Y)
A0QTP6	MSMEG_1918	Sensor histidine kinase	Cys421 not cons.	(K)TEQG**C$_{421}$**(+484)VTIK(A)
A0QWX7	OpcA (MSMEG_3100)	OpcA protein	Cys157 not cons.	(R)ITDATNGTDPLA**C$_{157}$**(+484)IK(S)
A0QQP8	Def (MSMEG_0832)	Peptide deformylase	Cys68 not cons.	(R)LFVYD**C$_{68}$**(+484)APTR(G)

DNA replication and transcription regulation

A0QRV8	MSMEG_1255	ATP-dependent DNA helicase, UvrD/REP family	Cys361 not cons.	(R)AGDGAAAA**C$_{361}$**(+484)ER(V)
A0QS66	RpoC (MSMEG_1368)	DNA-directed RNA polymerase subunit beta'	Cys1241 not cons.	(R)VLTDAAIN**C$_{1241}$**(+484)R(S)
A0R0Z8	Rnz2 (MSMEG_4568)	Ribonuclease Z	**Cys215 cons.**	(R)IRDI**C$_{215}$**(+484)DYHSSVQEAAATAAR(A)
A0QVZ3	IdeR (MSMEG_2750)	Iron-dependent repressor IdeR	**Cys102 cons.**	(R)LLVDVIGLPWEDVHAEA**C$_{102}$**(+484)R(W)
A0R217	MSMEG_4953	Putative transcriptional regulator	Cys21 not cons.	(R)LIDAAET**C$_{21}$**(+484)LR(A)
A0QP04	TetR (MSMEG_0227)	Transcriptional regulator, TetR family	Cys143 not cons.	(R)LTAILLGPEPGTA**C$_{143}$**(+484)R(V)

DNA replication and transcription regulation

A0R3Y5	EchA6 (MSMEG_5639)	Enoyl-CoA hydratase	Cys26 not cons.	(R)NALN**C$_{26}$**(+484)ELVDSLR(E)
A0QPV4	MSMEG_0531	Acyl-CoA dehydrogenase	Cys285 not cons.	(R)AAYEYALDYA**C$_{285}$**(+484)QR(E)
A0R461	MSMEG_5715	Luciferase-like protein	Cys83 not cons.	(K)LGQM**C$_{83}$**(+484)TAMSYR(N)
A0R5I9	MSMEG_6208	Acyl-CoA thioesterase	Cys21 not cons.	(R)DGDVF**C$_{21}$**(+484)IREPEPNTIER(L)
A0QTE7	AccD5 (MSMEG_1813)	Propionyl-CoA carboxylase beta chain	Cys356 not cons.	(R)VEGRPVGIVANQPTQFAG**C$_{356}$**(+484)LDINASEK(A)
A0R0B6	AccD6 (MSMEG_4329)	Acetyl/propionyl-CoA carboxylase (Beta subunit)	Cys294 not cons.	(R)LGG**C$_{294}$**(+484)LNSESAEK(S)

Enzymes involved in detoxification

A0R5M3	Adh2 (MSMEG_6242)	Alcohol dehydrogenase, iron-containing	**Cys398 cons.**	(R)AISEHIQDDW**C$_{398}$**(+484)TPGNPR(E)
A0R1V9	AhpC (MSMEG_4891)	Alkyl hydroperoxide reductase subunit C	**Cys61 cons. ; peroxidatic Cys**	(K)DFTFV**C$_{61}$**(+484)PTEIAAFGK(L)
A0QV23	OsmC (MSMEG_2421)	OsmC family protein	**Cys116 cons.**	(R)AVDQV**C$_{116}$**(+484)TVGR(T)
A0QXZ5	Tpx (MSMEG_3479)	Probable thiol peroxidase	**Cys60 cons. ; peroxidatic Cys**	(K)SVLLNIFPSVDTPV**C$_{60}$**(+484)ATSVR(T)

Proteins with other/unknown functions

A0R111	RsfS (MSMEG_4580)	Ribosomal silencing factor RsfS	Cys111 not cons.	(R)LWKD**C$_{111}$**(+484)PQVPVDLDHRGEASEDGAEAQE(-)
A0QSK6	RmlB (MSMEG_1512)	dTDP-glucose 4,6-dehydratase	Cys242 not cons.	(R)TYLIGAE**C$_{242}$**(+484)ER(N)
A0QV25	Smc (MSMEG_2423)	Chromosome partition protein Smc	Cys847 not cons.	(R)VAAAVSE**C$_{847}$**(+484)GR(Q)
A0QQA3	MSMEG_0683	Uncharacterized protein	Cys78 not cons.	(R)MYVHDGVVVAEQTIT**C$_{78}$**(+484)ADGSTAAGASAFR(V)
A0QQC2	MSMEG_0703	Uncharacterized protein	Cys26 not cons.	(R)ATGPDAPTL**C$_{26}$**(+484)EGWTTR(D)
A0QY84	MSMEG_3569	Uncharacterized protein	Cys18 not cons.	(R)ADDAW**C$_{18}$**(+484)AR(M)
A0R2Q6	MSMEG_5198	Carnitinyl-CoA dehydratase	Cys202 not cons.	(R)IT**C$_{202}$**(+484)NAPLAVQASK(R)
A0R3A6	PrpE (MSMEG_5404)	AMP-dependent synthetase and ligase	**Cys165 cons.**	(K)VVVAAS**C$_{165}$**(+484)GIEPTR(I)

Table S1. Summary of the discovered targets for protein S-mycothiolations in *Actinomycetes.* Selected S-mycothiolated proteins were identified under NaOCl stress in *Corynebacterium glutamicum, Corynebacterium diphtheriae* and *Mycobacterium smegmatis* according to previous studies (1,2,3). The S-mycothiolated proteins were identified using shotgun liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis based on a mass increase of 484 Da for MSH at Cys peptides. Conserved Cys residues were identified using the conserved domain database (CDD) (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and indicated in bold-face. The table lists the UniprotKB accession numbers (http://www.uniprot.org/), protein name (DIP-ID, MSMEG-ID), function, the S-thiolated Cys peptide sequence, and information about conservation of Cys residues according to CDD.

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Table S2. Summary of the discovered targets for protein *S*-bacillithiolations in *Bacillus* and *Staphylococcus* species.

Accession	Protein	Function	Conservation/function of Cys-SSB	Peptide-SSB Sequence
Bacillus subtilis [4]				
Metabolism of carbohydrates and amino acids				
P80877	MetE	Methionine synthase	**Cys647,730 cons. ; Zn-binding active site**	(R)FWVNPD$\mathbf{C_{730}}$(+396)GLK(T)
				(R)VPSTEEMYNIIVDALAV$\mathbf{C_{719}}$(+396)PTDR(F)
P42318	YxjG	methionine synthase homolog	**Cys346 cons. ; Zn-binding active site**	(R)YVSLDQLC$_{341}$(+IAM)LSPQ$\mathbf{C_{346}}$(+396)GFASTEEGNK(L)
P37487	PpaC	manganese-dependent inorganic pyrophosphatase	**Cys158 cons.**	(K)SPT$\mathbf{C_{158}}$(+396)TDQDVAAAK(E)
P35136	SerA	D-3-phosphoglycerate dehydrogenase (serine biosynthesis)	**Cys410 cons. in Bacillus species**	(K)ISSSESGYDN$\mathbf{C_{410}}$(+396)ISVK(V)
P39912	AroA	Phospho-2-dehydro-3-deoxyheptonate aldolase/ chorismate mutase	**Cys126 cons.**	(R)FIVGP$\mathbf{C_{126}}$(+396)AVESYEQVAEVAAAAK(K)
Metabolism of coenzymes and nucleotides				
P21879	GuaB	Inosine-5'-monophosphate dehydrogenase	**Cys308 cons. ; active site, Thioimidate intermediate**	(K)VGIGPGSI$\mathbf{C_{308}}$(+396)TTR(V)
Protein biosynthesis and quality control				
P18255	ThrS	Threonine--tRNA ligase 1	**Cys338 cons. ; Zn-binding,** Cys573 not cons.	(R)LQ$\mathbf{C_{573}}$(+396)EGLR(V)
O31678	QueF	NADPH-dependent 7-cyano-7-deazaguanine reductase	**Cys56 cons. ; Zn-binding active site**	(K)FNC$_{49}$(+IAM)PEFTSL$\mathbf{C_{56}}$(+396)PK(T)
P33166	Tuf	Elongation factor Tu	**Cys83 cons. in GTP binding site (82-86)**	(R)HYAHVD$\mathbf{C_{83}}$(+396)PGHADYVK(N)
Enzymes involved in detoxification				
P54170	YphP	UPF0403 protein, bacilliredoxin (reduction of protein-SSB)	**Cys53 cons. ; active site**	(K)AEGTTLVVVNSV$\mathbf{C_{53}}$(+396)GC$_{55}$(+IAM)AAGLAR(P)
O34777	OhrR	organic hydroperoxide resistance repressor	**Cys15 cons. ; redox sensing**	(K)LENQL$\mathbf{C_{15}}$(+396)FLLYASSR(E)
Bacillus amyloliquefaciens [4]				
Metabolism of carbohydrates and amino acids				
A7Z3U1	MetE (RBAM_013040)	Methionine synthase	**Cys730 cons. ; Zn-binding active site**	(R)FWVNPD$\mathbf{C_{730}}$(+396)GLK(T)
				(R)VPATEEIYQIIDDALEV$\mathbf{C_{719}}$(+396)PTDR(F)
A7Z657	SerA (RBAM_021220)	D-3-phosphoglycerate dehydrogenase (serine biosynthesis)	**Cys410 cons.**	(K)ISSNESGYDN$\mathbf{C_{410}}$(+396)ISVK(V)
A7Z7R9	AroA (RBAM_026870)	Phospho-2-dehydro-3-deoxyheptonate aldolase/ chorismate mutase	**Cys126 cons.**	(R)FIVGP$\mathbf{C_{126}}$(+396)AVESYEQVAEVAAAAK(K)
A7Z612	AroE (RBAM_020760)	3-phosphoshikimate 1-carboxyvinyltransferase	Cys79 not cons.	(K)GIDAL$\mathbf{C_{79}}$(+396)EPDSLLDVGNSGTTIR(L)
Metabolism of coenzymes and nucleotides				
A7Z0D1	GuaB (RBAM_000120)	Inosine-5'-monophosphate dehydrogenase	**Cys308 cons. ; active site, Thioimidate intermediate**	(K)VGIGPGSI$\mathbf{C_{308}}$(+396)TTR(V)
A7Z7N3	ThiI (RBAM_026510)	Probable tRNA sulfurtransferase (YtbJ homolog) (Thiamine biosynthesis)	Cys81 not cons.	(K)$\mathbf{C_{81}}$(+396)ESKLEDIK(K)
Enzymes involved in Fe-S cluster assembly				
F4EAC4	SufA (BAMTA208_16285)	Chaperone involved in Fe-S cluster assembly	**Cys102,104,120 cons ; Fe-S-cluster**	(K)NAGTPEE$\mathbf{C_{120}}$(+396)(-)
Enzymes involved in Cell wall biosynthesis				
A7Z1M1	Alr (RBAM_004980)	Alanine racemase 1	Cys63 not cons.	(K)AALEAGAS$\mathbf{C_{63}}$(+396)LAVAILDEAISLR(K)
Enzymes involved in detoxification				
A7Z8C1	YumC (RBAM_029160)	Ferredoxin--NADP reductase 2 ⍰	**Cys85 cons. ; probably active site**	(K)FDQTI$\mathbf{C_{85}}$(+396)LEQAVESVEK(Q)
Bacillus pumilus [4]				
Metabolism of carbohydrates and amino acids				
B4AEV7	MetE (BAT_2841)	Methionine synthase	**Cys730 Zn-binding active site**	(R)FWVNPD$\mathbf{C_{730}}$(+396)GLK(T)
A8FIT1	YwaA (BPUM_3500)	Branched-chain-amino-acid aminotransferase	Cys104 S-cysteinylated by diamide in *B.subtilis*	(R)L$\mathbf{C_{104}}$(+396)IPQIDTETVLEGLNELIR(I)
A8FGB2	AroA (BPUM_2621)	Phospho-2-dehydro-3-deoxyheptonate aldolase/ chorismate mutase	**Cys126 cons.**	(R)FIVGP$\mathbf{C_{126}}$(+396)AVESYEQVAEVAAAAK(Q)
B4AF61	MetI (BAT_2947)	Cystathionine gamma-synthase (CGS)	**Cys335 cons.**	(R)IANGV$\mathbf{C_{369}}$(+396)NK(L)
B4ANL1	LuxS (BAT_1217)	S-ribosylhomocysteine lyase (homocysteine biosynthesis)	**Cys84, 126 cons.: Cys84 catalytic; Cys126 Fe-binding site**	(R)F$\mathbf{C_{41}}$(+396)QPNK(Q)
A8FG49	CitZ (BPUM_2556)	Citrate (Si)-synthase II (TCA cyclus)	**Cys195 cons.**	(R)V$\mathbf{C_{195}}$(+396)VATLSDIYSGVTAAIGALK(G)
Metabolism of coenzymes and nucleotides				
B4AF80	ThiG (BAT_2967)	Thiazole synthase (Thiamine biosynthesis)	**Cys 92 cons.**	(K)VEVIG$\mathbf{C_{102}}$(+396)SR(S)
Protein biosynthesis and quality control				
B4AN33	Tuf (BAT_4036)	Elongation factor Tu	**Cys83 cons. in GTP binding site (82-86)**	(R)HYAHVD$\mathbf{C_{83}}$(+396)PGHADYVK(N)
Enzymes involved in detoxification				
B4AFT4	KatX2 (BAT_3178)	Catalase	no Cys cons.	(K)LLAI$\mathbf{C_{461}}$(+396)NFYR(A)
A8FCN5	YkuU (BPUM_1319)	2-Cys Peroxiredoxin YkuU	**Cys52, 169 cons.; C52 active site; Cys52-Cys169 catalytic disulfide**	(R)VLQALQTGGL$\mathbf{C_{169}}$(+396)PANWKPGQK(T)
Proteins with other/unknown functions				
A8FHX4	FliW (BPUM_3187)	Flagellar assembly factor FliW	Cys144 not cons.	(K)HLLEVASS$\mathbf{C_{144}}$(+396)
Bacillus megaterium [4]				
Metabolism of carbohydrates and amino acids				
D5DIR6	MetE (BMD_3527)	Methionine synthase	**Cys732 cons. ; Zn-binding active site**	(R)ALQVLDPALFWINPD$\mathbf{C_{732}}$(+396)GLK(T)
D5DER3	PpaC (BMD_2499)	manganese-dependent inorganic pyrophosphatase	**Cys158 cons. ; Thioimidate intermediate**	(K)SPT$\mathbf{C_{158}}$(+396)TDQDVAAAK(E)
Metabolism of coenzymes and nucleotides				
D5D924	GuaB (BMD_0013)	Inosine-5'-monophosphate dehydrogenase	**Cys308 cons. ; active site, Thioimidate intermediate**	(K)VGIGPGSI$\mathbf{C_{308}}$(+396)TTR(V)
Staphylococcus carnosus [4]				
Metabolism of carbohydrates and amino acids				
B9DN54	AroA (Sca_1344)	Phospho-2-dehydro-3-deoxyheptonate aldolase/ chorismate mutase	**Cys83, 125 cons.: Cys83 essentiell for catalysis; Cys125 Fe-binding site**	(K)SFIFGP$\mathbf{C_{125}}$(+396)SVESQEQVDK(V)
B9DN51	Ldh (Sca_1369)	L-lactate dehydrogenase	**Cys72 cons.**	(K)AGSYED$\mathbf{C_{72}}$(+396)SDADLVVITAGAPQKPGETR(L)
Metabolism of coenzymes and nucleotides				
B9DM03	GuaB (Sca_0049)	Inosine-5'-monophosphate dehydrogenase	**Cys307 cons. ; active site**	(K)VGIGPGSI$\mathbf{C_{307}}$(+396)TTR(I)
Q9RGS6	ThiM (Sca_1594)	Hydroxyethylthiazole kinase (Thiamine biosynthesis)	Cys258 not cons.	(R)IDSDAVAEN$\mathbf{C_{258}}$(+396)NLEEVK(-)
B9DN59	YtpR (Sca_1349)	similar to Phe-tRNA synthetase (YtpR homolog)	**Cys116,126,170 cons.**	(K)VNVGNEELQIV$\mathbf{C_{126}}$(+396)GAPNVEAGQK(V)
B9DM56	Adk (Sca_1714)	Adenylate kinase	**Cys 130, 133, 150 cons. ; Zn finger motif**	(K)VDGV$\mathbf{C_{150}}$(+396)DLDGGK(L)
Protein biosynthesis and quality control				
B9DKV8	Tuf (Sca_0207)	Elongation factor Tu	**Cys82 cons. in GTP binding site (81-85)**	(R)HYAHVD$\mathbf{C_{82}}$(+396)PGHADYVK(N)
B9DNK0	DnaK (Sca_1202)	Chaperone protein DnaK	no Cys cons.	(K)IIGIDLGTTNS$\mathbf{C_{15}}$(+396)VAVLEGDEPK(V)
B9DNK1	GrpE (Sca_1203)	Chaperone protein GrpE	no Cys cons.	(K)TYQAQ$\mathbf{C_{95}}$(+396)VLTDILPTIDNIER(A)
Enzymes involved in detoxification				
B9DNY0	YphP (Sca_1072)	UPF0403 protein, bacilliredoxin (reduction of protein-SSB)	**Cys54 cons. ; active site (CxC-motiv)**	(K)NVGKDETTFVVINST$\mathbf{C_{54}}$(+396)GC$_{56}$(+IAM)AAGLAR(P)
B9DJN8	YtxJ (Sca_0389)	DUF2847 protein, bacilliredoxin (reduction of protein-SSB)	**Cys30 cons. ; active site (TCIP-motiv)**	(K)HSNT$\mathbf{C_{30}}$(+396)PISANAYDQFNK(F)
Proteins with other/unknown functions				
B9DLW2	SceB (Sca_1790)	SceB precursor	**Cys166 cons**	(R)TSSGANYYTAGQ$\mathbf{C_{166}}$(+396)TYYAFDR(A)
B9DQ05	TypA (Sca_0735)	Putative GTP-binding protein family protein TypA (YlaG homolog)	Cys408 not cons.	(R)VQ$\mathbf{C_{408}}$(+396)EVPQENAGAVIESLGQR(K)
B9DN38	Sca_1381	Putative transaldolase	**Cys200 cons.**	(R)ELLNVIQADEIGADIIT$\mathbf{C_{200}}$(+396)PSGVISK(I)
B9DML0	Sca_1554	Putative ABC transporter ATP-binding protein	no Cys cons.	(R)QIENC$_{583}$(+IAM)EAEIEA$\mathbf{C_{590}}$(+396)EQK(I)
B9DMD5	Sca_1625	Putative aldehyde dehydrogenase family protein (similar to GbsA betaine aldehyde dehydrogenase)	**Cys279 cons. ; active site**	(K)VYNNTGQV$\mathbf{C_{279}}$(+396)TAGTR(T)
Staphylococcus aureus [5]				
Metabolism of carbohydrates and amino acids				
A8Z1A0_STAAT	Gap	Glyceraldehyde-3-phosphate dehydrogenase	**Cys151 cons. ; active site (catalytic Cys)**	(K)TIVFNTNHQELDGSETVVSGAS$\mathbf{C_{151}}$(+396)TTNSLAPVAK(V)
PPAC_STAAT	PpaC	Mn-dependent inorganic pyrophosphatase	**Cys110 cons.**	(R)IANFETAGPL$\mathbf{C_{110}}$(+396)YR(A)
Metabolism of coenzymes and nucleotides				
A8Z0R0_STAAT	GuaB	Inosine-5'-monophosphate dehydrogenase	**Cys307 cons. ; active site, Thioimidate intermediate**	(K)VGIGPGSI$\mathbf{C_{307}}$(+396)TTR(V)
Protein biosynthesis and quality control				
RL36_STAAT	RpmJ	50S ribosomal protein L36	**Cys 11 cons.**	(K)VRPSVKPI$\mathbf{C_{11}}$(+396)EK(C)
Proteins with other/unknown functions				
A8YY87_STAAT	AldA	Aldehyde dehydrogenase	**Cys 279 cons. ; active site (catalytic Cys)**	(K)VVNNTGQV$\mathbf{C_{279}}$(+396)TAGTR(V)

Table S2: Summary of the identified proteins with S-bacillithiolated peptides in *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus megaterium, Staphylococcus carnosus* and *Staphylococcus aureus*. Selected S-bacillithiolated proteins were identified in different Bacillus and Staphylococcus species under NaOCl stress according to previous studies (4,5). The S-bacillithiolated proteins were identified using shotgun liquid chromatography tandem mass spectrometry (LC-MS/MS) based on a mass increase of 396 Da for BSH at Cys peptides. Conserved Cys residues were identified using the conserved domain database (CDD) (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and indicated in bold-face. The table lists the UniprotKB accession numbers (http://www.uniprot.org/), protein name and locus tag, function, the S-thiolated Cys peptide sequence, and information about conservation of Cys residues according to CDD.

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