Quantitative phosphoproteomics reveals the role of protein arginine phosphorylation in the bacterial stress response

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Supplemental Information

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SUPPLEMENTAL LEGENDS:

Supplemental Table 1: Arginine-phosphorylated peptides identified in *B. subtilis ΔywlE*

The attached Supplemental Table 1 (in Excel format) depicts all identified arginine-phosphorylated peptides identified in the iTRAQ study of *B. subtilis ΔywlE*, grouped in accordance to funcional class. Identification scores were obtained from the database search using the MASCOT algorithm and an identification score above 16 was determined to give a false positive rate of < 1% on the PSM level. The phosphoRS site probability determines the likeliness of phosphorylation at the residue determined by the search algorithm. Individual site probabilities for each putative phospho-acceptor site in the indentified peptide are provided in the respective column. Relative protein and peptide abundance rations were determined from the area of the iTRAQ reporter ions in the corresponding HCD-MS/MS spectra. All phosphopeptide ratios were divided by the respecitve protein abundance ratios (listed in Table S5). Thereby, upregulation of the identified phosphorylations, independently of protein abundance changes, can be determined. Page two of this table contains the original peptide abundance ratios before normalization to the protein abundance as well as more identifcation details such as precursor mass accuracy. Identified Ser, Thr and Tyr phosphorylations are shown in page three of Supplemental Table 1.

Supplemental Table 4: GO terms of arginine phosphorylated proteins at an FDR cutoff of 5%

The proteins identified with phosphoarginine modification were subdued to GO term enrichment analysis using the Blast2GO software. The attached Supplemental Table 4 (in Excel format) lists the associated over- or underrepresented GO terms at a FDR of 5%.

Supplemental Table 5: Protein abundances upon heat shock or oxidative stress relative to non-stressed *B. subtilis* cells

The attached Supplemental Table 5 lists relative protein abundances upon heat shock or oxidative stress for all identified proteins in SCX experiment and in the phosphoproteomic samples were calculated from respective unphosphorylated peptides at an FDR rate of <1% for peptide identification. Those abundances were used to normalize the phosphorylation site abundance on the protein abundance in Supplemental Table 1.

Supplemental Table 2: Protein phosphorylations in *B. subtilis* wild type strain

Protein phosphorylation in wild type *B. subtilis* under non-stress conditions

Gene name	Protein			
rsbRB	RsbT co-antagonist protein rsbRB			
	Peptide sequence	**P-Site**	**MASCOT score**	**Peptide site probability**
	STALLPLVGDIDTER	S186	36	S(1): 0.0, T(2): 0.0, T(13): 100.0, R(15): 0.0
rsbV	Anti-sigma-B factor antagonist			
	DVSYMDSTGLGVFVGTFK	S56	41	S(3): 0.0, Y(4): 0.0, S(7): 100.0, T(8): 0.0, T(16): 0.0
ytcI	Uncharacterized acyl--CoA ligase ytcI			
	YLELLNRYK	S260	36	

Supplemental Table 3: Protein phosphorylation in *B. subtilis* Δ*mcsB*Δ*ywle*

Protein phosphorylation in *B. subtilis* Δ*mcsB*/Δ*ywle* under non-stress conditions

Gene name	**Protein**			
cotG	Spore coat protein G			
	Peptide sequence	**P-Site**	**MASCOT score**	**Peptide site probability**
	GHYSHSDIEEAVKSAKK	S15	69	H(2): 0.0, Y(3): 0.0, S(4): 0.0, H(5): 0.0, S(6): 0.0, S(14): 100.0
cotB	Spore coat protein B			
	SPGYSSSIKSSGK	S355	67	S(1): 0.0, Y(4): 0.0, S(5): 0.5, S(6): 0.5, S(7): 0.5, S(10): 97.9, S(11): 0.5
	SSGKQKEDYSYETIVR	S356	67	S(1): 0.3, S(2): 99.7, Y(9): 0.0, S(10): 0.0, Y(11): 0.0, T(13): 0.0, R(16): 0.0
rsbRB	RsbT co-antagonist protein rsbRB			
	STALLPLVGDIDTER	S186	58	S(1): 0.0, T(2): 0.0, T(13): 100.0, R(15): 0.0

Protein phosphorylation in *B. subtilis* Δ*mcsB*/Δ*ywle* upon heat shock

Gene name	**Protein**			
cotG	Spore coat protein G			
	Peptide sequence	**P-Site**	**MASCOT score**	**Peptide site probability**
	GHYSHSDIEEAVKSAK	S15	47	H(2): 0.0, Y(3): 0.0, S(4): 0.0, H(5): 0.0, S(6): 0.0, S(14): 100.0
	GHYSHSDIEEAVKSAKK	S15	28	H(2): 0.0, Y(3): 0.0, S(4): 0.0, H(5): 0.0, S(6): 0.0, S(14): 100.0
cotB	Spore coat protein B			
	SPGYSSSIKSSGK	S355	74	S(1): 0.0, Y(4): 0.0, S(5): 0.0, S(6): 0.0, S(7): 0.0, S(10): 99.6, S(11): 0.4
	SSGKQKEDYSYETIVR	S356	46	S(1): 50.0, S(2): 50.0, Y(9): 0.0, S(10): 0.0, Y(11): 0.0, T(13): 0.0, R(16): 0.0
rsbRB	RsbT co-antagonist protein rsbRB			
	STALLPLVGDIDTER	T186	60	S(1): 0.0, T(2): 0.0, T(13): 100.0, R(15): 0.0
	STALLPLVGDIDTERAK	T186	64	S(1): 0.0, T(2): 0.0, T(13): 100.0, R(15): 0.0
rsbV	Anti-sigma-B factor antagonist			
	DVSYMDSTGLGVFVGTFK	S56	56	S(3): 0.0, Y(4): 0.0, S(7): 98.8, T(8): 1.2, T(16): 0.0
ywqD	Tyrosine-protein kinase ywqD			
	HSEYGYYGTKDNFMQK	Y228	45	H(1): 0.0, S(2): 0.0, Y(4): 0.0, Y(6): 0.0, Y(7): 94.9, T(9): 5.1
atpA	ATP synthase subunit alpha			
	SIKAEEISTLIK	S1	50	S(1): 100.0, S(8): 0.0, T(9): 0.0
ycbU	Uncharacterized aminotransferase ycbU			
	ASETAGLLKKK	S323 orT325	29	S(2): 50.0, T(4): 50.0
yvyG	Uncharacterized protein yvyG			
	YIQAITQTEDDRIK	Y49	60	Y(1): 100.0, T(6): 0.0, T(8): 0.0, R(12): 0.0

Supplemental Table 6: Phosphopeptides identified in *B. subtilis* wild type submitted to YwlE inhibition with pervanadate and subsequent heat shock

Supplemental Table 6 shows identified arginine-phosphorylated peptides of a *B. subtilis* wild type cell lysate upon irreversible inhibition of YwlE activity by the cell membrane-permeable phosphatase inhibitor sodium pervanadate. Five minutes after adding the inhibitor (final concentration 400 µM), cells were exposed to heat shock at 50 °C and phosphosites were determined by LC-MS/MS without iTRAQ quantification. The corresponding unstressed sample did not yield arginine phosphorylations.

Gene name	Peptide sequence	pArg site	pArg identified in *B. subtilis ΔywlE*	MASCOT score	phosphoRS score	phosphoRS probability
Protein Quality Control						
clpC	EVESLIGRGQEMSQTIHYTPR	R70	Yes	53	61.2	0.980
	EVESLIGRGQEMSQTIHYTPR (MSO)	R70	Yes	34	134.4	0.999
groEL	GRNVVLEK	R35	Yes	28	3.5	1.000
Transcriptional Regulation						
ctsR	FTSERGYIVESK	R55	Yes	31	117.9	0988
hrcA	SAQPVGSRTLSK	R7	No	53	139.1	0.498
	THSSSGRTVPSEK	R64	Yes	28	24.7	0.746
Ribosome						
rplN	MIQQETRLK	R7	Yes	28	158.2	0.996
	MIQQETRLK (MSO)	R7	Yes	22	83.6	0.935
rpmGA	NNPDRVEFK	R29	Yes	34	162.0	1.000
	NNPDRVEFKK	R29	Yes	32	n.a.	n.a.
rpsH	IRNANMVR	R15	No	27	115.2	1.000
	YGQNNERVITGLK	R72	No	55	158.0	0.995
rplQ	AKELRSVVEK	R41	No	65	217.0	1.000
tufA	GTVATGRVER	R232	Yes	36	35.2	0.786
Other Proteins						
gudB	AADRNTGHTEEDKLDVLK	R5	Yes	42	323.2	0.989
	IFTGYRAQHNDSVGPTK	R69	Yes	57	n.a.	n.a.
	GGIRFHPNVTEK	R84	Yes	39	184.3	0.5
odhA	LTGQDSERGTFAQR	R624	Yes	52	132.2	0.926
rbsA	TASPETHARHLSGGNQQK	R392	Yes	42	n.a.	n.a.
ahpC	AAQYVRQNPGEVCPAK	R159	No	39	201.0	1.000