

SUPPLEMENTAL MATERIAL

MS/MS data acquired through WARP-LC were searched on MASCOT (Version 2.4.1) using ProteinScape (Version 3.0, Bruker Daltonics) with the search restricted to *Salmonella* on the UniProtKB/SwissProt database (status May 2013). This search was limited to tryptic peptides with variable methionine, histidine, and tryptophan oxidation, and MS/MS mass tolerance of ± 0.8 Da. The peptide mass tolerance was set to ± 100 ppm. The peptide charge was defined as +1 and two miscleavages were allowed. A peptide decoy database as well as percolator algorithm were used to improve significance of search results.

The analysis of the acquired data by searching the UniProtKB/SwissProt database yielded a number of proteins for each sample, ranging from 239 to 322 proteins. As a comparison, the number of protein peaks found by classical whole cell protein MALDI-TOF MS ranged only from 51 to 59.

Table S1 gives an overview of the biomarker peptides identified for the three subspecies *Salmonella enterica* subsp. *arizona*, *S. enterica* subsp. *enterica*, and *S. enterica* subsp. *houtenae* which were assigned to *Salmonella* proteins in the MASCOT search on the UniProtKB/SwissProt database. Of note, no biomarker peptides could be annotated to *S. enterica* subsp. *houtenae*, since the UniProtKB/SwissProt database contained only two protein entries for this subspecies at the time of the analysis, namely the guanine nucleotide exchange factor SopE and the inositol phosphate phosphatase SopB, to none of which a biomarker peptide could be annotated.

<i>Salmonella</i> subspecies	Assigned protein	Protein mass (kDa)	Biomarker peptide mass (Da)	Amino acid exchange (subspecies)	Search parameters (\pm ppm) ^o
<i>S. arizonae</i>	6-phospho-gluconolactonase	36.4	3,004.52 \pm 0.03	A \rightarrow C, Y \rightarrow H, A \rightarrow V (ent) A \rightarrow C, Y \rightarrow H, A \rightarrow V, E \rightarrow A (hou)	25 50 100
<i>S. arizonae</i>	60 kDa chaperonin	57.3	1,602.88 \pm 0.01	N \rightarrow T (ent*, hou*)	25
<i>S. arizonae</i>	ATP-dependent protease ATP-binding subunit HslU	49.6	2,889.48 \pm 0.00	V \rightarrow I, T \rightarrow A (ent*, hou*)	25 50 100
<i>S. arizonae</i>	Cell division protein ZipA homolog	36.1	2,457.00 \pm 0.02	DA \rightarrow EE (ent*) S \rightarrow A, DA \rightarrow EE (hou*)	25
<i>S. arizonae</i>	Lysine-tRNA ligase	57.6	2,923.32 \pm 0.05	Q \rightarrow L (ent, hou)	50
<i>S. arizonae</i>	Molecular chaperone DnaK	69.3	2,464.23 \pm 0.00	M \rightarrow T (ent*, hou*)	25
<i>S. enterica</i>	50S ribosomal protein L17	14.4	2,551.20 \pm 0.02	(\rightarrow) \rightarrow S, T \rightarrow A (ari*, hou*)	25 50 100
			2,567.19 \pm 0.02 (oxidized)	(\rightarrow) \rightarrow S, T \rightarrow A (ari*, hou*)	25 50 100
<i>S. enterica</i>	Flagella synthesis protein FlgN	16.0	3,103.55 \pm 0.01	D \rightarrow N, S \rightarrow A (ari) D \rightarrow N (hou)	25 50 100
<i>S. enterica</i>	Glutamine-fructose-6-phosphate aminotransferase [isomerizing]	66.8	2,722.40 \pm 0.02	A \rightarrow S (ari, hou)	25 50 100
<i>S. enterica</i>	Glutaredoxin-1 Grx1	9.9	1,114.49 \pm 0.01	K \rightarrow N (ari, hou)	25
<i>S. enterica</i>	H-NS histone family	15.5	1,979.88 \pm 0.01	MD \rightarrow IN (ari) D \rightarrow G (hou)	25
<i>S. enterica</i>	Nucleoside diphosphate kinase	15.5	2,424.11 \pm 0.02	L \rightarrow F (ari*, hou*)	25
<i>S. enterica</i>	Outer membrane protein A	38.4	3,540.76 \pm 0.03	V \rightarrow T, P \rightarrow A, T \rightarrow Y (ari) V \rightarrow A, GP \rightarrow (\rightarrow)Y (hou)	25 50 100
			3,956.00 \pm 0.04 (missed cleavage)	V \rightarrow T, P \rightarrow A, T \rightarrow Y (ari) V \rightarrow A, GP \rightarrow (\rightarrow)Y (hou)	25 50 100
<i>S. enterica</i>	Probable thiol peroxidase	18.0	2,379.23 \pm 0.01	S \rightarrow N (ari) T \rightarrow A (hou)	25
<i>S. enterica</i>	Transaldolase 1	35.1	3,048.54 \pm 0.02	H \rightarrow N (ari, hou)	25 50 100

TABLE S1 Complete list of biomarker peptides assigned to *Salmonella* proteins in the MASCOT search on the UniProtKB/SwissProt database. The peptide masses are given as the arithmetic mean calculated from the values of the three replicates of the subspecies with the according standard deviation. All numbers were rounded to the second digit after the coma.

*: Sequence analogous to biomarker peptide observed. (-): missing amino acid. °: for all searches S/N = 10. ari: *S. enterica* subsp. *arizonae*, ent: *S. enterica* subsp. *enterica*, hou: *S. enterica* subsp. *houtenae*.

<i>Salmonella</i> subspecies	Assigned protein	Protein mass (kDa)	Biomarker peptide mass (Da)	Amino acid exchange (subspecies)	Search parameters (\pm ppm) ^o
<i>S. arizonae</i>	6-phospho-gluconolactonase	36.4	3,004.52 \pm 0.03	A \rightarrow C, Y \rightarrow H, A \rightarrow V (ent) A \rightarrow C, Y \rightarrow H, A \rightarrow V, E \rightarrow A (hou)	25 50 100
<i>S. arizonae</i>	60 kDa chaperonin	57.3	1,602.88 \pm 0.01	N \rightarrow T (ent*, hou*)	25
<i>S. arizonae</i>	Aminoacyl-histidine dipeptidase	52.5	2,704.27 \pm 0.03	K \rightarrow T (ent*, hou)	25 50 100
<i>S. arizonae</i>	Arsenate reductase	13.4	1,420.81 \pm 0.00	E \rightarrow D (ent*) E \rightarrow D, G \rightarrow S (hou*)	25
<i>S. arizonae</i>	ATP-dependent protease ATP-binding subunit HslU	49.6	2,889.48 \pm 0.00	V \rightarrow I, T \rightarrow A (ent*, hou*)	25 50 100
<i>S. arizonae</i>	Cell division protein DamX	45.3	2,643.06 \pm 0.01	V \rightarrow D (ent*, hou*)	25 50 100
<i>S. arizonae</i>	Chromosomal replication initiation protein	49.9	2,235.13 \pm 0.01	P \rightarrow S (ent, hou)	25
<i>S. arizonae</i>	DNA-binding transcriptional regulator PhoP	25.7	1,482.89 \pm 0.01	L \rightarrow S (ent*, hou*)	25
<i>S. arizonae</i>	Ferric uptake regulator	17.0	3,291.42 \pm 0.01	Q \rightarrow H (ent) E \rightarrow D, Q \rightarrow H (hou)	25 50
<i>S. arizonae</i>	Fructose-bisphosphate aldolase	38.1	2,914.46 \pm 0.01	S \rightarrow A (ent, hou*)	25 50 100
<i>S. arizonae</i>	Heat shock protein	17.7	2,955.56 \pm 0.01	S \rightarrow L (ent, hou)	25 50 100
<i>S. arizonae</i>	Hydrogenase nickel incorporation protein HypB	31.4	3,764.78 \pm 0.11	K \rightarrow N, T \rightarrow A (ent•, hou)	100
<i>S. arizonae</i>	Hypothetical protein SARI_01500	34.0	3,305.78 \pm 0.00	I \rightarrow V (ent*, hou*)	25 50
<i>S. arizonae</i>	Hypothetical protein SARI_03463	10.5	2,231.05 \pm 0.00	H \rightarrow Y (ent*, hou)	25
<i>S. arizonae</i>	Isoprenoid biosynthesis protein	22.9	2,747.45 \pm 0.02	E \rightarrow D, A \rightarrow S (ent*) A \rightarrow S (hou*)	25
			3,117.72 \pm 0.01 (missed cleavage)	E \rightarrow D, A \rightarrow S (ent*) A \rightarrow S (hou*)	25

<i>S. arizonae</i>	Membrane protein	15.7	3,051.48 ± 0.02	L→I, T→A (ent*) L→I, M→V, T→A (hou*)	25 50 100
<i>S. arizonae</i>	Molecular chaperone DnaK	69.3	2,464.23 ± 0.00	M→T (ent*, hou*)	25
<i>S. arizonae</i>	N-acetyltransferase	18.6	1,011.45 ± 0.00	R→K (ent, hou)	25
<i>S. arizonae</i>	N-ethylmaleimide reductase	39.5	2,590.17 ± 0.02	K→E, E→A (ent*) E→A (hou*)	25
			2,789.30 ± 0.01 (missed cleavage)	K→E, E→A (ent*) E→A (hou*)	25
<i>S. arizonae</i>	NADH dehydrogenase subunit G	100.0	3,009.44 ± 0.01	H→Q (ent) A→T, D→G, H→Q (hou)	25 50 100
<i>S. arizonae</i>	Outer-membrane lipoprotein carrier protein	22.5	2,725.27 ± 0.01	S→A (ent, hou)	25 50 100
<i>S. arizonae</i>	Outer membrane porin C	41.2	2,433.06 ± 0.01	N→S (ent*, hou*)	25
<i>S. arizonae</i>	Outer membrane protein A	38.6	1,221.67 ± 0.00	N→D (ent*, hou*)	25
<i>S. arizonae</i>	Peptidyl-prolyl cis-trans isomerase SurA	47.2	3,771.96 ± 0.03	N→S, G→E (ent, hou*)	25 50 100
<i>S. arizonae</i>	Phosphoenolpyruvate-protein phosphotransferase	63.3	3,073.57 ± 0.01	FI→YL (ent*, hou*)	25 50 100
<i>S. arizonae</i>	Pyruvate dehydrogenase subunit E1	99.6	2,649.29 ± 0.03	V→I (ent*, hou*)	25 50 100
<i>S. arizonae</i>	Ribonuclease E	120.0	3,602.85 ± 0.04	T→V, I→V (ent*) T→A, I→V (hou) T→A, T→A, I→V (hou*)	25 50
<i>S. enterica</i>	Cell division protein DamX	45.5	3,153.62 ± 0.03	TT→AP, TA→KV (ari•) A→T, T→K, A→T (hou)	25 50 100
<i>S. enterica</i>	CRISPR system CASCADE complex protein CasC	38.7	2,377.21 ± 0.03	not found (ari) YE→FQ, ING→VRD, LNV→IPA, VK→IT (hou)	25 50 100
<i>S. enterica</i>	Flagella synthesis protein FlgN	16.0	3,103.55 ± 0.01	D→N, S→A (ari) D→N (hou*)	25 50 100
<i>S. enterica</i>	Formate acetyltransferase 1	85.0	2,250.99 ± 0.01	D→E, K→T (ari, hou)	25
<i>S. enterica</i>	Fumarate hydratase	51.8	2,460.30 ± 0.01	P→S (ari) L→M (hou*)	25 50 100
<i>S. enterica</i>	Glutaredoxin-1 Grx1	9.9	1,114.49 ± 0.01	K→N (ari•, hou)	25

<i>S. enterica</i>	Glycogen phosphorylase	84.1	2,666.28 ± 0.03	K→E (ari*, hou)	25
<i>S. enterica</i>	H-NS histone family	15.5	1,979.88 ± 0.01	MD→IN (ari*) D→G (hou*)	25
<i>S. enterica</i>	Isoprenoid biosynthesis protein	22.8	2,749.45 ± 0.01	D→E, S→A (ari*) D→E (hou*)	25 50 100
<i>S. enterica</i>	N-ethylmaleimide reductase	39.4	2,860.34 ± 0.01	E→K, A→E (ari) E→K (hou)	25 50
<i>S. enterica</i>	NlpB/DapX lipoprotein	36.9	2,843.33 ± 0.01	N→S (ari, hou*)	25 50
<i>S. enterica</i>	Nucleoside diphosphate kinase	15.5	2,424.11 ± 0.02	L→F (ari*, hou*)	25
<i>S. enterica</i>	Outer membrane protein A	38.4	3,540.76 ± 0.03	V→T, P→A, T→Y (ari*) V→A, GP→(-)Y (hou*)	25 50 100
<i>S. enterica</i>	Peptidyl-prolyl cis-trans isomerase PpiD	58.2	2,187.04 ± 0.00	V→A, V→T (ari*) V→T (hou*)	25
<i>S. enterica</i>	Probable thiol peroxidase	18.0	2,379.23 ± 0.01	S→N (ari*) T→A (hou*)	25
<i>S. enterica</i>	Protease specific for phage lambda cII repressor	45.6	2,736.21 ± 0.02	N→K (ari, hou)	25
			2,892.31 ± 0.02 (missed cleavage)	N→K (ari, hou)	25
<i>S. enterica</i>	Putative outer membrane protein	12.8	3,224.54 ± 0.02	L→F, S→R, S→I, N→Y (ari*) S→T (hou*)	25 50 100
<i>S. enterica</i>	Ribonuclease E	119.3	2,706.37 ± 0.00	S→T (ari*) P→T, S→T (hou*)	25 50 100
			3,046.42 ± 0.02	T→A, S→T (ari*) T→A, H→Y (hou*)	25 50 100
			3,586.85 ± 0.02	V→T, V→I (ari*) V→A (hou) V→A, T→A (hou*)	25
<i>S. houtenae</i>	2,3,4,5-tetrahydropyridine-2,6-carboxylate N-succinyltransferase	29.8	972.40 ± 0.00	D→E (ari*, ent*)	25
<i>S. houtenae</i>	60 kDa chaperonin	57.2	2,980.25 ± 0.04	A→G (ari*, ent*)	25
			3,336.50 ± 0.02 (missed cleavage)	A→G (ari*, ent*)	25 50 100

<i>S. houtenae</i>	Cell division protein DamX	45.6	1,925.13 ± 0.01	VA→AT, RP→ST, PAA→(−) (−) (−) (ari*) P→T (ent)	25 50
<i>S. houtenae</i>	Cell division protein MukB	170.0	1,453.78 ± 0.01	T→S (ari, ent)	25
<i>S. houtenae</i>	Cytochrome d ubiquinol oxidase subunit 3	15.0	1,812.85 ± 0.01	G→S (ari*, ent*)	25
<i>S. houtenae</i>	DNA-binding transcriptional regulator KdgR	29.9	1,881.78 ± 0.01	E→A (ari*, ent*)	25 50
<i>S. houtenae</i>	Elongation factor G	77.4	3,062.52 ± 0.02	D→E (ari, ent*)	25 50 100
<i>S. houtenae</i>	Flagella synthesis protein FlgN	16.0	3,102.61 ± 0.05	S→A (ari) N→D (ent*)	50 100
<i>S. houtenae</i>	Hydrogenase formation protein HypD	41.1	2,240.03 ± 0.01	H→R (ari*, ent)	25
<i>S. houtenae</i>	Isoprenoid biosynthesis protein	22.9	2,763.46 ± 0.02	S→A (ari*) E→D (ent*)	25
<i>S. houtenae</i>	NAD(P)H:quinone oxidoreductase, type IV	20.9	2,703.34 ± 0.04	E→D (ari*, ent*)	50 100
<i>S. houtenae</i>	NlpB/DapX lipoprotein	36.9	2,504.25 ± 0.01	D→E, V→P (ari) V→A (ent*)	25
<i>S. houtenae</i>	Outer membrane protein A	38.4	3,521.70 ± 0.02	A→T, Y→GA, T→Y (ari*) A→V, Y→GP (ent*)	25 50 100
<i>S. houtenae</i>	Sigma-E factor negative regulatory protein	24.2	2,662.39 ± 0.01	L→Q (ari*, ent*)	25 50 100
<i>S. houtenae</i>	Universal stress protein G	15.8	1,090.67 ± 0.00	S→T (ari*, ent*)	25
<i>S. houtenae</i>	Valyl-tRNA synthetase	108.1	1,056.65 ± 0.00	V→I (ari, ent*)	25

TABLE S2 Complete list of biomarker peptides assigned to *Salmonella* proteins in the MASCOT search on the NCBI database. The peptide masses are given as the arithmetic mean calculated from the values of the three replicates of the subspecies with the according standard deviation. All numbers were rounded to the second digit after the coma.

*: Sequence analogous to biomarker peptide observed. •: shorter or longer peptide sequence containing at least one relevant amino acid exchange was observed. (-): missing amino acid. °: for all searches S/N = 10. ari: *S. enterica* subsp. *arizona*, ent: *S. enterica* subsp. *enterica*, hou: *S. enterica* subsp. *houtenae*.