

Induction and identification of disulfide-intact and disulfide-reduced β -subunit of Shiga toxin 2 from *Escherichia coli* O157:H7 using MALDI-TOF-TOF-MS/MS and top-down proteomics

Clifton K. Fagerquist* and Omar Sultan

Western Regional Research Center, Agricultural Research Service,

U. S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710

Running title: Top-down proteomic ID of β -Stx2 by MALDI-TOF-TOF-MS/MS

**Correspondence to:* C. K. Fagerquist, Western Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710, USA. E-mail: clifton.fagerquist@ars.usda.gov. Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over other of a similar nature not mentioned. This article is a US Government work and is in the public domain in the USA.

Figure S-1

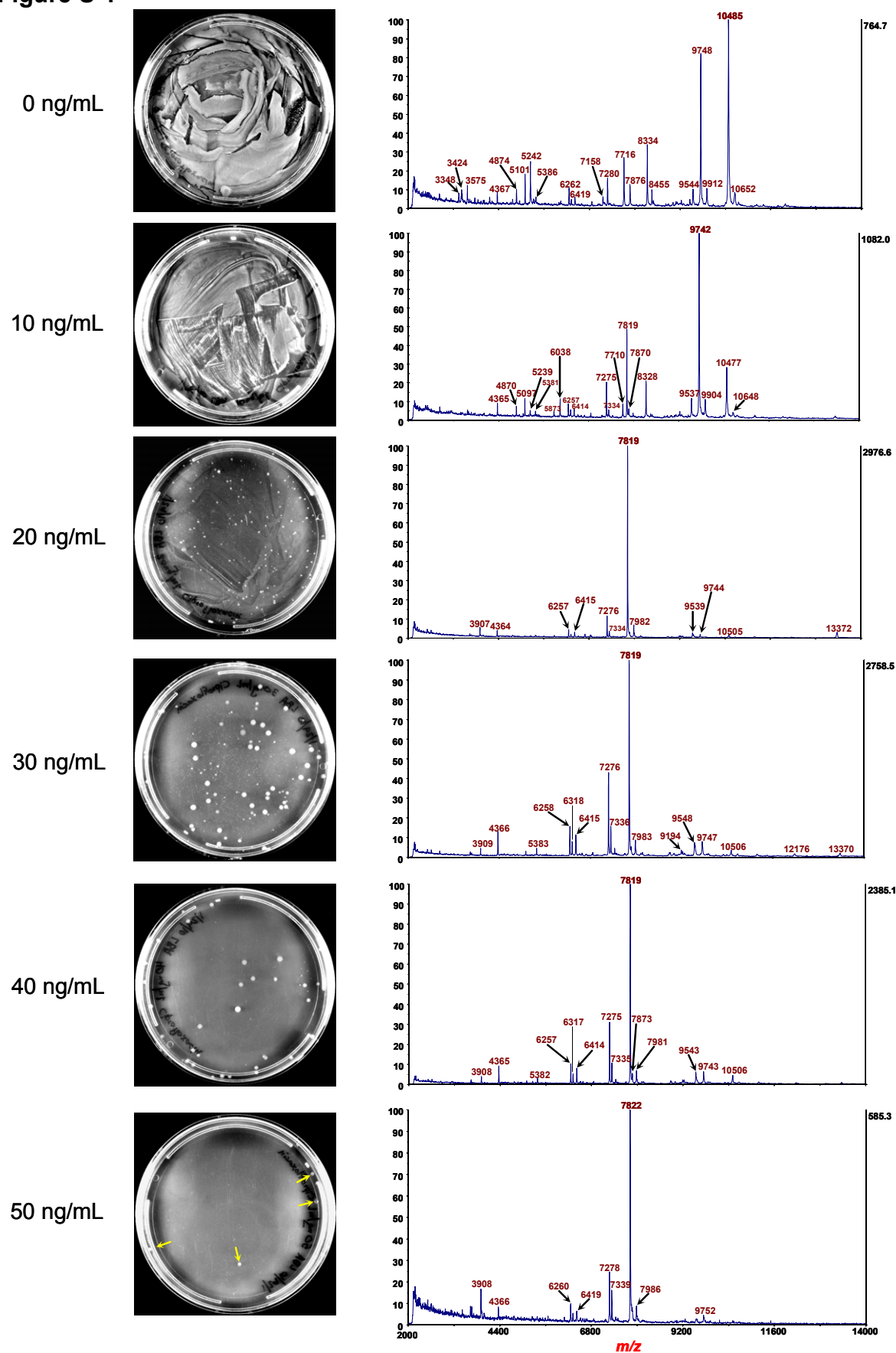


Figure S-2

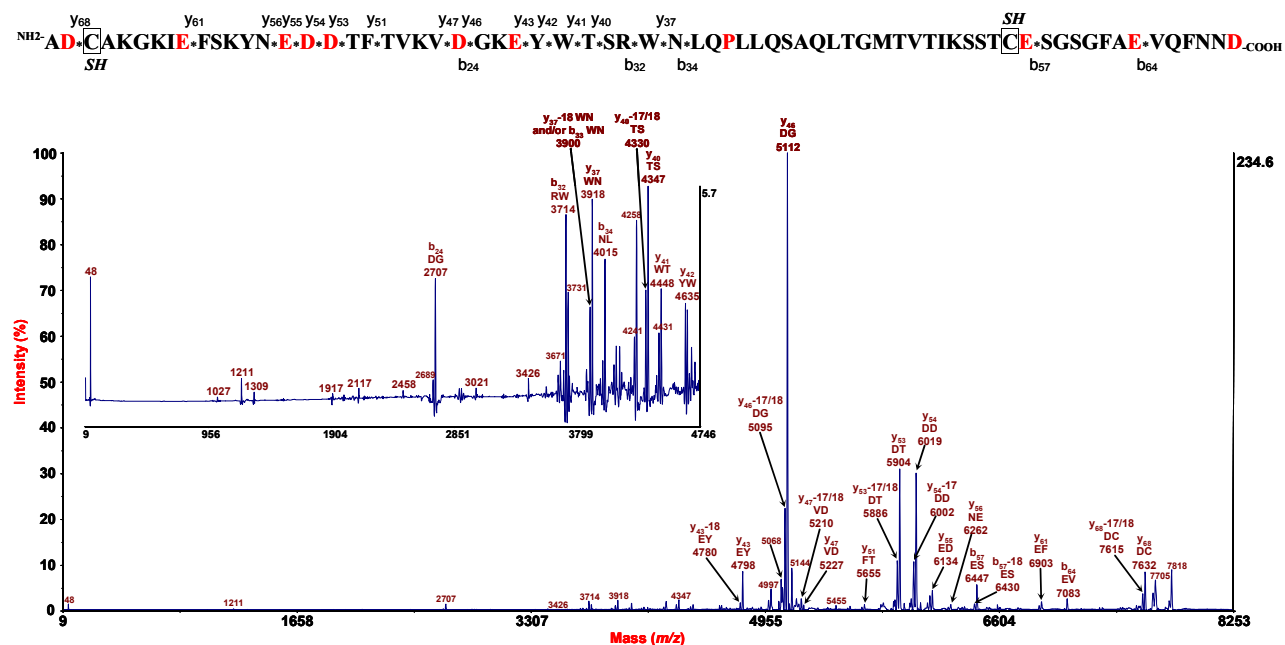
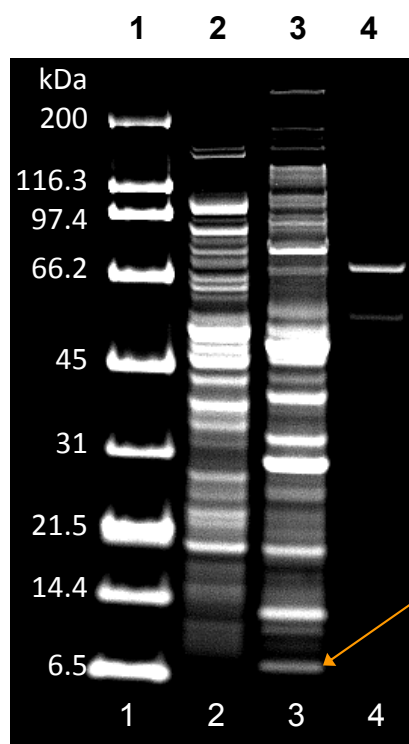


Figure S-3



Lane Assignments:

1. BioRad Broad Range Ladder
2. RM1272 LBA
3. RM1272 LBA + 20 ng/mL Cipro
4. Bovine serum albumin

β-subunit of Shiga toxin 2

Figure captions

Figure S-1: Images of bacterial growth of *E. coli* O157:H7 grown on LB agar plates supplemented with ng/mL concentrations of CP. The corresponding MALDI-TOF-MS spectrum (using caffeic acid) obtained of the bacterial cell lysate harvested from each plate.

Figure S-2: MS/MS of the precursor ion at $m/z \sim 7820$ from bacterial cell lysate (at the 20 ng/mL CP concentration) treated with dithiothreitol. Fragment ions matched to *in silico* fragment ions of the β -Stx2 sequence are indicated by the ion type/number as well as the two amino acid residues immediately adjacent to the site of backbone cleavage. The amino acid sequence of β -Stx2 showing sites of polypeptide cleavage (*) that correspond to the fragment ions observed.

Figure S-3: 1-D SDS-PAGE gel of proteins from bacterial cell lysate of *E. coli* O157:H7 (strain EDL933) harvested after overnight growth on solid LB agar supplemented with 20 ng/mL of ciprofloxacin (Lane 3). The gel band indicated by an arrow was identified by bottom-up proteomics as the β -subunit of Stx2 (**Tables S-4-A, S-4-B, S-5-A, and S-5-B**).

Table S-1-A (#94). The top 30 identifications of the biomarker ion at m/z 7819 analyzed by MS/MS (**Figure 2**) and top-down proteomics using a non-residue-specific MS/MS-to-*in silico* comparison. Comparison parameters: 2 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 92 fragment ion peaks above minimum intensity threshold; 4572 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score 123 seconds, P-value 470 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
43945	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43946	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43947	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43948	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43949	>tr B3ALI0 B3ALI0_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43950	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43951	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43942	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i> O111	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43960	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	26.09	1.0E-5
43943	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i> O157	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	26.09	1.0E-5
88446	>tr B1LVU5 B1LVU5_METRJ	<i>Methylobacterium radiotolerans</i> (strain ATCC 27329)	Putative uncharacterized protein PTM-Met Reduced MW = 7815.77	19.57	5.4E-3
88567	>tr A1WSS9 A1WSS9_VEREI	<i>Verminephrobacter eiseniae</i> (strain EF01-2)	Putative uncharacterized protein PTM-Met Reduced MW = 7811.89	19.57	7.1E-3
45053	>tr Q07I36 Q07I36_RHOP5	<i>Rhodopseudomonas palustris</i> (strain BisA53)	Transcriptional regulator, XRE family PTM-Met MW = 7814.03	19.57	
44507	>tr A9DT05 A9DT05_9FLAO	<i>Kordia algicida</i> OT-1	Putative uncharacterized protein PTM-Met MW = 7813.94		8.0E-3
88221	>tr A7JTQ2 A7JTQ2_PASHA	<i>Mannheimia haemolytica</i> PHL213	Putative uncharacterized protein PTM-Met Reduced MW = 7810.69	19.57	
45970	>tr A9KNF1 A9KNF1_CLOPH	<i>Clostridium phytofermentans</i> (strain ATCC 700394)	Flagellar FliB family protein MW = 7809.15		8.0E-3
44047	>tr A3UPI3 A3UPI3_VIBSP	<i>Vibrio enditus</i> 12B01	Putative uncharacterized protein PTM-Met MW = 7819.96	18.48	
45053	>tr Q07I36 Q07I36_RHOP5	<i>Rhodopseudomonas palustris</i> (strain BisA53)	Transcriptional regulator, XRE family PTM-Met MW = 7814.03		8.1E-3
44575	>tr B0ML79 B0ML79_9FIRM	<i>Eubacterium siraeum</i> DSM 15702	Putative uncharacterized protein PTM-Met MW = 7818.17	18.48	9.2E-3
87536	>tr B3XNW9 B3XNW9_LACRE	<i>Lactobacillus reuteri</i> 100-23	Putative uncharacterized protein PTM-Met Reduced MW = 7812.93	18.48	1.0E-2
46704	>tr Q53864 Q53864_STRCO	<i>Streptomyces coelicolor</i>	Truncated orfX near bldB MW = 7813.65	18.48	1.2E-2
44507	>tr A9DT05 A9DT05_9FLAO	<i>Kordia algicida</i> OT-1	Putative uncharacterized protein PTM-Met MW = 7813.94	18.48	
87743	>tr Q6MDU2 Q6MDU2_PARUW	<i>Protochlamydia amoebophila</i> (strain UWE25)	Putative uncharacterized protein PTM-Met Reduced MW = 7810.27		1.2E-2
44658	>tr B1MYS5 B1MYS5_LEUCK	<i>Leuconostoc citreum</i> (strain KM20)	Putative uncharacterized protein PTM-Met MW = 7826.21	18.48	
44562	>tr B0JMJ1 B0JMJ1_MICAN	<i>Microcystis aeruginosa</i> (strain NIES-843)	Putative uncharacterized protein PTM-Met MW = 7812.01		1.4E-2
44776	>tr A1WSS9 A1WSS9_VEREI	<i>Verminephrobacter eiseniae</i> (strain EF01-2)	Putative uncharacterized protein PTM-Met MW = 7810.89	18.48	1.5E-2
45970	>tr A9KNF1 A9KNF1_CLOPH	<i>Clostridium phytofermentans</i> (strain ATCC 700394)	Flagellar FliB family protein MW = 7809.15	18.48	
46042	>tr B0N9K1 B0N9K1_EUBSP	<i>Clostridium seindens</i> ATCC 35704	Putative uncharacterized protein MW = 7810.89		1.5E-2
89574	>tr Q0RCB2 Q0RCB2_FRAAA	<i>Frankia alni</i> (strain ACN14a)	Putative uncharacterized protein Reduced MW = 7821.96	18.48	
46050	>tr B0P8R9 B0P8R9_9FIRM	<i>Anaerotruncus colihominis</i> DSM 17241	Putative uncharacterized protein MW = 7810.89		1.5E-2
44562	>tr B0JMJ1 B0JMJ1_MICAN	<i>Microcystis aeruginosa</i> (strain NIES-843)	Putative uncharacterized protein PTM-Met MW = 7812.01	17.39	
44658	>tr B1MYS5 B1MYS5_LEUCK	<i>Leuconostoc citreum</i> (strain KM20)	Putative uncharacterized protein PTM-Met MW = 7826.21		1.6E-2
43996	>sp A4YB05 Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputcn32-3428 PTM-Met MW = 7815.86	17.39	1.7E-2
44003	>tr A2UYK4 A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200	Putative uncharacterized protein PTM-Met MW = 7815.86	17.39	1.7E-2
44753	>tr B2UZ0X B2UZ0X_CLOBA	<i>Clostridium botulinum</i> (strain Alaska E43 / Type E3)	Putative uncharacterized protein PTM-Met MW = 7812.59	17.39	
88221	>tr A7JTQ2 A7JTQ2_PASHA	<i>Mannheimia haemolytica</i> PHL213	Putative uncharacterized protein PTM-Met Reduced MW = 7810.69		1.9E-2
44893	>tr B5VYA7 B5VYA7_SPIMA	<i>Arthrorhiza maxima</i> CS-328	Putative uncharacterized protein PTM-Met MW = 7816.87	17.39	
89574	>tr Q0RCB2 Q0RCB2_FRAAA	<i>Frankia alni</i> (strain ACN14a)	Putative uncharacterized protein Reduced MW = 7821.96		1.9E-2
45376	>tr Q7UL98 Q7UL98_RHOB	<i>Rhodopirellula baltica</i> Candidatus Kuenenia stuttgartiensis	Putative uncharacterized protein PTM-Met MW = 7825.03	17.39	
46565	>tr Q1Q1Y6 Q1Q1Y6_9BACT		Putative uncharacterized protein MW = 7823.85		2.0E-2
45729	>tr A5GC90 A5GC90_GEOUR	<i>Geobacter uraniumreducens</i> (strain Rf4)	Putative uncharacterized protein MW = 7816.83	17.39	
44456	>tr A8FF50 A8FF50_BACP2	<i>Bacillus pumilus</i> (strain SAFR-032)	Putative uncharacterized protein yqzE PTM-Met MW = 7816.23		2.0E-2

Table S-1-B (#94). The top 30 identifications of the biomarker ion at m/z 7819 analyzed by MS/MS (**Figure 2**) and top-down proteomics using a D-, E-, P-specific MS/MS-to-*in silico* comparison. Comparison parameters: 2 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 92 fragment ion peaks above minimum intensity threshold; 4572 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score 59 seconds, P-value 487 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
43945	>tr[A7UQX3]A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43946	>tr[B2NM93]B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43947	>tr[B2P388]B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43948	>tr[B2PIT4]B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43949	>tr[B3ALI0]B3ALI0_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43950	>tr[B3BA09]B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43951	>tr[A0SEJ4]A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43942	>tr[A6MTT2]A6MTT2_EC0LX	<i>Escherichia coli</i> O111	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43960	>tr[Q6YI17]Q6YI17_EC0LX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	17.39	7.5E-9
43943	>tr[Q9RHP8]Q9RHP8_EC0LX	<i>Escherichia coli</i> O157	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	17.39	7.5E-9
44702	>tr[B1ZJP4]B1ZJP4_METPB	<i>Methylobacterium populi</i> (strain ATCC BAA-705)	Putative uncharacterized protein PTM-Met MW = 7809.52	15.22	
45788	>tr[A6CIU0]A6CIU0_9BACI	<i>Bacillus</i> SG-1	Putative uncharacterized protein MW = 7810.83		3.4E-4
89511	>tr[B3WYJ2]B3WYJ2_SHIDY	<i>Shigella dysenteriae</i> 1012	DNA-directed DNA polymerase Reduced MW = 7819.85	13.04	7.0E-4
43996	>sp[A4YB05]Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met MW = 7815.86	13.04	
89188	>tr[B1IHD8]B1IHD8_CLOBK	<i>Clostridium botulinum</i> (strain Okra / Type B1)	Acetyltransferase, gnat family Reduced MW = 7819.05		9.9E-4
44003	>tr[A2UYK4]A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200	Putative uncharacterized protein PTM-Met MW = 7815.86	13.04	
45820	>tr[A6TAD9]A6TAD9_KLEP7	<i>Klebsiella pneumoniae</i> sub pneumoniae	Putative uncharacterized protein ydhZ MW = 7824.21		1.4E-3
46107	>tr[A0QL74]A0QL74_MYCA1	<i>Mycobacterium avium</i> (strain 104)	Putative uncharacterized protein MW = 7813.62	11.96	
44386	>tr[A7A6P8]A7A6P8_BIFAD	<i>Bifidobacterium adolescentis</i> L2-32	Putative uncharacterized protein PTM-Met MW = 7825.21		1.6E-3
45788	>tr[A6CIU0]A6CIU0_9BACI	<i>Bacillus</i> SG-1	Putative uncharacterized protein MW = 7810.83	10.87	
88023	>tr[A4IKQ4]A4IKQ4_GEOTN	<i>Geobacillus thermotrophicans</i> (strain NG80-2)	Putative uncharacterized protein PTM-Met Reduced MW = 7813.06		2.0E-3
44562	>tr[B0JMJ1]B0JMJ1_MICAN	<i>Microcystis aeruginosa</i> (strain NIES-843)	Putative uncharacterized protein PTM-Met MW = 7812.01	10.87	2.6E-3
46445	>tr[B3WYJ2]B3WYJ2_SHIDY	<i>Shigella dysenteriae</i> 1012	DNA-directed DNA polymerase MW = 7818.84	10.87	
44646	>tr[B1IM44]B1IM44_CLOBK	<i>Clostridium botulinum</i> (strain Okra / Type B1)	DNA-binding protein PTM-Met MW = 7822.05		3.7E-3
46896	>tr[Q982R0]Q982R0_RHILO	<i>Rhizobium loti</i>	Msr8535 protein MW = 7817.79	10.87	
46107	>tr[A0QL74]A0QL74_MYCA1	<i>Mycobacterium avium</i> (strain 104)	Putative uncharacterized protein MW = 7813.62		4.8E-3
87461	>tr[Q0BCJ7]Q0BCJ7_BURCM	<i>Burkholderia ambifaria</i> (strain ATCC BAA-244)	Putative uncharacterized protein PTM-Met Reduced MW = 7809.69	10.87	
45893	>tr[A8A0A9]A8A0A9_ECOHS	<i>Escherichia coli</i> O9:H4 (strain HS)	Cold shock DNA-binding protein MW = 7814.95		5.0E-3
45562	>tr[A3Q7H5]A3Q7H5_MYCSJ	<i>Mycobacterium</i> (strain JLS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62	9.78	5.0E-3
45563	>tr[A1UN27]A1UN27_MYCSK	<i>Mycobacterium</i> (strain KMS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62	9.78	5.0E-3
45893	>tr[A8A0A9]A8A0A9_ECOHS	<i>Escherichia coli</i> O9:H4 (strain HS)	Cold shock DNA-binding protein MW = 7814.95	9.78	
87998	>tr[A3ZAX5]A3ZAX5_9SYNE	<i>Synechococcus</i> RS9917	Putative uncharacterized protein PTM-Met Reduced MW = 7825.87		5.3E-3
45116	>tr[B3WV16]B3WV16_EC0LX	<i>Escherichia coli</i> B171	Putative uncharacterized protein PTM-Met MW = 7822.03	9.78	
46820	>tr[Q7UW04]Q7UW04_RHOBA	<i>Rhodopirellula baltica</i>	Putative uncharacterized protein MW = 7819.58		5.7E-3
45530	>tr[A3PRG5]A3PRG5_RHOS1	<i>Rhodobacter aeroides</i> (strain ATCC 17029)	Putative uncharacterized protein PTM-24SigPep MW = 7816.12	9.78	
46445	>tr[B3WYJ2]B3WYJ2_SHIDY	<i>Shigella dysenteriae</i> 1012	DNA-directed DNA polymerase MW = 7818.84		7.1E-3
45589	>tr[A3HW11]A3HW11_9SPHI	<i>Algoriphagus</i> PR1	Glutamyl-tRNA synthetase MW = 7810.89	9.78	
87461	>tr[Q0BCJ7]Q0BCJ7_BURCM	<i>Burkholderia ambifaria</i> (strain ATCC BAA-244)	Putative uncharacterized protein PTM-Met Reduced MW = 7809.69		7.1E-3
45820	>tr[A6TAD9]A6TAD9_KLEP7	<i>Klebsiella pneumoniae</i> sub pneumoniae (strain ATCC 700721)	Putative uncharacterized protein ydhZ MW = 7824.21	9.78	
87747	>tr[Q6NIR7]Q6NIR7_CORDI	<i>Corynebacterium diphtheria</i>	Putative uncharacterized protein PTM-Met Reduced MW = 7812.21		7.2E-3
46328	>tr[B6FZ08]B6FZ08_9CLOT	<i>Clostridium hiranonis</i> DSM 13275	Putative uncharacterized protein MW = 7811.93	9.78	
89690	>tr[Q2SE56]Q2SE56_HAHCH	<i>Hahella chejuensis</i> (strain KCTC 2396)	Putative uncharacterized protein Reduced MW = 7817.62		7.2E-3
46507	>tr[Q0RC08]Q0RC08_FRAAA	<i>Frankia alni</i> (strain ACN14a)	Putative uncharacterized protein MW = 7811.75	9.78	
43996	>sp[A4YB05]Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met MW = 7815.86		7.4E-3
87393	>tr[B3PCZ5]B3PCZ5_CELJU	<i>Cellvibrio japonicus</i> (strain Ueda107)	Tetraacyldisaccharide-1-P 4-kinase PTM-Met Reduced MW = 7818.1	9.78	
44003	>tr[A2UYK4]A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200	Putative uncharacterized protein PTM-Met MW = 7815.86		7.4E-3

Table S-2-A (#95). The top 30 identifications of the biomarker ion at m/z 7819 analyzed by MS/MS (**Figure 2**) and top-down proteomics using a non-residue-specific MS/MS-to-*in silico* comparison. Fragment ions from asymmetric disulfide bond cleavage were removed. Comparison parameters: 2 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 84 fragment ion peaks above minimum intensity threshold; 4572 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score: 120 seconds; P-value: 384 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
43945	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43946	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43947	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43948	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43949	>tr B3AL10 B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43950	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43951	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43942	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i> O111	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43960	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	27.38	6.4E-6
43943	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i> O157	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	27.38	6.4E-6
45729	>tr A5GC90 A5GC90_GEOUR	<i>Geobacter uranireducens</i> (strain Rf4)	Putative uncharacterized protein MW = 7816.83	21.43	
88247	>tr A8FF50 A8FF50_BACP2	<i>Bacillus pumilus</i> (strain SAFR-032)	Putative uncharacterized protein yqzE PTM-Met Reduced MW = 7816.23		1.4E-3
44456	>tr A8FF50 A8FF50_BACP2	<i>Bacillus pumilus</i> (strain SAFR-032)	Putative uncharacterized protein yqzE PTM-Met MW = 7816.23	20.24	1.4E-3
88446	>tr B1LVU5 B1LVU5_METRJ	<i>Methylobacterium radiotolerans</i> (strain ATCC 27329)	Putative uncharacterized protein PTM-Met Reduced MW = 7815.77	20.24	
45729	>tr A5GC90 A5GC90_GEOUR	<i>Geobacter uranireducens</i> (strain Rf4)	Putative uncharacterized protein MW = 7816.83		1.7E-3
87743	>tr Q6MDU2 Q6MDU2_PARUW	<i>Protochlamydia amoebophila</i> (strain UWE25)	Putative uncharacterized protein PTM-Met Reduced MW = 7810.27	20.24	2.0E-3
87922	>sp A4YB05 Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met Reduced MW = 7819.89	20.24	3.1E-3
87929	>tr A2UYK4 A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200	Putative uncharacterized protein PTM-Met Reduced MW = 7819.89	20.24	3.1E-3
46704	>tr Q53864 Q53864_STRCO	<i>Streptomyces coelicolor</i>	Truncated orfX near bldB MW = 7813.65	20.24	4.7E-3
45129	>tr Q1NHN3 Q1NHN3_9SPHN	<i>Sphingomonas</i> SKA58	Putative uncharacterized protein PTM-Met MW = 7822.65	19.05	
88446	>tr B1LVU5 B1LVU5_METRJ	<i>Methylobacterium radiotolerans</i> (strain ATCC 27329)	Putative uncharacterized protein PTM-Met Reduced MW = 7815.77		4.7E-3
46116	>tr B1HPP7 B1HPP7_LYSSC	<i>Lysinibacillus aericus</i> (strain C3-41)	Putative uncharacterized protein MW = 7809.68	19.05	6.5E-3
88943	>tr A7N2V7 A7N2V7_VIBHB	<i>Vibrio harveyi</i> (strain ATCC BAA-1116)	Putative uncharacterized protein Reduced MW = 7814.86	19.05	6.5E-3
45045	>tr Q05R34 Q05R34_9SYNE	<i>Synechococcus</i> RS9916	Possible high light inducible protein PTM-Met MW = 7823.06	19.05	1.1E-2
45376	>tr Q7UL98 Q7UL98_RHOB	<i>Rhodopirellula baltica</i>	Putative uncharacterized protein PTM-Met MW = 7825.03	19.05	1.2E-2
44047	>tr A3UPI3 A3UPI3_VIBSP	<i>Vibrio enditus</i> 12B01	Putative uncharacterized protein PTM-Met MW = 7819.96	19.05	
44621	>tr B0VNN3 B0VNN3_ACIBS	<i>Acinetobacter baumannii</i> (strain SDF)	Putative uncharacterized protein PTM-Met MW = 7818.07		1.3E-2
44658	>tr B1MYS5 B1MYS5_LEUCK	<i>Leuconostoc citreum</i> (strain KM20)	Putative uncharacterized protein PTM-Met MW = 7826.21	19.05	
87935	>tr A3GKS8 A3GKS8_VIBCH	<i>Vibrio cholerae</i> NCTC 8457	Putative uncharacterized protein PTM-Met Reduced MW 7814.29		1.4E-2
45053	>tr Q07136 Q07136_RHOP5	<i>Rhodopseudomonas palustris</i> (strain BisA53)	Transcriptional regulator, XRE family PTM-Met MW = 7814.03	19.05	
88585	>tr A2PNF3 A2PNF3_VIBCH	<i>Vibrio cholerae</i> MAK 757	Putative uncharacterized protein PTM-Met Reduced MW = 7814.29		1.4E-2
44621	>tr B0VNN3 B0VNN3_ACIBS	<i>Acinetobacter baumannii</i> (strain SDF)	Putative uncharacterized protein PTM-Met MW = 7818.07	17.86	
46042	>tr B0N9K1 B0N9K1_EUBSP	<i>Clostridium scindens</i> ATCC 35704	Putative uncharacterized protein MW = 7810.89		1.5E-2
44893	>tr B5VYA7 B5VYA7_SPIMA	<i>Arthrorhiza maxima</i> CS-328	Putative uncharacterized protein PTM-Met MW = 7816.87	17.86	
46050	>tr B0P8R9 B0P8R9_9FIRM	<i>Anaerotruncus colihominis</i> DSM 17241	Putative uncharacterized protein MW = 7810.89		1.5E-2
45112	>tr Q1IH17 Q1IH17_PSEE4	<i>Pseudomonas entomophila</i> (strain L48)	Putative uncharacterized protein PTM-Met MW = 7813.77	17.86	
89927	>tr Q8GF48 Q8GF48_ZYMMO	<i>Zymomonas mobilis</i>	Putative uncharacterized protein Reduced MW = 7825.96		1.5E-2
45543	>tr Q20216 Q20216_RHOPB	<i>Rhodopseudomonas palustris</i> (strain BisB18)	Putative uncharacterized protein PTM-22SigPep MW = 7810.32	17.86	
44658	>tr B1MYS5 B1MYS5_LEUCK	<i>Leuconostoc citreum</i> (strain KM20)	Putative uncharacterized protein PTM-Met MW = 7826.21		1.5E-2
45561	>tr A1VQ77 A1VQ77_POLNA	<i>Polaromonas naphthalenivorans</i> (strain C.J2)	Conjugal transfer protein TrbC PTM-34SigPep MW = 7809.28	17.86	
45053	>tr Q07136 Q07136_RHOP5	<i>Rhodopseudomonas palustris</i> (strain BisA53)	Transcriptional regulator, XRE family PTM-Met MW = 7814.03		1.5E-2

Table S-2-B (#95). The top 30 identifications of the biomarker ion at m/z 7819 analyzed by MS/MS (**Figure 2**) and top-down proteomics using a D-, E-, P-residue-specific MS/MS-to-*in silico* comparison. Fragment ions from asymmetric disulfide bond cleavage were removed. Comparison parameters: 2 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 84 fragment ion peaks above minimum intensity threshold; 4572 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score: 58 seconds; P-value 381 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
43945	>tr[A7UQX3]A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43946	>tr[B2NM93]B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43947	>tr[B2P388]B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43948	>tr[B2PIT4]B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43949	>tr[B3AL10]B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43950	>tr[B3BA09]B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43951	>tr[A0SEJ4]A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43942	>tr[A6MTT2]A6MTT2_ECOLX	<i>Escherichia coli</i> O111	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43960	>tr[Q6YI7]Q6YI7_ECOLX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	19.05	1.9E-9
43943	>tr[Q9RHP8]Q9RHP8_ECOLX	<i>Escherichia coli</i> O157	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	19.05	1.9E-9
87922	>sp[A4YB05]Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met Reduced MW = 7819.89	15.48	
45788	>tr[A6CIU0]A6CIU0_9BACI	<i>Bacillus</i> SG-1	Putative uncharacterized protein MW = 7810.83		3.2E-5
87929	>tr[A2UYK4]A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200 GN=Sput200DRAFT-2556	Putative uncharacterized protein PTM-Met Reduced MW = 7819.89	15.48	
45820	>tr[A6TAD9]A6TAD9_KLEP7	<i>Klebsiella pneumoniae</i> sub pneumoniae	Putative uncharacterized protein ydhZ MW = 7824.21		3.2E-5
89511	>tr[B3WYJ2]B3WYJ2_SHIDY	<i>Shigella dysenteriae</i> 1012	DNA-directed DNA polymerase Reduced MW = 7819.85	13.10	1.1E-3
44702	>tr[B1ZJP4]B1ZJP4_METPB	<i>Methylobacterium populi</i> (strain ATCC BAA-705)	Putative uncharacterized protein PTM-Met MW = 7809.52	13.10	
87922	>sp[A4YB05]Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met Reduced MW = 7819.89		1.2E-3
45788	>tr[A6CIU0]A6CIU0_9BACI	<i>Bacillus</i> SG-1	Putative uncharacterized protein MW = 7810.83	13.10	
87929	>tr[A2UYK4]A2UYK4_SHEPU	<i>Shewanella putrefaciens</i> 200 GN=Sput200DRAFT-2556	Putative uncharacterized protein PTM-Met Reduced MW = 7819.89		1.2E-3
45820	>tr[A6TAD9]A6TAD9_KLEP7	<i>Klebsiella pneumoniae</i> sub pneumoniae	Putative uncharacterized protein ydhZ MW = 7824.21	13.10	
44562	>tr[B0JMJ1]B0JMJ1_MICAN	<i>Microcystis aeruginosa</i> (strain NIES-843)	Putative uncharacterized protein PTM-Met MW = 7812.01		1.3E-3
44562	>tr[B0JMJ1]B0JMJ1_MICAN	<i>Microcystis aeruginosa</i> (strain NIES-843)	Putative uncharacterized protein PTM-Met MW = 7812.01	11.90	
45562	>tr[A3Q7H5]A3Q7H5_MYCSJ	<i>Mycobacterium</i> (strain JLS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62		2.7E-3
46107	>tr[A0QL74]A0QL74_MYCA1	<i>Mycobacterium avium</i> (strain 104)	Putative uncharacterized protein MW = 7813.62	11.90	
45563	>tr[A1UN27]A1UN27_MYCSK	<i>Mycobacterium</i> (strain KMS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62		2.7E-3
46616	>tr[Q2LUP5]Q2LUP5_SYNAS	<i>Syntrophus aciditrophicus</i> (strain SB)	Hypothetical exported protein MW = 7825.24	10.71	
44601	>tr[B0S1X9]B0S1X9_FINM2	<i>Finexgoldia magna</i> (strain ATCC 29328)	Putative uncharacterized protein PTM-Met MW = 7821.01		3.5E-3
44601	>tr[B0S1X9]B0S1X9_FINM2	<i>Finexgoldia magna</i> (strain ATCC 29328)	Putative uncharacterized protein PTM-Met MW = 7821.01	10.71	
45396	>tr[Q82ZF5]Q82ZF5_ENTFA	<i>Enterococcus faecalis</i>	Putative uncharacterized protein PTM-Met MW = 7810.28		3.9E-3
45193	>tr[Q2Y1J4]Q2Y1J4_BRUA2	<i>Brucella abortus</i> (strain 2308)	Putative uncharacterized protein PTM-Met MW = 7824.20	10.71	
45553	>tr[Q121W7]Q121W7_POLSJ	<i>Polaromonas</i> (strain ATCC BAA-500)	Mercuric transport protein periplasmic component PTM-20SigPep MW = 7817.95		4.1E-3
45272	>tr[Q579Q1]Q579Q1_BRUA2	<i>Brucella abortus</i>	Putative uncharacterized protein PTM-Met MW = 7824.20	10.71	
88023	>tr[A4IKQ4]A4IKQ4_GEOTN	<i>Geobacillus thermodentificans</i> (strain NG80-2)	Putative uncharacterized protein PTM-Met Reduced MW = 7813.06		4.1E-3
45431	>tr[Q8FX88]Q8FX88_BRUSU	<i>Brucella suis</i>	Putative uncharacterized protein PTM-Met MW = 7824.20	10.71	
44110	>tr[A4MXS4]A4MXS4_HAEIN	<i>Haemophilus influenza</i> 22.1-21	Site-specific tyrosine recombinase XerD PTM-Met MW = 7812.03		4.6E-3
45457	>tr[Q8YB52]Q8YB52_BRUME	<i>Brucella melitensis</i>	Putative uncharacterized protein PTM-Met MW = 7824.20	10.71	
44111	>tr[A4MXS4]A4MXS4_HAEIN	<i>Haemophilus influenza</i> 22.1-21	Site-specific tyrosine recombinase XerD PTM-Met MW = 7812.03		4.6E-3
45562	>tr[A3Q7H5]A3Q7H5_MYCSJ	<i>Mycobacterium</i> (strain JLS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62	10.71	
44115	>tr[A4NW25]A4NW25_HAEIN	<i>Haemophilus influenzae</i> 22.4-21	Site-specific tyrosine recombinase XerD PTM-Met MW = 7812.03		4.6E-3
45563	>tr[A1UN27]A1UN27_MYCSK	<i>Mycobacterium</i> (strain KMS)	Putative uncharacterized protein PTM-35SigPep MW = 7811.62	10.71	
88948	>tr[A7V3H8]A7V3H8_BACUN	<i>Bacteroides uniformis</i> ATCC 8492	Putative uncharacterized protein Reduced MW = 7815.43		4.6E-3
45589	>tr[A3HW11]A3HW11_9SPHI	<i>Algoriphagus</i> PR1	Glutamyl-tRNA synthetase MW = 7810.89	10.71	
89690	>tr[Q2SE56]Q2SE56_HAHCH	<i>Haemella chejuensis</i> (strain KCTC 2396)	Putative uncharacterized protein Reduced MW = 7817.62		4.6E-3
46445	>tr[B3WYJ2]B3WYJ2_SHIDY	<i>Shigella dysenteriae</i> 1012	DNA-directed DNA polymerase MW = 7818.84	10.71	
46116	>tr[B1HPP7]B1HPP7_LYSSC	<i>Lysinibacillus aericus</i> (strain C3-41)	Putative uncharacterized protein MW = 7809.68		4.7E-3
46507	>tr[Q0RC08]Q0RC08_FRAAA	<i>Frankia alni</i> (strain ACN14a)	Putative uncharacterized protein MW = 7811.75	10.71	
87645	>tr[Q3ZX98]Q3ZX98_DEHSC	<i>Dehalococcoides</i> (strain CBDB1)	Putative uncharacterized protein PTM-Met Reduced MW = 7825.22		6.3E-3
43996	>sp[A4YB05]Y3428_SHEPC	<i>Shewanella putrefaciens</i> (strain ATCC BAA-453)	UPF0243 zinc-binding protein Sputc32-3428 PTM-Met MW = 7815.86	9.52	
46107	>tr[A0QL74]A0QL74_MYCA1	<i>Mycobacterium avium</i> (strain 104)	Putative uncharacterized protein MW = 7813.62		7.3E-3

Table S-3-A (#91): The top thirty identifications of the biomarker ion at m/z 7818 analyzed by MS/MS (**Figure S-2**) and top-down proteomics using a non-residue-specific MS/MS-to-*in silico* comparison. Comparison parameters: 1 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 63 fragment ion peaks above minimum intensity threshold; 4857 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score 106 seconds, P-value 221 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
87180	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Reduced shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87181	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87182	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87183	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87184	>tr B3AL10 B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87185	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87186	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Reduced shiga toxin II subunit B PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87177	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i> O111	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	47.62	3.4E-14
87178	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i> O157	Reduced shiga toxin II subunit B PTM-19SigPep MW = 7817.60	47.62	3.4E-14
87195	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Reduced shiga toxin 2 subunit B PTM-19SigPep MW = 7817.64	47.62	3.4E-14
43945	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43946	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43947	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43948	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43949	>tr B3AL10 B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43950	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43951	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43942	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i>	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	39.68	5.9E-10
43943	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	39.68	5.9E-10
43960	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	39.68	5.9E-10
46542	>tr Q1H238 Q1H238_METFK	<i>Methylobacillus flagellatus</i> (strain KT / ATCC 51484 / DSM 6875)	Putative uncharacterized protein MW = 7810.02	26.98	1.2E-4
88778	>tr A4YVK6 A4YVK6_BRASO	<i>Bradyrhizobium</i> (strain ORS278)	Putative uncharacterized protein Reduced MW = 7814.13	23.81	1.4E-3
44358	>tr A0NMI7 A0NMI7_9RHOB	<i>Labrenzia aggregata</i> IAM 12614	Putative uncharacterized protein PTM-Met MW = 7820.47	22.22	
45961	>tr A9GYH6 A9GYH6_9RHOB	<i>Roseobacter litoralis</i> Och 149	Putative uncharacterized protein MW = 7819.88		2.0E-3
44653	>tr A1K552 A1K552_AZOSB	<i>Azoarcus</i> (strain BH72)	Conserved hypothetical cytochrome oxidase maturation protein PTM-Met MW = 7816.91	22.22	
89913	>tr Q8CSZ1 Q8CSZ1_STAES	<i>Staphylococcus epidermidis</i> (strain ATCC 12228)	Putative uncharacterized protein Reduced MW = 7818.5		2.3E-3
45961	>tr A9GYH6 A9GYH6_9RHOB	<i>Roseobacter litoralis</i> Och 149	Putative uncharacterized protein MW = 7819.88	22.22	
44653	>tr A1K552 A1K552_AZOSB	<i>Azoarcus</i> (strain BH72)	Conserved hypothetical cytochrome oxidase maturation protein PTM-Met MW = 7816.91		2.7E-3
87840	>tr Q8KAD5 Q8KAD5_CHLTE	<i>Chlorobium tepidum</i>	Putative uncharacterized protein PTM-Met Reduced MW = 7817.11	22.22	
44358	>tr A0NMI7 A0NMI7_9RHOB	<i>Labrenzia aggregata</i> IAM 12614	Putative uncharacterized protein PTM-Met MW = 7820.47		3.4E-3
89008	>tr A8ZMN2 A8ZMN2_ACAM1	<i>Acaryochloris marina</i> (strain MBIC 11017)	Putative uncharacterized protein Reduced MW = 7826.03	22.22	
87840	>tr Q8KAD5 Q8KAD5_CHLTE	<i>Chlorobium tepidum</i>	Putative uncharacterized protein PTM-Met Reduced MW = 7817.11		4.4E-3
89913	>tr Q8CSZ1 Q8CSZ1_STAES	<i>Staphylococcus epidermidis</i> (strain ATCC 12228)	Putative uncharacterized protein Reduced MW = 7818.5	22.22	
89008	>tr A8ZMN2 A8ZMN2_ACAM1	<i>Acaryochloris marina</i> (strain MBIC 11017)	Putative uncharacterized protein Reduced MW = 7826.03		4.4E-3
44373	>tr A6Q252 A6Q252_NITSB	<i>Nitratiruptor</i> (strain SB155-2)	Putative uncharacterized protein PTM-Met MW = 7812.04	20.63	
46600	>tr Q2B7Z3 Q2B7Z3_9BACI	<i>Bacillus NRRL B-14911</i>	Putative uncharacterized protein MW = 7811.08		5.0E-3
44412	>tr A0QB09 A0QB09_MYCA1	<i>Mycobacterium avium</i> (strain 104)	Putative uncharacterized protein PTM-Met MW = 7817.97	20.63	
46721	>tr Q5KXH3 Q5KXH3_GEOKA	<i>Geobacillus kaustophilus</i>	Hypothetical conserved protein MW = 7817.79		5.0E-3

Table S-3-B (#91): The top thirty identifications of the biomarker ion at m/z 7818 analyzed by TOF-TOF-MS/MS (Figure S-2) and top-down proteomics using a D-, E-, and P-specific MS/MS-to-*in silico* comparison. Comparison parameters: 1 % minimum intensity threshold; $\pm m/z$ 1.0 fragment ion tolerance; ± 10 Da protein mass tolerance; 63 fragment ion peaks above minimum intensity threshold; 4857 *in silico* bacterial protein sequences compared. Algorithm computation time: USDA score 51 seconds, P-value 194 seconds.

In Silico ID	Identifier	Sample Name	Protein	USDA Score	P-value
87180	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Reduced shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87181	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87182	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87183	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87184	>tr B3AL10 B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87185	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87186	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Reduced shiga toxin II subunit B PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87177	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i> O111	Reduced shiga toxin 2 B subunit PTM-19SigPep MW = 7817.64	31.75	6.1E-16
87178	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i> O157	Reduced shiga toxin II subunit B PTM-19SigPep MW = 7817.60	31.75	6.1E-16
87195	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Reduced shiga toxin 2 subunit B PTM-19SigPep MW = 7817.64	31.75	6.1E-16
43945	>tr A7UQX3 A7UQX3_ECO57	<i>Escherichia coli</i> O157:H7	Shiga-like toxin II B subunit encoded by bacteriophage BP-933W (Shiga toxin 2 subunit B) PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43946	>tr B2NM93 B2NM93_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4196	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43947	>tr B2P388 B2P388_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4113	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43948	>tr B2PIT4 B2PIT4_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4076	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43949	>tr B3AL10 B3AL10_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4486	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43950	>tr B3BA09 B3BA09_ECO57	<i>Escherichia coli</i> O157:H7 str. EC4501	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43951	>tr A0SEJ4 A0SEJ4_ACIHA	<i>Acinetobacter haemolyticus</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43942	>tr A6MTT2 A6MTT2_ECOLX	<i>Escherichia coli</i>	Shiga toxin 2 B subunit PTM-19SigPep MW = 7815.62	22.22	2.6E-9
43943	>tr Q9RHP8 Q9RHP8_ECOLX	<i>Escherichia coli</i>	Shiga toxin II subunit B PTM-19SigPep MW = 7815.58	22.22	2.6E-9
43960	>tr Q6YI17 Q6YI17_ECOLX	<i>Escherichia coli</i> O157:Hneg	Shiga toxin 2 subunit B PTM-19SigPep MW = 7815.62	22.22	2.6E-9
45530	>tr A3PRG5 A3PRG5_RHOS1	<i>Rhodobacter aeroides</i> (strain ATCC 17029 / ATH 2.4.9)	Putative uncharacterized protein PTM-24SigPep MW = 7816.12	19.05	1.9E-4
44702	>tr B1ZJP4 B1ZJP4_METPB	<i>Methylobacterium populi</i> (strain BJ001)	Putative uncharacterized protein PTM-Met MW = 7809.52	14.29	
45689	>tr A4LP29 A4LP29_BURPS	<i>Burkholderia pseudomallei</i> 305	Putative uncharacterized protein MW = 7824.25		2.1E-4
45128	>tr Q1NG62 Q1NG62_9SPHN	<i>Sphingomonas</i> SKA58	Putative uncharacterized protein PTM-Met MW = 7817.05	14.29	
46640	>tr Q39VU1 Q39VU1_GEOMG	<i>Geobacter metallireducens</i> (strain ATCC 53774)	N-terminal methylation MW = 7819.11		3.8E-4
46721	>tr Q5KXH3 Q5KXH3_GEOKA	<i>Geobacillus kaustophilus</i>	Hypothetical conserved protein MW = 7817.79	14.29	
45128	>tr Q1NG62 Q1NG62_9SPHN	<i>Sphingomonas</i> SKA58	Putative uncharacterized protein PTM-Met MW = 7817.05		4.3E-4
89682	>tr Q2LUP5 Q2LUP5_SYNAS	<i>Syntrophus aciditrophicus</i> (strain SB)	Hypothetical exported protein Reduced MW = 7826.25	14.29	
46721	>tr Q5KXH3 Q5KXH3_GEOKA	<i>Geobacillus kaustophilus</i>	Hypothetical conserved protein MW = 7817.79		5.7E-4
44653	>tr A1K552 A1K552_AZOSB	<i>Azoarcus</i> (strain BH72)	Conserved hypothetical cytochrome oxidase maturation protein PTM-Met MW = 7816.91	12.70	
45289	>tr B4AW01 B4AW01_9CHRO	<i>Cyanobacter</i> PCC 7822	Putative uncharacterized protein PTM-Met MW = 7815.52		5.9E-4
44851	>tr B5J7C1 B5J7C1_9RHOB	<i>Octadecabacter antarcticus</i> 307	Putative uncharacterized protein PTM-Met MW = 7822.66	12.70	
87709	>tr Q5YVT5 Q5YVT5_NOCFA	<i>Nocardia farcinica</i>	Putative uncharacterized protein PTM-Met Reduced MW = 7816.97		6.1E-4
45689	>tr A4LP29 A4LP29_BURPS	<i>Burkholderia pseudomallei</i> 305	Putative uncharacterized protein MW = 7824.25	12.70	
44851	>tr B5J7C1 B5J7C1_9RHOB	<i>Octadecabacter antarcticus</i> 307	Putative uncharacterized protein PTM-Met MW = 7822.66		1.9E-3
46328	>tr B6FZ08 B6FZ08_9CLOT	<i>Clostridium hiranonis</i> DSM 13275	Putative uncharacterized protein MW = 7811.93	12.70	
45358	>tr Q72QZ9 Q72QZ9_LEPIC	<i>Leptora interrogans</i> serogroup Icterohaemorrhagiae serovar copenhageni	Putative uncharacterized protein PTM-Met MW = 7808.26		2.8E-3
87709	>tr Q5YVT5 Q5YVT5_NOCFA	<i>Nocardia farcinica</i>	Putative uncharacterized protein PTM-Met Reduced MW = 7816.97	12.70	
45425	>tr Q8F4T7 Q8F4T7_LEPIN	<i>Leptora interrogans</i>	Putative uncharacterized protein PTM-Met MW = 7808.26		2.8E-3

One-dimensional polyacrylamide gel electrophoresis (1-D PAGE) and in-gel tryptic digest

Approximately 2 μ L of bacterial cells were harvested from solid agar plates after overnight growth and transferred to individual microcentrifuge tubes containing 300 μ L of HPLC grade water (Burdick and Jackson) and processed as previously described. Bacterial cells were grown on Luria-Bertani agar in the absence or presence of 20 ng/mL ciprofloxacin. Denaturing gel electrophoresis was performed on the samples using the supplier's recommended protocol. Briefly, 16.25 μ L of cell lysate was reduced and applied to separate lanes of a 1.0 mm NuPAGE Novex 4-12% Bis-Tris mini-gel (NuPAGE, Invitrogen, Carlsbad, CA), followed by electrophoresis in MES SDS running buffer (Invitrogen, Carlsbad, CA) at a constant voltage (200 V) for 40 minutes. A third sample consisting of bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO) was also reduced and applied to the gel as a positive control to assess trypsin digest efficiency. The gel was stained with a ruthenium staining solution (SYPRO Ruby, Invitrogen, Carlsbad, CA) using the supplier's procedure and imaged. A band having high fluorescent signal intensity was detected at approximately ~8 kDa for cells grown in the presence of antibiotic. This band was absent in the cell lysate of cells grown in the absence of antibiotic. The relevant bands were excised and in-gel digested with porcine trypsin (Promega, Madison, WI) using standard protocols (DigestPro, Intavis, Langenfeld, Germany).

Bottom-up proteomic analysis

Twenty μ L of each tryptic digest were separated by nano-HPLC (LC Packings/Dionex, Sunnyvale, CA) and analyzed by MS and MS/MS using a Q-Star-Pulsar I (Applied Biosystems, Foster City, CA). Peptides (and their corresponding proteins) were identified by analysis of MS and MS/MS data using MASCOT and the following parameters: database: NCBI nr; taxonomy: bacteria or viruses; enzyme: trypsin; missed cleavages: 3; fixed modifications: carbamidomethyl (C); variable modifications: oxidation (M); peptide charge: +2, +3, +4.

Table S-4-A: Summary of bottom-up proteomic identifications of excised gel band shown in **Figure S-3** using a MASCOT search, taxonomy: "bacteria". Stx identifications in bold red.

Sample	Name	Organism	Mass (Da)	Mascot Score	Coverage (%)	Queries Matched
β -Stx2	Murein lipoprotein	Escherichia coli O157:H7 EDL933	8375	329	48	7
	Shiga toxin subunit B	Escherichia coli	9816	245	41	4
	Shiga toxin II subunit B	Escherichia coli	9992	208	39	4
	Transcriptional regulator HU subunit alpha	Escherichia coli O157:H7 EDL933	9529	117	27	2
	Cold shock-like protein	Escherichia coli	8626	103	27	2
	Variant Shiga-like toxin II	Escherichia coli	8738	70	41	2
	50S ribosomal protein L30	Escherichia coli O157:H7 EDL933	6538	59	15	1

Table S-4-B: Summary of bottom-up proteomic identifications of excised gel band shown in **Figure S-3** using a MASCOT search, taxonomy: "viruses". Stx identifications in bold red.

Sample	Name	Organism	Mass (Da)	Mascot Score	Coverage (%)	Queries Matched
β -Stx2	Shiga toxin subunit B	Enterobacteria phage 933W	9982	245	39	5
	Shiga toxin 2, subunit B	Escherichia coli O157:H7 str. EC4113	9965	157	39	5
	Shiga toxin 2e B-subunit	Enterobacteria phage phiP27	9758	94	19	3

Table S-5-A: Bottom-up peptide/protein identifications of excised gel band shown in **Figure S-3** using a MASCOT search, taxonomy: "bacteria". Stx identifications boxed in red.

Mascot Score: 329 Queries Matched: 7
Protein ID: Murein lipoprotein [Escherichia coli O157:H7 EDL933]
Calculated Mr = 8375; Calculated pI = 9.30

1 MKATKLV LGA VILGSTLLAG CSSNAK**IDQL SSDVQTLNAK VDQLSNDVNA**
51 **MRSQVQAQK DAARANQRLD** NMAKYRK

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
623.8000	1245.5854	1245.5949	-0.0095	1	31	34	1	U	R.SDVQAQKDDAAR.A
689.3183	1376.6220	1376.6354	-0.0134	0	98	6.5e-06	1	U	K.VDQLSNDVNAMR.S +
Oxidation (M)									
459.8908	1376.6507	1376.6354	0.0153	0	(49)	0.51	1	U	K.VDQLSNDVNAMR.S +
Oxidation (M)									
766.3699	1530.7252	1530.7889	-0.0638	0	122	2.9e-08	1	U	K.IDQLSSDVQTLNAK.V
511.2725	1530.7957	1530.7889	0.0068	0	(71)	0.0033	1	U	K.IDQLSSDVQTLNAK.V
693.0000	2075.9781	2075.9906	-0.0125	1	52	0.27	1	U	
K.VDQLSNDVNAMRSDVQAQK.D + Oxidation (M)									
652.1000	2604.3709	2604.2198	0.1511	2	27	78	1	U	
K.VDQLSNDVNAMRSDVQAQKDDAAR.A + Oxidation (M)									

Mascot Score: 245 Queries Matched: 4
Protein ID: Shiga toxin subunit B [Escherichia coli]
Calculated Mr = 9816; Calculated pI = 5.30

1 MKKMFMVLF ALASVNAMAA DCAK**GKIEFS KYNEDDTFTV** KVDGKEYWTS
51 **RXLQPLLQS AQLTGMTVTI** KSSTXESGSG FAEGQFNND

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
616.2816	1230.5487	1230.5405	0.0082	0	64	0.019	1		K.YNEDDTFTVK.V
612.6333	1834.8782	1834.8625	0.0157	1	62	0.027	1		K.IEFSKYNEDDTFTVK.V
674.3537	2020.0394	2019.9789	0.0604	2	48	0.59	1		K.GKIEFSKYNEDDTFTVK.V
729.7174	2186.1304	2186.1617	-0.0312	0	76	0.00094	1	U	
R.DNLQPLLQSAQLTGMTVTIK.S + Oxidation (M)									

Mascot Score: 208 Queries Matched: 4
Protein ID: Shiga toxin II subunit B [Escherichia coli]
Calculated Mr = 9992; Calculated pI = 5.30

1 MKKMFMVLF ALASVNAMAA DCAK**GKIEFS KYNEDDTFTV** KVDGKEYWTS
51 **RWNLQPLLQS AQLTGMTVXI** KSSTCESGSG **FAEVQFNND**

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
616.2816	1230.5487	1230.5405	0.0082	0	64	0.019	1		K.YNEDDTFTVK.V
612.6333	1834.8782	1834.8625	0.0157	1	62	0.027	1		K.IEFSKYNEDDTFTVK.V
645.9257	1934.7552	1934.7589	-0.0037	0	34	11	1		K.SSTCESGSGFAEVQFNND.-
674.3537	2020.0394	2019.9789	0.0604	2	48	0.59	1		K.GKIEFSKYNEDDTFTVK.V

Mascot Score: 117 Queries Matched: 2
Protein ID: Transcriptional regulator HU subunit alpha [Escherichia coli O157:H7 EDL933]
Calculated Mr = 9529; Calculated pI = 9.57

1 MNK**TQLIDVI AEKAELSKTQ** AKA**ALESTLA AITESLKEGD** AVQLVGFGTF
51 KVNHRARTG RNPQTGKEIK IAAANVPAFV SGKALKDAVK

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
----------	----------	----------	-------	------	-------	--------	------	--------	---------

565.3321 1128.6497 1128.6390 0.0107 0 54 0.17 1 U K.TQLIDVIAEK.A
506.6241 1516.8505 1516.8348 0.0157 0 64 0.015 1 U K.AALESTLAAITESLK.E

Mascot Score: 103 Queries Matched: 2
Protein ID: Cold shock-like protein [Escherichia coli]
Calculated Mr = 8626; Calculated pI = 9.16

1 MSIFHVKVIL MSKIKGNVKW FNESK**GFGFI** **TPEDGSK**DVF VHFSAIQTNQ
51 FKTLAGQQR**V** **EFEITNGAKG** PSAANVIAL

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
554.2945	1106.5745	1106.5608	0.0137	0	55	0.12	1	U	R.VEFEITNGAK.G
627.8053	1253.5961	1253.5928	0.0033	0	48	0.57	1	U	K.GFGFITPEDGSK.D

Mascot Score: 70 Queries Matched: 2
Protein ID: Variant Shiga-like toxin II [Escherichia coli]
Calculated Mr = 8738; Calculated pI = 5.26

1 ALVSVNAMAA DCAKGK**IEFS** **KYNENDTFTV** KVAGKEYWTS RWNLQPLLQS
51 AQLTGMTVTI **KSSTCESGSG** **FAEVQFNND**

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
612.6333	1834.8782	1833.8785	0.9997	1	35	12	6	U	K.IEFSKYNENDTFTVK.V
645.9257	1934.7552	1934.7589	-0.0037	0	34	11	1		K.SSTCESGSGFAEVQFNND.-

Mascot Score: 59 Queries Matched: 1
Protein ID: 50S Ribosomal protein L30 [Escherichia coli O157:H7 EDL933]
Calculated Mr = 6538; Calculated pI = 10.96

1 MAKTIKITQT RSAIGRLPKH **KATLLGLGLR** RIGHTVERED TPAIRGMINA
51 VSFMVKVEE

Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
457.3026	912.5906	912.5756	0.0149	0	59	0.049	1	U	K.ATLLGLGLR.R

Table S-5-B: Bottom-up peptide/protein identifications of excised gel band shown in **Figure S-3** using a MASCOT search, taxonomy: “viruses”. Stx identifications boxed in red.

Mascot Score: 245 Queries Matched: 5 Protein ID: Shiga toxin subunit B [Enterobacteria phage 933W] Calculated Mr = 9982; Calculated pI = 5.30									
1 MKKMFMVLF ALASVNAMAA DCAKGKIEFS KYNEDDTFTV KVDGKEYWTS 51 RWNLQPLLQS AQLTGMTVTI KSSTCESGSG FAEVQFNND									
Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
404.7345	807.4545	807.4490	0.0054	1	38	0.84	1		K.GKIEFSK.Y
616.2816	1230.5487	1230.5405	0.0082	0	64	0.0027	1	U	K.YNEDDTFTVK.V
612.6333	1834.8782	1834.8625	0.0157	1	62	0.0039	1	U	K.IEFSKYNEDDTFTVK.V
645.9257	1934.7552	1934.7589	-0.0037	0	34	1.5	1		K.SSTCESGSGFAEVQFNND.-
674.3537	2020.0394	2019.9789	0.0604	2	48	0.13	1	U	K.GKIEFSKYNEDDTFTVK.V

Mascot Score: 157 Queries Matched: 5 Protein ID: Shiga toxin 2, subunit B [Escherichia coli O157:H7 str. EC4113] Calculated Mr = 9965; Calculated pI = 7.74									
1 MKKMFMVLF ALVSVNAMAA DCAKGKIEFS KYNENDTFTV KVAGKEYWTS 51 RWNLQPLLQS AQLTGMTVTI KSSTCESGSG FAEVQFNND									
Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
404.7345	807.4545	807.4490	0.0054	1	38	0.84	1		K.GKIEFSK.Y
616.2816	1230.5487	1229.5564	0.9922	0	20	62	4	U	K.YNENDTFTVK.V
612.6333	1834.8782	1833.8785	0.9997	1	35	1.8	2	U	K.IEFSKYNENDTFTVK.V
645.9257	1934.7552	1934.7589	-0.0037	0	34	1.5	1		K.SSTCESGSGFAEVQFNND.-
674.3537	2020.0394	2018.9949	1.0445	2	30	9.2	2	U	K.GKIEFSKYNENDTFTVK.V

Mascot Score: 94 Queries Matched: 3 Protein ID: Shiga toxin 2e B-subunit [Enterobacteria phage phiP27] Calculated Mr = 9758; Calculated pI = 9.34									
1 MKKMFIAVLF ALVSVNAMAA DCAKGKIEFS KYNEDNTFTV KVSGREYWTN 51 RWNLQPLLQS AQLTGMTVTI ISNTCSSGSG FAQVKFN									
Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
404.7345	807.4545	807.4490	0.0054	1	38	0.84	1		K.GKIEFSK.Y
612.6333	1834.8782	1833.8785	0.9997	1	29	7.9	3	U	K.IEFSKYNEDNTFTVK.V
674.3537	2020.0394	2018.9949	1.0445	2	28	15	3	U	K.GKIEFSKYNEDNTFTVK.V