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Proteomic and bioinformatic analysis of outer membrane proteins of the protobacterium *Bartonella henselae* (Bartonellaceae)

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ABSTRACT. *Bartonella henselae*, an infectious agent causing cat-scratch disease and vasculoproliferative disorders in humans, is a fastidious facultative intracellular pathogen. The outer membrane proteins of *B. henselae* are key molecules that play a primary role in host-cell interactions. We isolated *B. henselae* outer membrane proteins, using the ionic detergent N-lauroyl sarcosine sodium salt and sodium carbonate, purification by two-dimensional (2-D) gel electrophoresis, and protein identification using mass spectrometry. Treatment with buffers containing ASB-14 and ZWITTERGENT 3-10 increased solubilization of *B. henselae* proteins, particularly proteins with basic p*I*. Three hundred and sixty-

eight spots were detected from the sarcosine-insoluble outer membrane fraction; 94 distinct protein species were identified from 176 spots. In the outer membrane fraction from carbonate incubation, 471 spots were calculated and 259 spots were identified, which included 139 protein entries. There were six outer membrane proteins in the sarcosine-insoluble outer membrane fraction compared with nine outer membrane proteins from samples subjected to carbonate incubation. We used bioinformatic analysis to identify 44 outer membrane proteins by prediction of their domains and tertiary structures and documented the potential virulence factors. We established the 2-D reference maps of the outer membrane subproteome of *B. henselae* using the two different extraction methods, which were partly complementary to each other. Sodium carbonate extraction isolated low-abundance and basic proteins better than the lauroyl sarcosine sodium salt extraction, which enriched high-abundance porins.

Key words: *Bartonella henselae*; Outer membrane protein; 2-D gel electrophoresis; MS; Bioinformatics

INTRODUCTION

Bartonella spp is a genus of aerobic, fastidious Gram-negative, facultative intracellular bacilli. Nine of the 21 species identified cause the various *Bartonella* diseases in human: *B. bacilliformis*, *B. elizabethae*, *B. henselae*, *B. quintana*, *B. koehlerae*, *B. grahamii*, *B. vinsonii* subsp *Berkhoffii*, and *B. vinsonii* subsp *arupensis*. Cat scratch disease (CSD) is a typically benign and self-limiting illness, mostly caused by *B. henselae. B. henselae* is detected in association with a wide range of wild and domestic feline hosts and has worldwide distribution. Wild and domestic cats serve as the natural reservoir for *B. henselae*, which establish long-term infections in erythrocytes typically without inducing disease symptoms. If transmitted to humans, who are only an incidental host, *B. henselae* may cause CSD, bacteremia with fever, bacillary angiomatosis and peliosis, endocarditis, and neuroretinitis.

In recent years, studies of *Bartonella* pathogenesis have taken a major step forward. With regard to the pathogenicity factors of *Bartonella*, studies point to outer membrane proteins (OMPs) of *Bartonella* as being important, where they are essential for attachment, invasion, and survival within host cells, as seen in other Gram-negative human pathogenic bacteria (Dehio, 2004). Since OMPs are the interface between the bacterium and the host cells, the outer membrane (OM) and individual OMPs are targeted in developments of diagnostic markers and vaccines against many bacterial diseases. The sarcosine-insoluble OM fraction of *B. henselae* and *B. quintana* was examined in more detail. By means of two-dimensional gel electrophoresis (2-DE) combined with matrix-assisted laser desorption-ionization time-of-flight-mass spectrometry (MALDI-TOF-MS), seven OMPs of *B. henselae* and ten OMPs of *B. quintana* were previously identified (Rhomberg et al., 2004; Boonjakuakul et al., 2007). Two-dimensional gel electrophoresis is the main platform used in proteomics and has been used in studies on profiling of membrane proteins. Although membrane proteins have been underrepresented in 2-DE gels due to difficulties in extracting and solubilizing them in the isoelectrofocusing (IEF) sample buffer, comparatively high amounts of soluble OMPs, which are potential candidates for diagnosis and vaccination, have been represented in 2-DE gels.

In recent years, advances in the solubilization of intractable proteins have prompted the proteomic analysis of OMPs, especially in various bacteria including *Escherichia coli* (Molloy et al., 2000), *Aeromonas salmonicida* (Ebanks et al., 2005) and *Dickeya dadantii* (Babujee et al., 2007). Because of the importance of OMPs for the molecular basis of pathogenesis and diagnosis and advances of 2-DE technique, we focused on these fractions and developed a technique for 2-DE of *Bartonella* OMPs using carbonate incubation compared with sodium lauryl sarcosine incubation for evaluating the differentiation of the two OMP extraction methods, followed by improved solubilization conditions for array by 2-DE using the zwitterionic detergent ZWITTERGENT 3-10, ASB-14 and immobilized pH gradients. In addition, by exploiting the genome sequence of *B. henselae* strain Houston-1, we scanned *in silico* for proteins predicted to be localized at the cell surface and compared predicted and experimentally identified *B. henselae* OMPs.

MATERIAL AND METHODS

Strains and culture conditions

B. henselae strain Houston-1 (ATCC 49882) was routinely grown on tryptic soy agar containing 5% defibrinated sheep blood (TSB agar) in a humidified atmosphere with 5% CO_2 at 37°C and harvested for protein preparation after 6 days.

Sample preparation of *Bartonella* OMPs

Isolation of cell membranes by N-lauroyl sarcosine sodium salt (LSS) method

The isolation of cell membranes using the LSS (Merck, Germany) method was performed according to Rhomberg's modified procedure (Rhomberg et al., 2004). In brief, 5×10^8-1×10^9 colony-forming units/mL bacteria were harvested from 6-10 TSB agar plates, washed in low-salt phosphate-buffered saline (3 mM KCl, 1.5 mM KH_2PO_4, 68 mM NaCl, 9 mM NaH_2PO_4), and pelleted twice by centrifugation at 2500 *g* for 10 min at 4°C. The pellet was resuspended in 4 mL hyperosmolar buffer (0.2 M Tris, pH 8.0, 0.5 M sucrose, 250 µg/mL lysozyme, 1 mM EDTA) and incubated at 4°C for 1 h. Subsequently, one tablet protease inhibitor cocktail (Roche, Germany) was added, and bacteria were lysed by repeated sonication using a Bandelin HD3200 (single bursts at a 40% amplitude level for no longer than 5 s with cooling in ice-cold water to avoid heat denaturation), until the solution turned translucent. Cell debris was removed by centrifugation for 30 min at 2500 *g* at 4°C. The supernatant was then cleared by centrifugation in a HITACHI CP80MX ultracentrifuge with a P55AT-784 rotor for 90 min at 100,000 *g* at 4°C. The resulting total membrane pellet was resuspended in a lysis buffer (10 mM HEPES, pH 7.4, 1% (w/v) lauryl sarcosine), incubated at room temperature for 20 min, and pelleted by ultracentrifugation under the same conditions. The lauryl sarcosine-insoluble pellet that contains the outer membrane-peptidoglycan complex was washed twice in 10 mM HEPES, pH 7.4, to remove residual detergent and centrifuged at 25,000 *g* for 30 min at 4°C; the washed pellet was then stored at 70°C.

Isolation of cell membranes by sodium carbonate (Na_2CO_3) method

The isolation of cell membranes using the Na_2CO_3 method was performed according

to the procedure modified by Molloy et al. (2000). Briefly, harvesting and washing of bacteria were performed as in the preceding procedure. The pellet was resuspended in 10 mL 50 mM Tris-HCl buffer, pH 7.5, supplemented with the protease inhibitor cocktail. Bacteria were lysed by repeated sonication using a Bandelin HD3200 (single bursts at a 40% amplitude level for no longer than 5 s with cooling in ice-cold water to avoid heat denaturation), until the solution turned translucent, and the unbroken cells were removed by centrifugation at 2500 *g* for 10 min at 4°C. The supernatant was diluted with ice-cold 0.1 M sodium carbonate, pH 11, to a final volume of 50 mL and stirred slowly for 1 h at 4°C. The carbonate-treated membranes were collected by ultracentrifugation in a HITACHI CP80MX P55AT-784 rotor at 100,000 *g* for 1 h at 4°C. The supernatant was discarded and the membrane pellet was resuspended and washed in 2 mL 50 mM Tris-HCl buffer, pH 7.5. The pellet was collected by centrifugation at 100,000 *g* for 20 min (HITACHI CP80MX P55AT-784 rotor) at 4°C.

Protein solubilization and quantitation

Outer membranes were resuspended in the solubilization buffer (5 M urea, 2 M thiourea, 2% CHAPS, 1% ZWITTERGENT 3-10, 1% ASB-14, 1% DTT). To avoid precipitation of urea, the suspension was then centrifuged at 16,000 *g* for 15 min at 23°C. The supernatant containing solubilized OMPs was collected, protein content quantified by the 2-D QUANT Kit (Amersham Biosciences, USA), and immediately used or stored at -70°C.

Two-dimensional electrophoresis

Isoelectric focusing electrophoresis

Proteins were separated in the first dimension using the IPGphor™ isoelectric focusing system (Amersham Biosciences) employing immobilized pH gradient strips (Amersham Biosciences), according to Amersham Biosciences manuals. The OMP samples were focused to their isoelectric points using 24 cm Immobiline™ DryStrip gels, pH 3-10 (Amersham Bioscience) under the following conditions: 30 V for 5 h, 60 V for 6 h, 100 V for 1 h, 300 V for 1 h, 600 V for 1 h, 1000 V for 1 h, and 8000 V for 11 h. For the sarcosine-insoluble OMP fraction, 600 μg protein was loaded, and for the OMP of sodium carbonate preparations, 800 μg was loaded.

Following isoelectric focusing, the proteins were reduced and bound to sodium dodecyl sulfate (SDS) by equilibrating each strip for 15 min in 10 mL SDS equilibration buffer (50 mM Tris-HCl, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.007% (w/v) bromophenol blue) containing 100 mg dithiothreitol (added fresh before use). A second equilibration step in SDS equilibration buffer containing 480 mg iodoacetamide (added fresh before use) instead of dithiothreitol was performed.

SDS-PAGE

After equilibration, the immobilized pH gradient strips were loaded onto 12.5% (w/v) homogeneous acrylamide gels (1 mm thickness × 24 cm width × 19 cm length), sealed with 0.5% (w/v) agarose. Protein samples that contained 600 μg LSS and 800 μg Na_2CO_3 of the solubilized membrane proteins were separated by electrophoresis with the Ettan DALT II System (Amersham Biosciences) at 2.5 W/gel for 30 min at 20°C, and then, the conditions were

changed to 18 W/gel and the gels run until the bromophenol blue dye migrated to about 1 cm from the bottom of the gel. The gels were kept in the fixing solution (dehydrated alcohol:glacial acetic acid:deionized water; 4:1:5) for 30 min, stained with Coomassie brilliant blue G-250 (Neuhoff et al., 1985) (Bio-Rad, USA) overnight, and destained with 1% glacial acetic acid.

Analysis of gels

Individual gels were visualized using an image scanner (Amersham Biosciences) with a transparency adapter using an 8-bit grayscale, 400 dpi. Images were analyzed using the ImageMaster™ 2-D Platinum 5.0 software.

Protein enzymolysis

All protein spots of stained gels were excised and gel digestion performed as previously described (Zou et al., 2006). The gel pieces were washed with 100 µL 25 mM ammonium bicarbonate, pH 8.0 (Fluka, USA) for 20 min, followed by washing with 50 µL 30% acetonitrile (Sigma, USA) containing 0.1 M ammonium bicarbonate until the color disappeared, and vacuum freeze-dried. Lyophilized samples were reconstituted in digestion buffer consisting of 25 mM ammonium bicarbonate and 3 µL trypsin solution containing 20 µg/mL trypsin (Sigma) and incubated at 37°C for 16 h. Supernatants were collected and gel pieces extracted twice with a 40-µL extraction buffer containing 50% acetonitrile and 5% trifluoroacetic acid (Fluka) for 60 min each time. The peptide containing supernatant was transferred to a new microtiter plate and pre-frozen at -80°C for 2 h and then dried under vacuum.

MALDI-TOF-MS

Lyophilized samples were dissolved in a 2-µL solution containing deionized water, 50% acetonitrile and 0.5% trifluoroacetic acid, and 0.5 µL of the solubilized proteins was mixed with 0.5 µL matrix (saturated solution of α-cyano-4-hydroxyl-cinnamic acid; Sigma) on a sample plate; the droplet was then left to dry at room temperature. Protein identifications were carried out using a 4700 MALDI-TOF/TOF-MS (Applied Biosystems, Foster City, CA, USA). The mass spectra were obtained using the 4000 Series Explorer™ software, version 3.0, in the positive ion reflector mode with a mass accuracy of approximately 20 ppm. The MALDI-MS was equipped with a 200-Hz frequency-tripled Nd:YAG laser operating at a wavelength of 355 nm. The MS spectra were acquired in the mass range between 800 and 4000 Da, using 1500 laser shots.

Database searching

The experimental MS data were matched to the corresponding virtual peptide mass database derived from the GPS Explorer™ v3.6 software (Applied Biosystems) and the Mascot v2.1 software (Matrix Science, London, UK) search protein database and NCBInr database (ftp://ftp.ncbi.nih.Gov/blast/db/FAST/nr.gz) to produce an experimental data set consisting of peptide mass fingerprints. One missed tryptic cleavage, variable modified carboxymethyl, and oxidation were selected. Data were searched at 100 ppm peptide mass tolerance. Identifications with a GPS confidence interval greater than 95% were accepted.

In silico analysis

Theoretical mass, p*I* and grand average of hydropathy (GRAVY) values of OMP candidates were calculated using the ProtParam tool (http://www.expasy.ch/tools/protparam.html) (Gasteiger et al., 2005). SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/) was used to hypothesize N-terminal secretory signal peptides of the identified proteins. HMMTOP version 2.0 (Tusnády and Simon, 2001) (http://www.enzim.hu/hmmtop/index.html) was used to predict transmembrane helices in the identified proteins. Predicted Subcellular Localization of OMPs of *B. henselae* strain Houston-1 was downloaded from the databases of PSORTdb (http://db.psort.org/) (Rey et al., 2005) and Proteome Analyst PA-GOSUB 2.5 (http://www.cs.ualberta.ca/~bioinfo/PA/GOSUB/) (Lu et al., 2004). Potential domains were identified by use of InterPro (http://www.ebi.ac.Uk/interpro/) and Pfam 24.0 (http://pfam.sanger.ac.uk/). Secondary and tertiary structure predictions were performed using SOPMA (Geourjon and Deleage, 1995), MODBASE (http://modbase.compbio.ucsf.edu/ModWeb20-html/modweb.html) (Pieper et al., 2009) and SWISS-MODEL (http://swissmodel.expasy.org//SWISS-MODEL.html) (Arnold et al., 2006).

RESULTS

2-DE of LSS method and the *in silico* analysis of protein assignment

Spot pattern and resolution of the two independent gels from two replicate samples were highly reproducible (Appendix Figure 1). In total, about 368 protein spots were exhibited on the 2-DE gels, and 176 spots belonging to *B. henselae* were verified by MS and searching NCBI nr databases for the *B. henselae* ATCC 49882 genome sequence (Alsmark et al., 2004) (Figure 1).

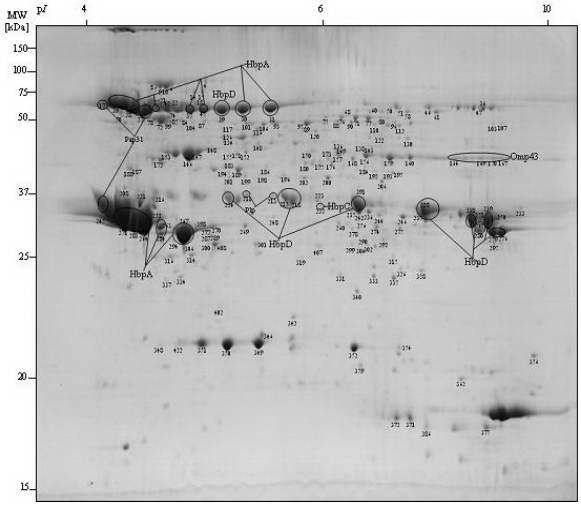

Figure 1. Sarcosine insoluble *Bartonella henselae* Houston-1 outer membrane proteins (OMPs) separated by 2-DE using pH 3-10 IPG and 12.5% SDS-PAGE. Proteins were stained with Coomassie brilliant blue G-250; spots were excised individually and then identified by peptide mass fingerprints (PMF). Each number indicates a protein for which a PMF identity was obtained; these numbers correspond to the protein identities shown in Appendix Table 1 and Appendix Table 2. Proteins in circles are annotated as OMPs.

The 176 spots (predicted p*I*s ranging from 4.81 to 9.33 and molecular mass ranging from 15.64 to 80.6 kDa) representing 94 different proteins belonged to 6 OMPs, 6 periplasmic (PP) and inner membrane proteins (IMPs), 59 cytoplasmic proteins (CPs) and 23 proteins with unknown subcellular localization. Of the identified proteins, 16 proteins possessed a GRAVY-value >0, indicating that some hydrophobic proteins were also isolated by this approach, including malate dehydrogenase, a hydrophobic protein with the highest GRAVY value (0.311) (Appendix Table 1).

To further characterize the proteins with unknown subcellular localization, *in silico* predictions by means of SignalP, HMMTOP, Pfam, and PA-SUB were carried out. There were only 7 proteins whose location could be predicted, including 5 CPs, 1 IMP and 1 PP (Appendix Table 2).

2-DE of Na_2CO_3 method and the *in silico* analysis of protein assignment

Spot pattern and resolution of the two independent gels from two replicate samples were highly reproducible (Appendix Figure 2). In total, about 471 protein spots were detected and 259 spots belonging to *B. henselae* were verified by MS and searching NCBI nr databases for the *B. henselae* ATCC 49882 genome sequence (Alsmark et al., 2004) (Figure 2). The 259 spots (predicted p*I*s ranging from 4.78 to 9.75 and molecular mass ranging from 15.7 to 89.0 kDa) representing 139 different proteins belonged to 9 OMPs, 8 PP and IMPs, 87 CPs and 35 proteins with unknown subcellular localization. Of the identified proteins, 28 proteins possessed a GRAVY-value >0, indicating that some hydrophobic proteins were also isolated by this approach, including malate dehydrogenase, a hydrophobic protein with the highest GRAVY value (0.311) (Appendix Table 3).

By using SignalP, HMMTOP, Pfam, and PA-SUB for analysis of the proteins with unknown subcellular localization, there were 8 proteins with signal peptides and 15 proteins

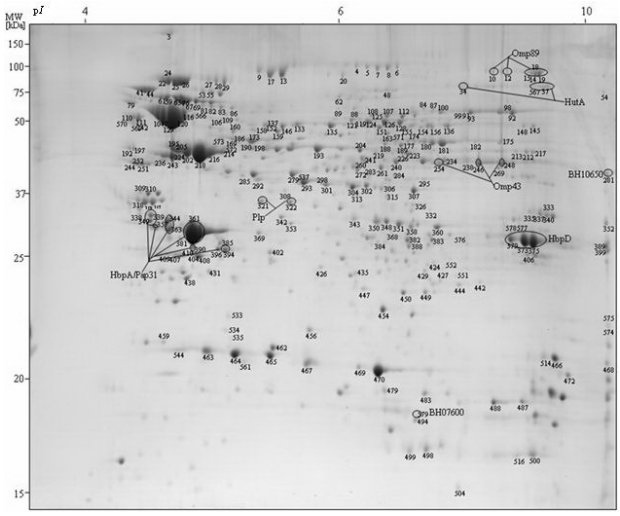

Figure 2. Carbonate insoluble *Bartonella henselae* Houston-1 outer membrane proteins (OMPs) separated by 2-DE using pH 3-10 IPG and 12.5% SDS-PAGE. Proteins were stained with Coomassie brilliant blue G-250; spots were excised individually and then identified by peptide mass fingerprints (PMF). Each number indicates a protein for which a PMF identity was obtained; these numbers correspond to the protein identities shown in Appendix Table 3 and Appendix Table 4. Proteins in circles are annotated as OMPs.

with 1 to 3 transmembrane helices in these proteins, of which 7 were assigned to CPs, IMP and PP (Appendix Table 4).

Comparison of MS identification results between LSS and Na_2CO_3 methods

In summary, six OMPs, including HbpA, HbpD, HbpC, Omp43, Pap31, and Plp, were identified using the LSS method. Besides, HbpA, HbpD, Omp43, Pap31, Plp, HutA, Omp89, BH10650, and BH07600 were extracted by using the Na_2CO_3 method. The differences between the two OMP purification methods are listed in Table 1.

Table 1. Comparison of mass spectrometry identification results between LSS and Na_2CO_3 methods.

Subcellular location	LSS method specific	Na_2CO_3 method specific	Common	Total
Outer membrane	1	4	5	10
Periplasm and inner membrane	2	5	4	11
Cytoplasm	9	37	49	95
Unknown	9	20	14	43
Total	21	66	72	159

LSS = N-lauroyl sarcosine sodium salt method; Na_2CO_3 = sodium carbonate method.

Characteristics of *B. henselae* OMPs identified

We identified a total of 10 OMPs, including HbpA, HbpD, HbpC, Pap31, Omp43, Plp, HutA, Omp89, BH10650, and BH07600, using the LSS and Na_2CO_3 methods (Table 2). Many of the OMPs were resolved into more than two protein species, although in some cases only a single-charged species was observed (e.g., HbpC), while for others (e.g., HbpA), multiple-charged protein species existed. Furthermore, 2-DE of LSS showed that two clusters of mass for HbpA and HbpD (25-30 and 68 kDa) existed.

To investigate potential functions of these OMPs of *B. henselae*, the Pfam database was searched for accessory domains. The proteins HbpA, HbpD, HbpC, Pap31, and Omp43 have features of porins. Plp is peptidyl-prolyl cis-trans isomerase, containing isomerase and rotamase domains. HutA, heme receptor, was found to have a tonB-dependent receptor plug domain.

Omp89, 798 AA, was an OMP of unknown function of *B. henselae*. The Pfam database indicated that there were five surface antigen variable number repeat domains (39-106AA, 107-184AA, 187-275AA, 278-357AA, 360-433AA) in the N-terminus of the amino acid sequence and one surface antigen domain (463-798AA) in the C-terminus. It exhibited 74-91 and 56-58% identity to the corresponding protein of *Bartonella* spp and *Brucella* spp, respectively. The second structure prediction using SOPMA exhibited 24.94% alpha helix, 26.69% extended strand, 6.52% beta turn, and 41.85% random coil.

BH07600, 159AA, was a putative uncharacterized protein with no functional annotation in Uniprot, and one SmpA_OmlA hit in the Pfam database. It is an OM lipoprotein exhibiting 78-84% amino acid identity to the homologous protein of *Bartonella* spp (based on the whole genome sequence of *B. bacilliformis*, *B. tribocorum*, *B. quintana*, and *B. grahamii*, available in the database) and 55-56% to that of *Brucella* spp. The second structure prediction exhibited 13.84% alpha helix, 30.82% extended strand, 11.95% beta turn, and 43.40% random coil by consensus prediction from multiple alignments.

Table 2. List of *Bartonella henselae* OMPs identified in this study by PMF using MALDI-TOF-MS and subsequent database query, including *in silico* analysis by assignment of Pfam domains (Pfam), predictions of *N*-terminal signal peptides (SignalP), predictions of subcellular localization (PSORTb, PA-SUB), and calculation of GRAVY.

Gene	Spot ID	Sequence coverage (%)	UniprotKB annotations					Predicted		Experimental		GRAVY[a] (P)[b]	PSORTb (P)[b]	PA-SUB model	Pfam E-value[c]	Pfam (P)[d]	SignalP site (P)[e]	Cleavage
			Locus	Accession No.	Protein	Cellular component	Molecular function	Mass	p*I*	Mass (kDa)	p*I*							
hutA	34/37/ 54/567	23/50/ 30/39	BH04970	Q6G471	Heme receptor precursor	Cell outer membrane	Receptor	82612.4	9.34	82.5	7.27, 8.80	-0.611	OM (10.00)	OM (100%)	TonB-dependent Receptor Plug Domain (PF07715)	7.00E-19	Yes (1)	28/29 (0.999)
omp89	10/12/14/ 15/18/19	37/33/65/ 56/63/60	BH06280	Q8VQ24	Outer membrane protein	Outer membrane	UNA	88985.9	9.01	93.2	7.87, 8.12, 8.72	-0.255	OM (10.00)	OM (100%)	Surface antigen variable number repeat (PF07244)	3.50E-11	Yes (0.933)	28/29 (0.324)
omp43	246/248/ 254	42/58/51	BH12500	Q9L7A9	Omp43 precursor	Membrane	Porin activity	44224.4	8.53	43.5	8.20-8.60	-0.254	OM (9.93)	OM (100%)	Porin_2 (PF02530)	3.70E-171	Yes (1)	22/23 (0.953)
UNA	281	57	BH10650	Q6G2V1	Outer membrane protein	Outer membrane	Transporter activity	48299.3	9.71	41.8	10.4	-0.075	OM (10.00)	OM (100%)	Outer membrane efflux protein (PF02321)	2.00E-26	Yes (0.994)	36/37 (0.994)
plp	321/322	43/54	BH02000	Q6G5U1	Peptidyl-prolyl cis-trans isomerase	UNK	Isomerase, Rotamase	35596.8	5.82	37.9	5.59, 5.73	-0.442	OM	OM (99.6%)	Rotamase (PF00639)	6.10E-38	Yes (1)	24/25 (0.999)
pap31	349/367/ 394	45/45/44	JC6528	UPI0000 17CA3D	31 K major protein, Pap31 - *B. henselae* phage	Cell outer membrane	Porin activity	30074.7	5.37	32.7, 31.0, 27.6	4.88, 5.37, 5.40	-0.207	OM (9.93)	OM (100%)	Porin_2 (PF02530)	9.80E-88	Yes (1)	22/23 (0.997)
pap31	363	39	AAK97307	Q94M28	Pap31	UNK	UNA	25663.7	4.90	30.24	4.75	-0.125	OM (9.94)	OM (100%)	Porin_2 (PF02530)	4.40E-58	Yes (0.997)	15/16 (0.994)
hbpA	339/357/ 390/344/ 361/338/ 363	39/45/ 44/37/ 38/37/ 37	BH02560	Q6G4S7	Hemin-binding protein a	Cell outer membrane, integral to membrane	Porin activity	29916.6	5.37	29.5-68.7	4.80, 5.56, 5.71	-0.205	OM (9.93)	OM (100%)	Porin_2 (PF02530)	5.10E-91	Yes (1)	22/23 (0.997)
hbpD	373/375/ 379/389/ 577/578	52/55/ 54/46/ 45/37	BH04810	Q6G487	Hemin-binding protein d	Cell outer membrane, integral to membrane	Porin activity	30269.7	9.06	31.2-68.7	5.44, 5.8, 6.19, 7.10, 8.10, 8.60	-0.158	OM (9.45)	OM (100%)	Porin_2 (PF02530)	3.40E-14	Yes (1)	22/23 (0.996)
UNA	579	54	BH07600	Q6G3K7	Putative uncharacterized protein	Outer membrane	UNA	17635.4	9.12	18.3	6.53	-0.025	Unknown	OM (100%)	Outer membrane lipoprotein (PF04355)	1.10E-19	Yes (0.987)	30/31 (0.560)
hbpC	232	32	BH02550	Q6G4S8	Hemin-binding protein c	Cell outer membrane	Porin activity	29956.3	8.95	35.5	5.98	-0.064	OM 10.00)	OM (100%)	Porin_2 (PF02530)	2.50E-17	Yes (1)	20/21 (0.996)

[a]Grand average of hydropathy (Kyte/Doolittle). [b]Designations of subcellular localization based on PSORT-B. Numbers refer to localization probability (from 0 to 10). [c]Expectation value. [d]Designations of signal peptide predictions based on SignalP. Numbers refer to probability assigned to the identification of a signal peptide (from 0 to 1). [e]Designations of cleavage site of signal peptide based on SignalP. Numbers refer to probability assigned to the cleavage site of a signal peptide (from 0 to 1). UNA = unannotated gene; UNK = unknown; OM = outer membrane.

BH10650, 440AA, was a bona fide OMP of *B. henselae*, which was identified as a presumptive outer membrane efflux protein (the OEP family) with Pfam (2 OEP domain hits), related to the type I secretion OMP, TolC of *Escherichia coli*. It exhibited 62-89% and 50% identity to the corresponding protein of *Bartonella* spp and *Brucella* spp. The second structure prediction exhibited 69.77% alpha helix, 5.23% extended strand, 2.05% beta turn, and 22.95% random coil.

Analysis of the *in silico* OM subproteome of *B. henselae*

PSORTdb (Rey et al., 2005), Proteome Analyst PA-GOSUB 2.5 (Szafron et al., 2004) and UniProt were interrogated to retrieve all *B. henselae* proteins annotated as OMPs (including hypothetical proteins) (Table 3). Twenty-nine OMPs were predicted by PSORTdb, except for DNA topoisomerase I, which was a chromosome component, and Plp and BH07600 were not predicted but detected in our study. There were 38 predicted OMPs using PA-GOSUB, and a total of 44 proteins were located on the outer membrane after removing the repetitive proteins in the two predicted results and UniProt. Of the 44 OMPs with p*I* 4.94-10, the predicted molecular mass ranged from 11.70 kDa for VirB3 to 323.67 kDa for BadA1, and there were 7 of these proteins with predicted molecular mass >100 kDa and 19 OMPs with predicted p*I* >9. Forty-one OMPs were predicted to be hydrophilic using the ProtParam tool, and 29 gene products were predicted by SignalP to possess an N-terminal secretion signal. To further substantiate this analysis, *in silico* predictions by means of Pfam, MODBASE, and SWISS-MODEL were carried out. Table 3 lists the 44 predicted OMPs, whose putative function was predicted based on domain and homology search results, including adhesion-associated proteins (e.g., BadA1 (Riess et al., 2007), filamentous hemagglutinin, etc.), autotransporters in the type V secretion systems (e.g., BH13140, BH13180, etc.), type IV secretion system protein VirB3, tonB-dependent receptor (HutA), porins (e.g., HbpA, HbpB, HbpC, and HbpD), hemolysin activation protein Hec, peptidoglycan-associated lipoprotein Pal transferase (BH11350), and others.

DISCUSSION

The bacterial OMPs mediating adhesion and host immune evasion between *Bartonella* and its hosts are critical targets of the host's immune response, often having diagnostic relevance, and are potential candidate antigens for vaccine developments. To date, there are only two studies that identified *Bartonella* surface proteins, including OMPs, using proteomic methods, namely 2-DE and MALDI-TOF-MS (Rhomberg et al., 2004; Boonjakuakul et al., 2007). These two reports mostly focused on the OM compartment prepared with lauryl sarcosine in order to identify potential pathogenicity factors of *B. henselae* (Rhomberg et al., 2004) and to identify the predominant *B. quintana* antigens (Boonjakuakul et al., 2007). Seven proteins (of 53 proteins analyzed) and 10 proteins (of 60 proteins analyzed), respectively, were identified as prototypical OMPs; however, PSORTb predicts a significantly higher number of OMPs in *B. henselae*. Thus, our aims were to develop a better method for the isolation of *Bartonella* OMPs and to further describe the OM subproteome of *B. henselae* based on 2-DE gels. Molloy et al. (2000) identified 21 of 26 (80%) of the predicted integral OMPs of *E. coli* that are annotated in SWISS-PROT release 37 using sodium carbonate extraction, which was the more efficient method for the isolation of *Bartonella* OMPs.

We analyzed the OM proteome of *B. henselae* by using the sarcosine- and carbonate-insoluble OM fraction (Figure 3). We identified six sarcosine-insoluble OMPs including HbpA,

Table 3. OMP list of bioinformatic analysis using PSORTdb, Proteome Analyst PA-GOSUB 2.5 and the proteins annotated as OMPs in UniProt.

Origins	Protein	Gene	Locus	Subcellular localization	Mass	p*I*	GRAVY	SignalP/ SecretomeP score	Molecular function/ biological process	Pattern (Pfam,ID)/ Tertiary (ModBase,ID)	Function of homologue
Psortb	Surface protein / *Bartonella* adhesin	*badA1*	BH01510	Outer membrane	323674.3	5.19	-0.533	-/0.954	Pathogenesis	Hep_Hag (PF05658), HIM (PF05662)/ left-handed beta-roll (1p9ha)	Collagen-binding, cell adhesion
	Filamentous hemagglutinin	*fhaB1*	BH06550	Outer membrane	280386.7	6.22	-0.28	-/0.905	Binding	Fil_haemagg (PF05594), Haemagg_act (PF05860)/beta-helix (1rwr)	Cell adhesion, type v secretion
	Filamentous hemagglutinin	*fhaB4*	BH07150	Outer membrane	278994.6	5.71	-0.302	-/0.934	Binding	Fil_haemagg (PF05594), Haemagg_act (PF05860)/beta-helix (1rwr)	Cell adhesion, type v secretion
	Filamentous hemagglutinin	*fhaB2*	BH06670	Outer membrane	278069	5.75	-0.297	-/0.935	Binding	Fil_haemagg (PF05594), Haemagg_act (PF05860)/beta-helix (1rwr)	Cell adhesion, type v secretion
	Hypothetical protein BH05490	UNA	BH05490	Outer membrane	138970.7	5.0	-0.332	-/0.921	UNK	Pertactin (PF03212),Autotransporter (PF03797)/ pertussis beta helix (1daba)	Cell adhesion
	Hypothetical genomic island protein	UNA	BH06590	Outer membrane	99331.2	6.34	-0.147	-/0.865	UNK	Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter
	Hypothetical protein BH09990	UNA	BH09990	Outer membrane	95450.4	4.94	-0.983	-/0.891	UNK	SPOR (PF05036)/transmembrane, inner membrane	Bacterial cell division protein
	Hypothetical protein BH05510	UNA	BH05510	Outer membrane	95156.1	8.44	-0.413	1/0.939	Cell adhesion	Pertactin (PF03212), Autotransporter (PF03797)/ pertussis beta helix (1daba)	Cell adhesion
	Probable surface protein	UNA	BH13140	Outer membrane	94913.9	8.71	-0.317	1/0.926	UNK	Autotransporter beta-domain (PF03797/ Transmembrane beta-barrels (1uyn)	Autotransporter
	Hypothetical protein BH13180	UNA	BH13180	Outer membrane	94426.9	8.56	-0.391	1/0.947	UNK	Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter
	Hypothetical protein BH05500	UNA	BH05500	Outer membrane	93092.5	7.65	-0.383	1/0.926	UNK	Pertactin (PF03212), Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter
	Outer membrane protein	*omp89*	BH06280	Outer membrane	88985.9	9.01	-0.255	0.933/-	UNK	bac_surface_ag (PF01103), Surf_Ag_VNR (PF07244)/2qdzA	Protein transport
	Heme receptor precursor	*hutA*	BH04970	Outer membrane	82612.4	9.34	-0.611	1/0.893	Receptor	TonB_dep_Rec (PF00593), Plug (PF07715) maltoporin (2fcp)	tonB-dependent receptor, ion transport
	Hypothetical protein	UNA	BH00450	Outer membrane	71579.9	9.47	-0.133	0.732/ 0.474	UNK	bac_surface_ag (PF01103)/UNK	Protein transport
	Hemolysin activation protein hec	UNA	BH07920	Outer membrane	66848.0	9.31	-0.138	-/0.582	UNK	POTRA domain (PF08479), ShlB (PF03865)/ beta barrel (2qdz)	Protein transport
	Hemolysin activator protein hec	UNA	BH06660	Outer membrane	66818.9	9.16	-0.131	-/0.715	UNK	POTRA domain (PF08479), ShlB (PF03865)/ beta barrel (2qdz)	Protein transport
	Hemolysin activator protein hec	UNA	BH07140	Outer membrane	66788.8	9.16	-0.135	-/0.605	UNK	POTRA domain (PF08479), ShlB (PF03865)/ beta barrel (2qdz)	Protein transport
	Hemolysin activator protein hec	UNA	BH06540	Outer membrane	65813.7	9.0	-0.137	-/0.614	UNK	POTRA domain (PF08479), ShlB (PF03865)/ beta barrel (2qdz)	Protein transport
	Hypothetical protein BH04050	UNA	BH04050	Outer membrane	62928.0	9.54	-0.171	0.793/0.15	UNK	UNK	UNK

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Table 3. Continued.

Origins	Protein	Gene	Locus	Subcellular localization	Mass	p*I*	GRAVY	SignalP/ SecretomeP score	Molecular function/ biological process	Pattern (Pfam,ID)/ Tertiary (ModBase,ID)	Function of homologue
	Hypothetical protein BH15480	UNA	BH15480	Outer membrane	54338.9	9.77	-0.198	0.735/0.283	Lipid binding, transport	Outer membrane efflux protein (PF02321)/ beta-barrel (1wp1)	Lipid binding, transporter activity
	Outer membrane protein	UNA	BH10650	Outer membrane	48299.3	9.71	-0.075	0.994/0.193	Transporter activity	Outer membrane efflux protein (PF02321)/ alpha helical barrel, beta barrel (1ek9)	Transport
	Omp43 precursor	UNA	BH12500	Outer membrane	44224.4	8.53	-0.254	1/0.867	Porin activity	Porin_2 (PF02530)/UNK	UNK
	Hemin binding protein b	*hbpB*	BH02570	Outer membrane	41403.2	9.25	-0.304	1/0.949	Porin activity	Porin_2 (PF02530)/beta barrel (1p4t)	UNK
	Hemin binding protein d	*hbpD*	BH04810	Outer membrane	30269.7	9.06	-0.158	0.994/0.887	Porin activity	Porin_2 (PF02530)/beta barrel (1p4t)	UNK
	Hemin binding protein c	*hpbC*	BH02550	Outer membrane	29956.3	8.95	-0.064	1/0.883	Porin activity	Porin_2 (PF02530)/beta barrel (1p4t)	UNK
	Hemin binding protein a	*hbpA*	BH02560	Outer membrane	29916.6	5.37	-0.205	1/0.946	Porin activity	Porin_2 (PF02530)/beta barrel (1p4t)	UNK
	Filamentous hemagglutinin	*fhaB6*	BH07930	Outer membrane	29562.3	9.36	0.231	1/-	Binding	Beta-helix (1rwr)	Cell adhesion, type v secretion
	Peptidoglycan-associated lipoprotein	*pal*	BH14790	Outer membrane	19369	8.9	-0.047	0.658/-	Lipoprotein	OmpA (PF00691)/*E. coli* Pal Protein (1oap)	Peptidoglycan binding, tol system
PA-GOSUB	Peptidyl-prolyl cis-trans isomerase	*plp*	BH02000	Outer membrane	35596.8	5.82	-0.442	1/-	Isomerase, Rotamase	Rotamase (PF00639)/piC-type peptidyl-prolyl cis-trans isomerase domain (3gpkA)	Peptidyl-prolyl cis-trans isomerase activity, isomerase activity
	Surface protein	UNA	BH01500	Outer membrane	16819.7	5.17	-0.437	-/0.827	UNK	Trimeric autotransporter (2gr8)	Adhesion, pathogenesis
	Outer membrane lipoprotein	UNA	BH01770	Outer membrane	13230.1	7.64	-0.061	0.910/ 0.068	Lipoprotein	UNK	UNK
	Expressed protein	UNA	BH05430	Outer membrane	90180.5	9.08	-0.442	0.999/0.598	Response to organic substance, outer membrane	OstA-like protein (PF03968), OstA_C (PF04453)	UNK
	Filamentous hemagglutinin	*fhaB3*	BH06750	Outer membrane	60592.9	5.79	-0.502	-/0.952	UNK	UNK	UNK
	Putative uncharacterized protein	UNA	BH07600	Outer membrane	17635.4	9.12	-0.025	0.987/ 0.090	UNK	SmpA_OmlA (PF04355)/2pxg	UNK
	Filamentous hemagglutinin	*fhaB7*	BH07940	Outer membrane	70211.1	5.93	-0.07	-/0.752	Binding	Fil_haemagg (PF05594) beta-helix (1rwr)	Adhesins, type v secretion hemagglutinin
	Competence lipoprotein comL	*comL*	BH11160	Outer membrane	34079.1	9.53	-0.437	0.699/0.210	Lipoprotein	OM_YfiO/ UNK	UNK
	Transmembrane protein	UNA	BH11350	Outer membrane	18454.5	9.52	0.114	0.873/0.293	Transmembrane	Transglycosylase SLT domain (PF01464)/ tetratricopeptide-like helical (1qsaA)	Transferase
	Putative uncharacterized protein	UNA	BH13020	Outer membrane	89053.5	6.24	-0.397	0.964/0.940	UNK	UNK	UNK

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Table 3. Continued.

Origins	Protein	Gene	Locus	Subcellular localization	Mass	pI	GRAVY	SignalP/ SecretomeP score	Molecular function/ biological process	Pattern (Pfam.ID)/ Tertiary (ModBase.ID)	Function of homologue
	Putative uncharacterized protein	UNA	BH13160	Outer membrane	85622.8	8.8	-0.321	1/0.878	Outer membrane	Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter, type V pathway
	Putative uncharacterized protein	UNA	BH14510	Outer membrane	42210.9	9.64	-1.091	-/0.716	UNK	UNK	UNK
	Outer membrane lipoprotein	UNA	BH16590	Outer membrane	19595.2	8.79	-0.097	0.747/0.923	Lipoprotein	OMP_19 (PF06604)/UNK	UNK
UniProt	Probable surface protein	UNA	BH13030	Outer membrane	214343.4	4.96	-0.239	0.963/0.934	UNK	Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter, type V pathway
	Acidic repeat protein	*arp*	BH13120	Outer membrane	155788.2	4.95	-0.455	0.951/0.918	UNK	Autotransporter beta-domain (PF03797)/ Transmembrane beta-barrels (1uyn)	Autotransporter, type V pathway
	Type IV secretion system protein virB3	*virB3*	BH13270	Outer membrane	11704.7	10	0.076	0.170/0.100	Virulence determinant, T4SS	UNK	UNK

The list was subdivided into 3 sections: the OMPs from Psortdb, the OMPs from Proteome Analyst PA-GOSUB 2.5, the OMPs from UniProt. UNK = unknown; UNA = unannotated gene.

HbpD, HbpC, Omp43, Pap31, and Plp. Sodium carbonate treatment resulted in the isolation of four additional proteins, including Omp89, HutA, BH10650, and BH07600 (Figure 3). Different 2-DE maps were obtained for OM proteins extracted using the LSS and carbonate methods. LSS OM protein fraction was enriched with the Hbp proteins, which are the products of the *hbpCAB/D* locus. HbpA and HbpD had the highest abundance among all identified OMPs and were detected as a cluster of proteins with different molecular mass of 25-30 kDa, corresponding to studies by Rhomberg et al. (2004) and Chenoweth et al. (2004); however, the higher molecular mass forms of 68 kDa were also expressed. The carbonate method provided better conditions to detect the alkaline protein fraction, including Omp89, HutA, BH10650, and BH07600, which were alkaline proteins except for the common OMPs with the LSS methods.

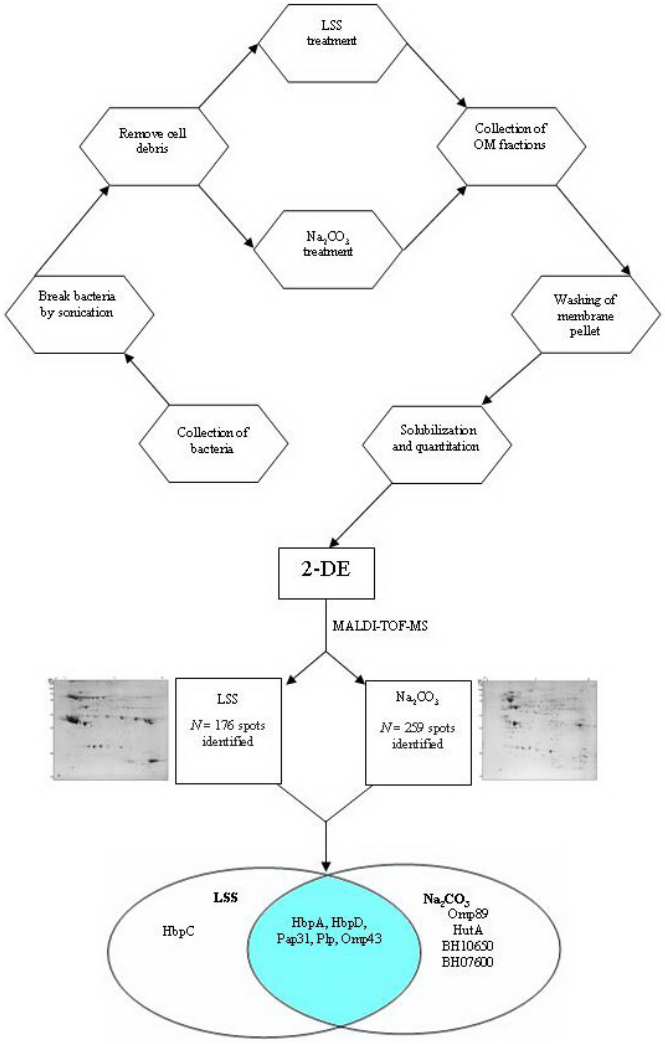

Figure 3. Workflow for sample preparation and numbers (*N*) of identified proteins and outer membrane proteins (OMPs) of *Bartonella henselae.*

The hemin-binding proteins, HbpA, HbpD and HutA, cell adhesion-associated protein Omp43 (Burgess et al., 2000), Omp89 and Plp have previously been identified in the sarcosine-insoluble OM fraction of *B. henselae* (Rhomberg et al., 2004). Hbp family members, which are homologous to *B. henselae* Pap31 (Zimmermann et al., 2003), *Brucella* Omp31, *Agrobacterium tumefaciens* Omp25 and *Neisseria meningitides* opacity proteins, contain eight β-barrel transmembrane domains and four extracellular loops (Minnick et al., 2003). HbpA plays an active role in hemin acquisition (Carroll et al., 2000), and the other Hbp family members constitute a compensatory iron-acquisition system important for *B. henselae* survival (Burgess et al., 2000). Omp43, which is a porin, and OM antigen and Omp89, which appeared to produce the strongest humoral response (Chenoweth et al., 2004), as the three major putative Fn-binding proteins, may play an important role in *B. henselae* adherence to and invasion of host cells (Dabo et al., 2006). We identified two hypothetical proteins, BH07600 and BH10650. BH07600, an OM lipoprotein, is possibly involved in maintaining the structural integrity of the cell envelope (Ochsner et al., 1999). BH10650 is a putative outer membrane efflux protein, which forms trimeric channels involved in the export of a variety of substrates in Gram-negative bacteria, such as *E. coli* (Higgins et al., 2004). Outer membrane protein TolC is the prototypical OM channel component involved in multi-drug resistant and type I secretion, which belongs to the resistance-nodulation-cell division protein family involved in the transport of various drugs across the cell envelope in bacteria; it exhibits broad substrate specificity and acts like an efflux pump (Yang et al., 2003; Hernandez-Mendoza et al., 2007). Antibiotic substrates of the drug efflux pump include erythromycin, tetracycline, chloramphenicol, ampicillin, and rifampin (Okusu et al., 1996; Zgurskaya and Nikaido, 1999), which are the preferred antibiotic treatments for *Bartonella* infections. *Bartonella* are susceptible to many antibiotics *in vitro* (including β-lactams, aminoglycosides, chloramphenicol, tetracyclines, macrolides, and rifampicin) (Maurin et al., 1995). Conversely, clinical data indicate that the bacteria may be more resistant *in vivo*. For *Bartonella*, in which potential mechanisms of drug efflux are not known, the other antibiotic resistance systems such as tetracycline resistance protein and macrolide-specific efflux proteins were identified by *in silico* genome analysis of *Bartonella* (Biswas et al., 2008), suggesting that some drug resistance systems including BH10650 may play an important role *in vivo* or in overexpressions.

One of the major problems encountered in membrane protein studies are the difficulty to solubilize these hydrophobic proteins using solutions compatible with their subsequent separation, digestion, and/or identification by MS analysis (Tan et al., 2008). The application of zwitterionic detergents such as ZWITTERGENT 3-10 and ASB-14 significantly increased the solubilization of the OMPs in the IEF sample buffer and resulted in the identification of more protein spots, especially in the alkaline p*I* range. Although a higher yield of extracted proteins was detected on the 2-DE gels, their MS identification may be incomplete due to i) the inherent lack of trypsin cleavage sites in these proteins, ii) poor accessibility to proteases in these regions, iii) tendency of the hydrophobic domains to aggregate and precipitate after the removal of SDS, and iv) difficulties in extracting these proteins (Tan et al., 2008). It has been well documented that cytoplasmic and periplasmic components and inner membrane proteins are present as contaminants in the OM preparation. This partially reflects their surface localization (e.g., ATPase, chaperon GroEL, FAD, NADH/NADPH-proteins), as well as the tight association between the inner and outer membranes or a technical artifact. The other components from the *Bartonella* cells besides OMPs were shown on the 2-DE profiles as we

increased the sample amounts compared with the previous studies (Molloy et al., 2000; Rhomberg et al., 2004) to detect more low-abundance OMPs.

Despite all this, the inherent limitation of solubility of membrane proteins in 2-DE (Jungblut et al., 2010) rendered our results with distinct differences relative to the OMPs from the *in silico* genome analysis. There were 44 OMPs predicted by the two predictor programs along with the Uniprot. There could have been multi-transmembrane alpha helix or β-barrel proteins in the 162 hydrophobic proteins from the predicted 540 proteins with unknown localization (data not shown). Therefore, it is possible that more *B. henselae* OMPs will be identified when optimized methods for their isolation become available.

Among the 44 predicted OMPs, 7 OMPs with molecular mass ≥100 kDa, including adhesin BadA, filamentous hemagglutinin and autotransporters, and 6 OMPs with p*I* ≥9.5, including VirB3, ComL and hypothetical proteins, which exceeded the resolution capacity of the pH 3-10 IPG strip, were lost from the 2-DE maps. For the other hypothetical proteins, further study is required to understand the lack of their detection by 2-DE. Monitoring of mRNA expression may determine if these proteins are transcribed and processed, and if they could be therefore expected on 2-DE gels. A membrane proteome is the entire complement of membrane proteins present in a cell at a given condition and time. Synthesis of proteins will be induced or repressed in the situation of growing on the artificial culture medium, especially for intracellular bacteria pathogens (Cash, 2006), and therefore, the actual number of proteins, which are the sort of proteins associated with adhesion and invasion observed on a 2-DE gel, is expected to be much lower. For settling the problems of 2-DE in separating membrane proteins, various alternative techniques in gel-based (e.g., blue native-PAGE [BN-PAGE], clear-native-PAGE [CN-PAGE], enzyldimethyl-*n*-hexadecylammonium chloride [BAC], and SDS/SDS [dSDS]-PAGE) and LC-based (free-flow electrophoresis [FFE] and multidimensional protein identification technology [Mud- PIT]) separation platforms have been employed in membrane proteomics.

We established a 2-DE map of the outer membrane subproteome of *B. henselae* using the sodium carbonate extraction method and the lauryl sarcosine extraction method. We identified a total of 10 proteins present in the OM (HbpA, HbpC, HbpD, Pap31, HutA, OMP89, OMP43, Plp, and two novel proteins BH10650 and BH07600) of *B. henselae.* To complement the inventory of the OMPs, a bioinformatic approach was performed with two programs, immunogenic proteins, adhesion-associated proteins, type I secretion system-associated protein outer membrane efflux protein, type IV secretion system-associated proteins VirB (Schulein et al., 2005), type V secretion system-associated protein autotransporters, type VI secretion-associated protein Pal and hemin-binding surface proteins, thus maximizing the number of protein species in the OM proteome. Although the analysis of detection and identification of membrane proteins remains a difficult task, 2-DE was used to display the soluble and highly abundant OMPs. These findings may help in the further characterization of *B. henselae* OMPs immunogenic candidates. Some of these proteins merit further investigations because of their immunodominant properties, conservation across serotypes, structure and functions, and *in vivo* expression in hosts.

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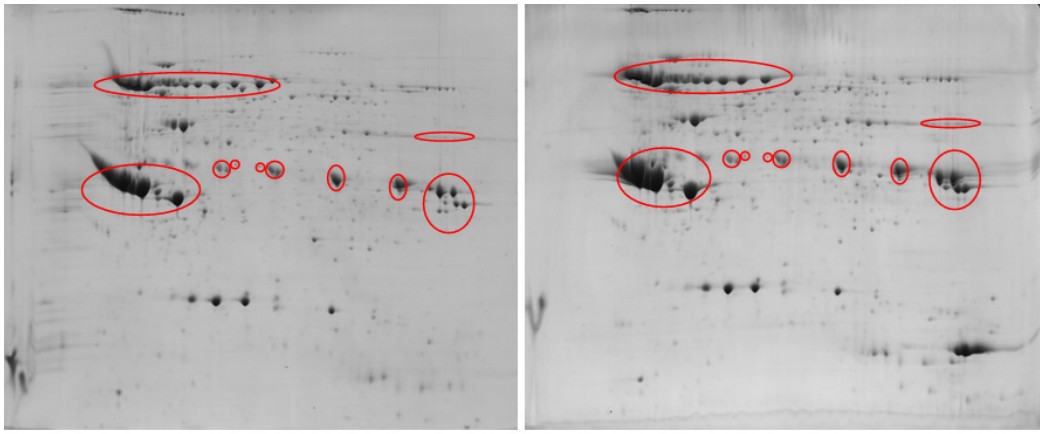
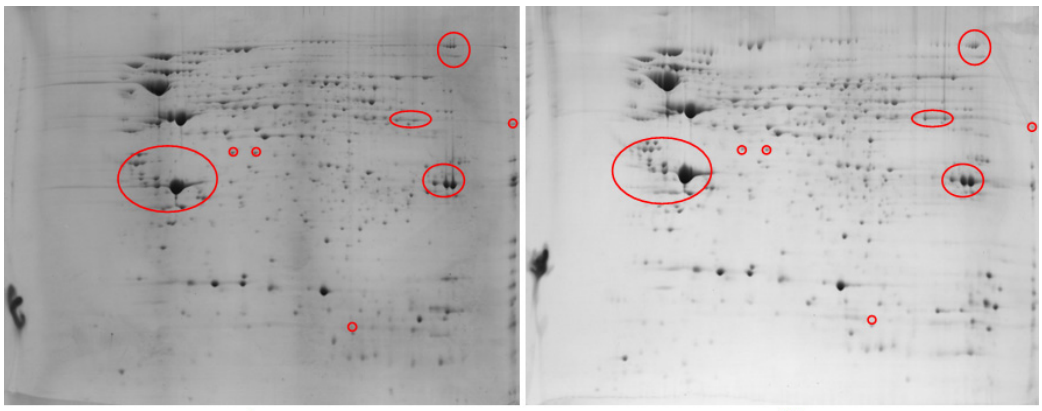
APPENDIX



Appendix Figure 1. Sarcosine-insoluble *Bartonella henselae* Houston-1 outer membrane proteins (OMPs) separated by 2-DE, using pH 3-10 IPG and 12.5% SDS-PAGE. Proteins were stained with Coomassie brilliant blue G-250. A and B were performed under the same conditions. Red circles indicate proteins annotated as OMPs.



Appendix Figure 2. Carbonate-insoluble *Bartonella henselae* Houston-1 outer membrane proteins (OMPs) separated by 2-DE, using pH 3-10 IPG and 12.5% SDS-PAGE. Proteins were stained with Coomassie brilliant blue G-250. A and B were performed under the same conditions. Proteins in red circles are annotated as OMPs.

Appendix Table 1. List of the locations and functions of sarcosine-insoluble *Bartonella henselae* Houston-1 proteins identified in this study by PMF using MALDI-TOF-MS.

Gene	Spot ID	Locus	UniprotKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function / Biological process	Mass	pI		
tuf	153/147	AAG24621	Q9F653	Elongation factor EF-Tu	Intracellular	Elongation factor	38255.5	4.94	-0.145	Cytoplasmic (9.97)
lpdA	90	AAR21288	Q67B06	Dihydrolipoyl dehydrogenase	Cytoplasm	FAD binding, dihydrolipoyl dehydrogenase activity	49371.8	6.05	0.143	Cytoplasmic (9.97)
hrcA	180	BH00550	Q6G564	Heat-inducible transcription repressor hrcA	UNA	Transcription repressor activity	39415.6	5.79	-0.200	Cytoplasmic (8.96)
rpsA	42/21/22	BH00930	Q6G5H4	30S ribosomal protein S1	Ribosome	RNA binding, structural constituent of ribosome	62779.6	5.18	-0.354	Cytoplasmic (8.96)
typA	14/15	BH01700	Q6G4X9	GTP-binding protein typA	Intracellular	GTP binding, GTPase activity	67181.0	5.30	-0.294	Cytoplasmic (9.26)
galU	256	BH01750	Q6G4X4	UTP-glucose-1-phosphate uridylyltransferase	UNA	UTP: glucose-1-phosphate uridylyltransferase activity	33226.3	6.56	-0.173	Cytoplasmic (8.96)
prfA	152/155	BH01970	Q6G5T7	Peptide chain release factor 1	Cytoplasm	Translation release factor activity, codon specific	40426.0	5.53	-0.402	Cytoplasmic (9.97)
pnp	4	BH02100	Q6G5F8	Polynucleotide phosphorylase/ polyadenylase	Cytoplasm	3'-5'-exoribonuclease activity, RNA binding	80601.8	5.36	-0.225	Cytoplasmic (9.97)
UNA	359	BH02210	Q6G4W1	Transcriptional regulator	UNA	Sequence-specific DNA binding	15644.1	6.34	-0.475	Cytoplasmic (8.96)
dnaJ2	233	BH04300	Q6G4D6	Heat shock protein DnaJ	UNA	Heat shock protein binding, unfolded protein binding	33238.3	9.33	-0.611	Cytoplasmic (9.97)
fabI2	275/274	BH04310	Q6G4D5	Enoyl-[acyl-carrier-protein] reductase	UNA	Binding,enoyl-[acyl-carrier-protein] reductase (NADH) activity	29349.1	6.54	0.025	Cytoplasmic (9.26)
ribA	176/175	BH04330	Q6G4D3	3,4-Dihydroxy-2-butanone 4-phosphate synthase	UNA	3,4-dihydroxy-2-butanone-4-phosphate synthase activity, hydrolase activity	40942.1	5.94	-0.146	Cytoplasmic (8.96)
tldD	122	BH04380	Q6G4C8	tldD protein	UNA	Peptidase	50764.8	6.33	-0.296	Cytoplasmic (9.26)
UNA	269	BH04780	Q6G490	Two-component response regulator	UNA	DNA binding, two-component response regulator activity	26068.5	5.46	-0.303	Cytoplasmic (9.97)
pyrE	333	BH05050	Q6G463	Orotate phosphoribosyltransferase	UNA	Magnesium ion binding, orotate phosphoribosyltransferase activity	20856.1	6.53	0.103	Cytoplasmic (8.96)
rplI	337/334	BH05290	Q6G449	50S ribosomal protein L9	Ribosome	rRNA binding, structural constituent of ribosome	22899.8	5.17	-0.478	Cytoplasmic (9.97)
fabF1	141	BH05370	Q6G441	3-oxoacyl-[acyl-carrier-protein] synthase II	UNA	Transferase activity, transferring acyl groups other than amino-acyl groups	44693.8	5.61	-0.024	Cytoplasmic (9.97)
pdhC	96	BH05770	Q6G403	Dihydrolipoamide acetyltransferase (E2)	Pyruvate dehydrogenase complex	Dihydrolipoyllysine-residue acetyltransferase activity, protein binding	47754.9	7.16	-0.112	Cytoplasmic (9.26)
ntrX	101/106	BH05850	Q6G3Z5	Nitrogen regulation protein	Intracellular	ATP binding, nucleoside-triphosphatase activity, transcription factor activity	50539.9	5.62	-0.206	Cytoplasmic (9.97)
tig	99/85	BH05940	Q6G3Y8	Trigger factor	UNA	Peptidyl-prolyl cis-transisomerase activity	53189.8	5.14	-0.539	Cytoplasmic (8.96)
gatB	84	BH05950	Q6G3Y7	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase subunit B	UNA	Glutaminyl-tRNA synthase (glutamine-hydrolyzing) activity	55499.2	5.17	-0.356	Cytoplasmic (8.96)
tufI	146/148	BH06020	Q8KHX9	Elongation factor Tu	Cytoplasm	GTP binding, GTPase activity, translation elongation factor activity	42839.2	5.30	-0.166	Cytoplasmic (9.97)
nusG	346	BH06050	Q6G3Y0	Transcription antitermination protein nusG	UNA	Transcription elongation regulator activity	20152.7	5.61	-0.335	Cytoplasmic (8.96)
frr	340	BH06250	Q6G5C6	Ribosome-recycling factor	Cytoplasm	Translational termination	20652.8	6.18	-0.358	Cytoplasmic (9.97)
lpxD	192/195/ 193	BH06290	Q8VQ23	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	UNA	Acyltransferase activity	36813.0	6.66	0.136	Cytoplasmic (8.96)
rpiA	318	BH06410	Q6G3V6	Ribose-5-phosphate isomerase A	UNA	Ribose-5-phosphate isomerase activity	25145.2	4.97	0.249	Cytoplasmic (8.96)
ibpA2	422/349/351/ 183/198/199/ 201/328/ 350	BH07300	Q6G5C1	Small heat shock protein	UNA	Response to stress	19073.5	5.56	-0.707	Cytoplasmic (8.96)
fabF2	134	BH07440	Q6G3M3	3-oxoacyl-[acyl-carrier-protein] synthase	UNA	Catalytic activity	45213.3	5.45	-0.092	Cytoplasmic (9.26)
accC	120	BH08050	Q6G3G8	Biotin carboxylase	UNA	ATP binding, biotin binding, ligase activity	49396.3	5.88	-0.102	Cytoplasmic (8.96)
nifS1	143	BH08610	Q6G3C2	Nitrogenase co-factor synthesis protein nifS	UNA	Cysteine desulfurase activity, pyridoxal phosphate binding	46509.7	6.34	-0.209	Cytoplasmic (8.96)

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Appendix Table 1. Continued.

Gene	Spot ID	Locus	UniprotKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function / Biological process	Mass	pI		
nuoE	302	BH08910	Q6G392	NADH dehydrogenase I,E subunit	UNA	NAD or NADH binding, electron carrier activity, oxidoreductase activity	24961.8	6.45	-0.327	Cytoplasmic (8.96)
glpX	189/186	BH10020	Q6G312	Glycerol-inducible protein	UNA	Glycerol metabolic process	35141.2	5.53	-0.078	Cytoplasmic (8.96)
ppiB1	348	BH10090	Q6G306	Peptidyl-prolyl cis-transisomerase	UNA	Peptidyl-prolyl cis-transisomerase activity	18849.5	5.03	-0.314	Cytoplasmic (9.26)
glnA2	117	BH10160	Q6G2Z9	Glutamine synthetase	Cytoplasm	Glutamate-ammonia ligase activity	52640.3	5.47	-0.315	Cytoplasmic (9.97)
recA	194	BH10230	Q6G2Z3	Protein recA	Cytoplasm	ATP binding, DNA-dependent ATPase activity, damaged DNA binding	37515.8	5.40	-0.054	Cytoplasmic (8.96)
rpoA	187/188	BH10270	Q9FDC7	DNA-directed RNA polymerase subunit alpha	UNA	Nucleotidyltransferase, Transferase	37648.5	4.85	-0.236	Cytoplasmic (9.97)
adk	335	BH10300	Q6G2Y6	Adenylate kinase	Cytoplasm	ATP binding, adenylate kinase activity	21467.4	6.63	-0.193	Cytoplasmic (9.97)
fusA	6/10/9/151	BH10540	Q8KQB3	Elongation factor G	Cytoplasm	GTP binding, GTPase activity, translation elongation factor activity	76284.9	5.12	-0.233	Cytoplasmic (9.97)
xthA1	278	BH10570	Q6G2V9	Exodeoxyribonuclease III	Intracellµlar	DNA binding, endonuclease activity	30111.3	6.18	-0.553	Cytoplasmic (9.97)
pcm2	316	BH10660	Q6G2V0	Protein-L-isoaspartate (D-aspartate) O-methyltransferase	UNA	Protein-L-isoaspartate (D-aspartate) O-methyltransferase activity	24398.8	5.14	0.303	Cytoplasmic (9.26)
murE	110/91	BH11290	Q6G2Q0	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	Cytoplasm	ATP binding, acid-amino acid ligase activity	52821.6	6.61	-0.099	Cytoplasmic (9.97)
ctrA	407	BH12050	Q6G2J6	Cell cycle transcriptional regulator CtrA	UNA	DNA binding, two-component response regµlator activity	26279.9	5.90	-0.058	Cytoplasmic (9.97)
gcvT	168/173	BH12840	Q6G2E9	Aminomethyltransferase	Cytoplasm	Aminomethyltransferase activity, transaminase activity	40529.1	6.21	0.010	Cytoplasmic (9.97)
groEL	70/72/78/74	BH13530	O33963	Chaperonin GroEL	Cytoplasm	ATP binding, unfolded protein binding	57589.5	5.10	-0.041	Cytoplasmic (9.97)
fumC	130	BH13550	Q6G294	Fumarate hydratase	Tricarboxylic acid cycle enzyme complex	Fumarate hydratase activity	50119.6	6.84	-0.139	Cytoplasmic (9.26)
atpD	87/95/ 88/104	BH15320	Q6G1W9	ATP synthase subunit beta	Plasma membrane	Hydrolase	56559.4	5.44	-0.082	Cytoplasmic (8.96)
purH	60	BH15970	Q6G5S5	Bifunctional purine biosynthesis protein purH	UNA	Hydrolase, transferase	57560.8	6.41	0.053	Cytoplasmic (8.96)
sucC	172	BH16560	Q6G1M1	Succinyl-CoA ligase [ADP-forming] subunit beta	UNA	Ligase	42790.3	5.07	-0.035	Cytoplasmic (8.96)
mdh	196	BH16570	Q6G1M0	Malate dehydrogenase	UNA	L-malate dehydrogenase activity, binding	33642.8	5.75	0.311	Cytoplasmic (9.26)
rho	126	BH16700	Q6G1K7	Transcription termination factor rho	Proton-transporting two-sector ATPase complex, catalytic domain	Hydrolase	47371.9	5.47	-0.206	Cytoplasmic (8.96)
pdhA	200	BH05750	Q8L1Z6	Pyruvate dehydrogenase E1 component alpha subunit	Intracellular membrane-bounded organelle	Pyruvate dehydrogenase (acetyl-transferring) activity	38009.1	6.04	-0.354	Cytoplasmic membrane (9.46)
UNA	408	BH08630	Q6G3C0	ATP-dependent transporter	UNA	ATP binding, ATPase activity	27835.7	5.61	-0.071	Cytoplasmic membrane (8.02)
asd	184	BH12890	Q6G2E5	Aspartate-semialdehyde dehydrogenase	Cytoplasm	NADP or NADPH binding, aspartate-semialdehyde dehydrogenase activity	37069.6	6.20	-0.036	UNK
ftsZ	18	BH11180	O69074	Cell division protein FtsZ	Cytoplasm	GTP binding, GTPase activity	62352.3	5.25	-0.256	UNK
pepA	138	BH02040	Q6G5G4	Leucyl aminopeptidase	Intracellular	Aminopeptidase, hydrolase, protease	50247.4	6.31	-0.106	UNK
rplJ	356	BH06080	Q6G3X7	50S ribosomal protein L10	Ribosome	Ribonucleoprotein, ribosomal protein	18154.9	9.56	0.084	UNK
rpsB	246	BH06220	Q6G5C9	30S ribosomal protein S2	Small ribosomal subunit	Ribonucleoprotein, ribosomal protein	28363.4	6.66	-0.238	UNK

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Appendix Table 1. Continued.

Gene	Spot ID	Locus	UniprotKB annotations			Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function / Biological process	Mass	pI		
rplY	314	BH11850	Q6G2L2	50S ribosomal protein L25/general stress protein Ctc	Ribosome	Ribonucleoprotein, ribosomal protein	22750.9	5.18	-0.175	UNK
accA	235/242	BH16340	Q6G1P1	Acetyl-CoA carboxylase, carboxyl transferase alpha subunit	Cytoplasm	Ligase, transferase	34948.9	6.27	-0.292	UNK
degP	46/61	BH04770	P54925	Probable periplasmic serine protease DO-like	Periplasm	Hydrolase, protease, serine protease	54113.3	8.56	-0.21	Periplasmic (9.44)
hrA2	103/107	BH10250	Q6G2Z1	Serine protease	UNA	Protein binding, serine-type endopeptidase activity	50562.1	9.02	0.026	Periplasmic (9.44)
UNA	300	BH06370	Q6G3W0	ABC transporter, periplasmic amino acid-binding protein	UNA	Transporter activity	28179.4	5.54	-0.166	Periplasmic (10.00)
atpA	93/89	BH15340	Q6G1W7	ATP synthase subunit alpha	Cell inner membrane, cell membrane	Hydrolase	55511.6	5.91	-0.061	UNK
pstB	292	BH02460	Q6G4T6	Phosphate import ATP-binding protein pstB	Inner membrane, cell membrane	Hydrolase	28030.2	6.46	-0.157	UNK
Pap31	17/27/245	JC6528	UPI0000 17CA3D	31 K major protein, Pap31 - *Bartonella henselae* phage	Cell outer membrane	Porin activity	30074.7	5.37	-0.207	OM (9.93)
hbpA	25/28/30/31/37/ 39/45/208/271/ 277/283/289/306	BH02560	Q6G4S7	Hemin-binding protein a	Cell outer membrane, integral to membrane	Porin activity	29916.6	5.37	-0.205	OM (9.93)
hbpD	29/36/47/212/ 218/224/228/ 237/238/239/ 250/262/276/ 279/280/391	BH04810	Q6G487	Hemin-binding protein d	Cell outer membrane, integral to membrane	Porin activity	30269.7	9.06	-0.158	OM (9.45)
hbpC	232	BH02550	Q6G4S8	Hemin-binding protein c	Cell outer membrane	Porin activity	29956.3	8.95	-0.064	OM (10.00)
ompA3	170	BH12500	Q9L7A9	Omp43 precursor	Membrane	Porin activity	44224.4	8.53	-0.254	OM (9.93)
plp	211	BH02000	Q6G5U1	Peptidyl-prolyl cis-transisomerase	UNA	Isomerase Rotamase	35596.8	5.82	-0.442	Outer membrane integral membrane protein

UNA = unannotated; UNK = unknown.

Appendix Table 2. Analysis of sarcosine-insoluble *Bartonella henselae* Houston-1 proteins with subcellular location unknown identified in this study by PMF using MALDI-TOF-MS.

Gene	Spot ID	Locus	UniProtKB annotations			Predicted		GRAVY	PA-SUB (P)	Pfam model (ID)	Pfam E-value	Signal (P)	HMMTOP
			Accession No.	Protein name	Molecular function	Mass	pI						
guaB	71/77/75/80/76/68	BH01800	Q6G4W9	Inosine-5'-monophosphate dehydrogenase	IMP dehydrogenase activity	52816.6	6.29	0.015	No positive predictions	IMPDH (PF00478)	2.3E-225	No	0
UNA	221	AAL13209	Q93FN9	35 kDa protein	UNA	31632.1	4.97	-0.558	No positive predictions	Collar (PF07484)	5.9E-22	No	0
UNA	159/160	BH14010	Q6G265	Expressed protein	UNA	41031.7	6.98	-0.258	No positive predictions	Tubulin (PF00091)	4.1E-88	No	1
parA1	319	BH13200	Q6G2C5	parA protein	Cobyrinic acid a, c-diamide synthase activity	22269.6	5.81	0.222	Cytoplasm (60.9%)	DUF1256 (PF06866)	0.92	No	0
UNA	398	BH14040	Q6G262	Hypothetical protein BH14040	UNA	29426.6	5.27	-0.765	No prediction	CbiA (PF01656)	1.1E-32	No	0
UNA	236	BH15840	Q6G1R7	sapC-related protein	UNA	31188.7	6.51	-0.467	No prediction	Glyco_hydro_57 (PF03065)	0.68	No	0
sucD	206	BH16550	Q6G1M2	Succinyl-CoA ligase [ADP-forming] subunit alpha	Ligase	30991.7	6.47	0.001	Cytoplasm (100%)	SapC (PF07277)	1.0E-42	No	0
UNA	354/352	BH13580	Q6G291	Expressed protein	Oxidoreductase activity	19696.3	6.13	-0.388	No positive predictions	CoA_binding (PF02629)	4.1E-43	No	1
UNA	304	BH12430	Q6G2G4	Glutathione S-transferase	Transferase activity	26422.1	6.18	-0.246	Cytoplasm (91.5%)	Redoxin (PF08534)	1.1E-37	No	0
ssb4	342	BH10130	Q6G302	Single-stranded DNA-binding protein	Single-stranded DNA binding	19312.2	5.75	-0.828	Cytoplasm (99.8%)	GST_N (PF02798)	5.9E-05	No	0
UNA	290	BH08320	Q6G3E1	Hydrolase	Hydrolase activity	28223.5	6.31	0.052	Extracellular (98.9%), periplasm (99%)	Abhydrolase_1 (PF00561)	3.3E-15	No	1
UNA	149	BH11640	Q6G5L6	Aspartate aminotransferase	Aminotransferase, transferase	44066.6	5.97	-0.163	Cytoplasm (100%)	Aminotran_1_2 (PF00155)	1.8E-100	No	1
UNA	287	BH12780	Q6G5P1	Ferredoxin-NADP reductase	Oxidoreductase	30935.6	5.39	-0.090	Inner membrane (100%), Cytoplasm (91.7%)	FAD_binding_6 (PF00970), NAD_binding (PF00175)	0.00016	No	0
UNA	295	BH14450	Q6G224	Phage related protein	DNA binding, nuclease activity	27012.8	9.05	-0.376	Cytoplasm (57.2%)	YqaJ viral recombinase family (PF09588)	9.8E-38	No	0
UNA	392	BH16120	Q6G1R2	Putative uncharacterized protein	UNA	16494.2	8.76	-0.685	No prediction	DUF55 (PF01878)	5.0E-61	No	0
gshB	223	BH02420	Q6G4U0	Glutathione synthetase	ATP binding, glutathione synthase activity	35335.5	5.88	-0.136	No prediction	GSH-S_ATP (PF02955), GSH-S_N (PF02951)	4.5E-28	No	0
UNA	136	BH02500	Q6G4T3	Aminopeptidase	Aminopeptidase activity	45251.1	5.96	-0.081	No prediction	Peptidase_M29 (PF02073)	3.8E-200	No	0
UNA	216	BH04920	Q45197	Phage related protein	UNA	36140.6	5.01	-0.524	No prediction	Phage Tail Collar Domain (PF07484)	5.9E-24	No	0

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Appendix Table 2. Continued.

Gene	Spot ID	Locus	UniProtKB annotations		Predicted		GRAVY	PA-SUB (P)	Pfam model (ID)	Pfam E-value	Signal (P)	HMMTOP
			Accession no.	Protein name	Molecular function	Mass	pI					
UNA	50/51	BH08800	Q6G3A3	Putative uncharacterized protein	Hydrolase activity	61313.1	6.56	-0.087	No prediction	Lactamase_B (PF00753)	3.4E-16	No	1
etfA	248	BH12300	Q6G2H6	Electron transfer flavoprotein alpha-subunit	FAD binding, electron carrier activity	32444.2	5.57	0.138	No prediction	ETF (PF01012)	2.70E-33	No	0
UNA	379	BH13950	Q6G271	Putative uncharacterized protein	UNA	10006.2	6.97	-0.399	No prediction	PfamB PB002926	3.40E-41	No	0
UNA	294	BH14810	Q6G5P2	UPF0082 protein BH14810	UNA	26993.6	5.1	-0.401	No prediction	Domain of unknown function DUF28 (PF01709)	3.60E-148	No	0
htrA3	159	BH10940	Q6G2T2	Serine protease	Hydrolase, protease	54858.8	6.31	-0.150	Periplasm (100%)	Trypsin (PF00089)	3.9e-31	Yes (1)	3

UNA = unannotated.

Appendix Table 3. List of the locations and functions of carbonate-insoluble *Bartonella henselae* Houston-1 proteins identified in this study by PMF using MALDI-TOF-MS.

Gene	Spot ID	Locus	UniProtKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function	Mass	pI		
rho	169/173/573	BH16700	Q6G1K7	Transcription termination factor Rho	Proton-transporting two-sector ATPase complex, catalytic domain	Hydrolase	47401.6	5.46	-0.206	Cytoplasmic (8.96)
mdh	292/293	BH16570	Q6G1M0	Malate dehydrogenase	UNA	L-malate dehydrogenase activity, binding	33664.1	5.75	0.311	Cytoplasmic (9.26)
sucC	224	BH16560	Q6G1M1	Succinyl-CoA ligase [ADP-forming] subunit beta	UNA	Ligase	42817.2	5.07	-0.035	Cytoplasmic (8.96)
sucB	193	BH16530	Q6G1M4	Dihydrolipoamide acetyltransferase	Oxoglutarate dehydrogenase complex	Acyltransferase, transferase	43640.3	5.99	-0.071	Cytoplasmic (9.26)
purH	108/107/112	BH15970	Q6G5S5	Bifunctional purine biosynthesis protein purH	UNA	Hydrolase, transferase	57597.0	6.41	0.053	Cytoplasmic (8.96)
ftsE	551	BH15420	Q6G1V9	Cell division protein ftsE	UNA	ATP binding, ATPase activity	24587.7	7.15	0.020	Cytoplasmic membrane (7.88)
gap	302	BH15080	Q8L201	Glyceraldehyde-3-phosphate dehydrogenase	UNA	Oxidoreductase	36177.2	6.04	0.069	Cytoplasmic (9.97)
fbaB	313/315	BH15060	Q8L207	Fructose-bisphosphate aldolase	UNA	Lyase	37211.8	6.35	-0.038	Cytoplasmic (8.96)
clpB	9/17/13	BH14110	Q6G255	ATP-dependent clp protease, ATP-binding subunit clpB	Cytoplasm	Chaperone, hydrolase, protease	96059.8	5.63	-0.369	Cytoplasmic (9.97)
fumC	181	BH13550	Q6G294	Fumarate hydratase	Ttricarboxylic acid cycle enzyme complex	Fumarate hydratase activity	50151.2	6.83	-0.139	Cytoplasmic (9.26)
groL	104/111/129/ 120/140	BH13530	O33963	60 kDa chaperonin	Cytoplasm	Chaperone	57625.1	5.1	-0.041	Cytoplasmic (9.97)
gcvT	261/257	BH12840	Q6G2E9	Aminomethyltransferase	Cytoplasm	Aminotransferase, transferase	40554.7	6.21	0.01	Cytoplasmic (9.97)
gpmA	441/442	BH12450	Q8L1Z7	2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase	UNA	Isomerase	23252.4	7.95	-0.467	Cytoplasmic (8.96)
UNA	432	BH11740	Q6G2M3	Putative uncharacterized protein	UNA	Hydrolase activity	24439.7	6.09	-0.239	Cytoplasmic (8.96)
murE	156/154/155	BH11290	Q6G2Q0	UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase	Cytoplasm	Ligase	52854.4	6.61	-0.099	Cytoplasmic (9.97)
pcm2	407	BH10660	Q6G2V0	Protein-L-isoaspartate (D-aspartate)O-methyltransferase	UNA	Methyltransferase, Transferase	24414.4	5.14	0.303	Cytoplasmic (9.26)
fusA	24/22/25/26	BH10540	Q8KQB3	Elongation factor G	Cytoplasm	Elongation factor	76333.1	5.12	-0.233	Cytoplasmic (9.97)
rpsC	430	BH10450	Q6G2X1	30S ribosomal protein S3	Small ribosomal subunit	Ribonucleoprotein, ribosomal protein	26685.7	9.75	-0.467	Cytoplasmic (8.96)
rplE	575	BH10390	Q6G2X7	50S ribosomal protein L5	UNA	Ribonucleoprotein, ribosomal protein	21185.6	9.67	-0.349	Cytoplasmic (8.96)
adk	449	BH10300	Q6G2Y6	Adenylate kinase	Cytoplasm	Kinase, transferase	21480.9	6.63	-0.193	Cytoplasmic (9.97)
rpoA	244/251/252	BH10270	Q9FDC7	DNA-directed RNA polymerase subunit alpha	UNA	Nucleotidyltransferase, transferase	37672.1	4.85	-0.236	Cytoplasmic (9.97)
ppiB1	459	BH10090	Q6G306	Peptidyl-prolyl cis-transisomerase	UNA	Isomerase, rotamase	18861.3	5.03	-0.314	Cytoplasmic (9.26)
UNA	402	BH08630	Q6G3C0	ATP-dependent transporter	UNA	ATP binding, ATPase activity	27853.3	5.61	-0.071	Cytoplasmic membrane (8.02)
nifS1	226	BH08610	Q6G3C2	Nitrogenase co-factor synthesis protein nifS	UNA	Transferase	46539.1	6.34	-0.209	Cytoplasmic (8.96)
accc	168/166	BH08050	Q6G3G8	Acetyl-CoA carboxylase biotin carboxylase subunit	UNA	ATP binding, biotin binding, ligase activity	49427.4	5.88	-0.102	Cytoplasmic (8.96)

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Appendix Table 3. Continued.

Gene	Spot ID	Locus	UniProtKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function	Mass	p*I*		
glyA	177/180	BH07540	Q6G3L3	Serine hydroxymethyltransferase	Cytoplasm	Transferase	47780.5	6.55	-0.154	Cytoplasmic (9.97)
fabF2	186	BH07440	Q6G3M3	3-Oxoacyl-(acyl carrier protein) synthase II	UNA	Transferase	45241.2	5.45	-0.092	Cytoplasmic (9.26)
ibpA2	464/465/463	BH07300	Q6G5C1	Small heat shock protein	UNA	Stress response	19085.2	5.56	-0.707	Cytoplasmic (8.96)
rpiA	404/409	BH06410	Q6G3V6	Ribose-5-phosphate isomerase A	UNA	Isomerase	25161.2	4.97	0.249	Cytoplasmic (8.96)
lpxD	295	BH06290	Q8VQ23	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	UNA	Acyltransferase, transferase	36836.2	6.66	0.136	Cytoplasmic (8.96)
frr	454	BH06250	Q6G5C6	Ribosome-recycling factor	Cytoplasm	Translational termination	20665.7	6.18	-0.358	Cytoplasmic (9.97)
tsf	317/318/319	BH06230	Q6G5C8	Elongation factor Ts	Cytoplasm	Elongation factor	32374.8	4.93	0.015	Cytoplasmic (9.26)
UNA	342	BH06210	Q6G5D0	Hypothetical protein BH06210	UNA	UNA	31272.6	5.82	-0.31	Cytoplasmic (8.96)
rplA	399	BH06070	Q6G3X8	50S ribosomal protein L1	Large ribosomal subunit	Repressor, ribonucleoprotein, ribosomal protein	24649.6	9.63	-0.056	Cytoplasmic (8.96)
nusG	462	BH06050	Q6G3Y0	Transcription antitermination protein nusG	UNA	Transcription elongation regulator activity	20165.1	5.61	-0.335	Cytoplasmic (8.96)
tuf1	195/202/205/ 216/214/218	BH06020	Q8KHX9	Elongation factor Tu	Cytoplasm	Elongation factor	42866	5.3	-0.166	Cytoplasmic (9.97)
gatB	566	BH05950	Q6G3Y7	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase subunit B	UNA	Ligase	55534.1	5.17	-0.356	Cytoplasmic (8.96)
tig	116	BH05940	Q6G3Y8	Trigger factor	UNA	Chaperone, isomerase, rotamase	53222.7	5.14	-0.539	Cytoplasmic (8.96)
clpX	572	BH05890	Q6G3Z2	ATP-dependent Clp protease ATP-binding subunit clpX	UNA	Chaperone	46795.7	5.57	-0.15	Cytoplasmic (8.96)
ntrX	146/152	BH05850	Q6G3Z5	Nitrogen regulation protein	Intracellular	ATP binding, transcription factor activity	50571.1	5.62	-0.206	Cytoplasmic (9.97)
pdhC	136	BH05770	Q6G403	Dihydrolipoamide acetyltransferase (E2)	UNA	Acyltransferase, transferase	47785.1	7.17	-0.112	Cytoplasmic (9.26)
pdhB	569/110/142	BH05760	Q6G404	Pyruvate dehydrogenase subunit beta	UNA	Catalytic activity	49303.5	4.81	0.006	Cytoplasmic (8.96)
pdhA	304/301	BH05750	Q8L1Z6	Pyruvate dehydrogenase E1 component, alpha subunit	Intracellular membrane-bounded organelle	Oxidoreductase	38033	6.04	-0.354	Cytoplasmic membrane (9.46)
kdsA	346	BH05710	Q6G409	2-Dehydro-3-deoxyphosphooctonate aldolase	Cytoplasm	Transferase	30508.9	6.24	-0.065	Cytoplasmic (8.96)
fabF1	199/198	BH05370	Q6G441	3-Oxoacyl-(acyl carrier protein) synthase II	UNA	Transferase	44721.7	5.61	-0.024	Cytoplasmic (9.97)
rpsF	488/489/487	BH05320	Q6G446	30S ribosomal protein S6	UNA	Ribonucleoprotein, ribosomal protein	15848.1	8.86	-0.699	Cytoplasmic (9.97)
rplI	438	BH05290	Q6G449	50S ribosomal protein L9	Ribosome	Ribonucleoprotein, ribosomal protein	22913.7	5.17	-0.478	Cytoplasmic (9.97)
dnaB	106/109	BH05270	Q6G450	Replicative DNA helicase	UNA	Helicase, hydrolase	55335.7	5.42	-0.328	Cytoplasmic (8.96)
pyrC1	163	BH05160	Q6G5H1	Dihydroorotase	UNA	Hydrolase	49238.1	6.14	-0.254	Cytoplasmic (8.96)
pyrE	450/447	BH05050	Q6G463	Orotate phosphoribosyltransferase	UNA	Glycosyltransferase, transferase	20869.1	6.53	0.103	Cytoplasmic (8.96)
UNA	369	BH04780	Q6G490	Two-component response regulator	UNA	DNA binding, two-component response regulator activity	26084.8	5.46	-0.303	Cytoplasmic (9.97)
UNA	431	BH04520	Q6G4B4	Phosphatase	UNA	Catalytic activity	25597.4	5.36	0	Cytoplasmic (8.96)
tldD	571/174	BH04380	Q6G4C8	tldD protein	UNA	UNA	50796.4	6.33	-0.296	Cytoplasmic (9.26)
tlyA	552	BH04360	Q6G4D0	Hemolysin-like protein	UNA	RNA binding	27053.3	6.86	0.12	Cytoplasmic (8.96)
ribA	272	BH04330	Q6G4D3	3,4-Dihydroxy-2-butanone 4-phosphate synthase	UNA	Hydrolase, lyase	40967.8	5.94	-0.146	Cytoplasmic (8.96)
abl2	382/384/383	BH04310	Q6G4D5	Enoyl-(acyl carrier protein) reductase	UNA	Binding,enoyl-[acyl-carrier-protein] reductase (NADH) activity	29367.7	6.54	0.025	Cytoplasmic (9.26)

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Appendix Table 3. Continued.

Gene	Spot ID	Locus	UniProtKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function	Mass	p*I*		
dnaJ2	564/352/340/297/333	BH04300	Q6G4D6	Heat shock protein DnaJ	UNA	Heat shock protein binding, unfolded protein binding	33258.7	9.33	-0.611	Cytoplasmic (9.97)
UNA	274/228	BH02700	Q6G4R3	2'-Deoxycytidine 5'-triphosphate deaminase	UNA	UNA	40664.6	5.88	-0.111	Cytoplasmic (8.96)
UNA	479	BH02210	Q6G4W1	Transcriptional regulator	UNA	Sequence-specific DNA binding	15653.8	6.35	-0.475	Cytoplasmic (8.96)
UNA	435	BH02180	Q6G4W4	UPF0090 protein BH02180	UNA	UNA	24846.6	6.54	-0.358	Cytoplasmic (8.96)
nusA	79	BH02170	Q6G4W5	Transcription elongation factor NusA	UNA	RNA binding, nucleotide binding, protein binding, transcription factor activity	59077.8	4.78	-0.34	Cytoplasmic (8.96)
pnp	29/27/28	BH02100	Q6G5F8	Polyribonucleotide nucleotidyltransferase	Cytoplasm	Nucleotidyltransferase, transferase	80652.3	5.36	-0.225	Cytoplasmic (9.97)
prfA	232	BH01970	Q6G5T7	Peptide chain release factor 1	Cytoplasm	Translation release factor activity, codon specific	40450.9	5.53	-0.402	Cytoplasmic (9.97)
galU	241/368	BH01750	Q6G4X4	UTP-glucose-1-phosphate uridylyltransferase	UNA	Nucleotidyltransferase, transferase	33247.4	6.55	-0.173	Cytoplasmic (8.96)
typA	55/53	BH01700	Q6G4X9	GTP-binding protein typA	IntracellµLar	GTP binding, GTPase activity	67222.7	5.3	-0.294	Cytoplasmic (9.26)
obgE	288	BH01560	Q6G4Z2	GTPase ObgE	IntracellµLar	GTP binding	37296.4	8.69	-0.313	Cytoplasmic (8.96)
fabB	188/189	BH01280	Q6G516	3-oxoacyl-(acyl carrier protein) synthase I	UNA	Transferase	43504.3	6.19	-0.134	Cytoplasmic (9.97)
dnaN	236/243	BH01190	Q6G525	DNA polymerase III subunit beta	Cytoplasm	DNA-directed DNA polymerase, Nucleotidyltransferase, Transferase	41024.9	5.07	0.001	Cytoplasmic (8.96)
acnA	4/5/7/8/6	BH01160	Q6G528	Aconitate hydratase	UNA	4 iron, 4 sulfur cluster binding	98552.5	6.22	-0.212	Cytoplasmic (9.26)
maeB	20	BH01010	Q6G541	Malic enzyme	UNA	Oxidoreductase	84603.1	5.87	-0.108	Cytoplasmic (8.96)
rpsA	59/69/76/67/61/63	BH00930	Q6G5H4	30S ribosomal protein S1	Ribosome	Ribonucleoprotein, ribosomal protein	62818.1	5.18	-0.354	Cytoplasmic (8.96)
dnaK	44/41	BH00650	Q6G554	Chaperone protein dnaK	UNA	Chaperone	68261.2	4.93	-0.397	Cytoplasmic (9.97)
hrcA	273	BH00550	Q6G564	Heat-inducible transcription repressor hrcA	UNA	Repressor	39439.8	5.79	-0.200	Cytoplasmic (8.96)
prfC	89/88	BH00250	Q6G589	Peptide chain release factor 3	Cytoplasm	GTP binding, GTPase activity, translation elongation factor activity	59271.6	5.9	-0.320	Cytoplasmic (9.97)
lpdA	150/151	AAR21288	Q67B06	Dihydrolipoyl dehydrogenase	Cytoplasm	Oxidoreductase	49403	6.05	0.143	Cytoplasmic (9.97)
serS	570	BH05610	Q6G419	Seryl-tRNA synthetase	Cytoplasm	Aminoacyl-tRNA synthetase, ligase	48034.8	5.74	-0.335	Cytoplasmic (10.00)
accA	348/343	BH16340	Q6G1P1	Acetyl-CoA carboxylase, carboxyl transferase alpha subunit	Cytoplasm	acetyl-CoA carboxylase activity, transferase activity	34948.9	6.27	-0.292	UNK
accD	580	BH00340	Q6G581	Acetyl-co-enzyme A carboxylase carboxyltransferase beta chain	Acetyl-CoA carboxylase complex	Acetyl-CoA carboxylase activity, transferase activity	33903.3	8.49	-0.089	UNK
asd	283/284	BH12890	Q6G2E5	Aspartate-semialdehyde dehydrogenase	Cytoplasm	NADP or NADPH binding, aspartate-semialdehyde dehydrogenase activity	37069.6	6.2	-0.036	UNK
rpsB	358/360	BH06220	Q6G5C9	30S ribosomal protein S2	Small ribosomal subunit	Structural constituent of ribosome	28363.4	6.66	-0.238	UNK
rplJ	468	BH06080	Q6G3X7	50S ribosomal protein L10	Ribosome	Structural constituent of ribosome	18154.9	9.56	0.084	UNK
rplY	408/410	BH11850	Q6G2L2	50S ribosomal protein L25	Ribosome	5S rRNA binding, structural constituent of ribosome	22750.9	5.18	-0.175	UNK
rplF	574	BH10360	Q6G2Y0	50S ribosomal protein L6	Ribosome	rRNA binding, structural constituent of ribosome	19558.6	9.66	-0.480	UNK
pyrH	427	BH06240	Q6G5C7	Uridylate kinase	Cytoplasm	ATP binding, UMP kinase activity	25147	7.88	0.284	UNK
pdxJ	424	BH10900	Q3V7Q9	Pyridoxal phosphate biosynthetic protein PdxJ	Cytoplasm	Catalytic activity	27099	6.45	-0.141	UNK
ftsZ	192/197	BH11180	O69074	Cell division protein FtsZ	Cytoplasm	GTP binding, GTPase activity	62313.6	5.25	-0.256	UNK
dnaJ1	238	BH00660	Q6G553	Chaperone protein dnaJ	Cytoplasm	Chaperone	42221.9	8.58	-0.703	UNK

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Appendix Table 3. Continued.

Gene	Spot ID	Locus	UniProtKB annotations				Predicted		GRAVY	PSORTb (Probability)
			Accession No.	Protein name	Cellular component	Molecular function	Mass	*pI*		
livJ	279	BH08290	Q6G3E4	Branched chain aa ABC transporter, periplasmic ligand-binding protein	UNA	UNA	41187.3	6.18	-0.104	Periplasmic (9.76)
ugpB	269	BH01870	Q6G5J2	Glycerol-3-phosphate-binding periplasmic protein	UNA	Transporter activity	48847.6	8.49	-0.293	Periplasmic (9.76)
tolB	219/223	BH14840	Q6G5R6	Protein tolB	Periplasm	Protein transport, transport	50140.9	7.08	-0.328	Periplasmic (9.76)
ybeJ	576	BH10580	Q6G2V8	Amino-acid ABC transporter binding protein	Outer membrane-bounded periplasmic space	Transporter activity	33815.8	8.6	-0.296	Periplasmic (9.76)
UNA	396	BH06370	Q6G3W0	ABC transporter, periplasmic amino acid-binding protein	Outer membrane-bounded periplasmic space	Transporter activity	28179.4	5.54	-0.166	Periplasmic (10.00)
hrx42	145/148	BH10250	Q6G2Z1	Hydrolase, protease	UNA	Hydrolase, protease	50562.1	9.02	0.026	Periplasmic (9.44)
atpA	135/133/137	BH15340	Q6G1W7	ATP synthase subunit alpha	Cell inner membrane, cell membrane	Hydrolase	55511.6	5.91	-0.061	UNK
UNA	429	BH02760	Q6G4Q8	Putative ABC transporter ATP-binding protein BH02760	Cell inner membrane, cell membrane	Hydrolase	25552.8	6.53	-0.004	Cytoplasmic (9.12)
hutA	34/37/54/567	BH04970	Q6G47I	Heme receptor precursor	Cell outer membrane	Receptor	82612.4	9.34	-0.611	OM (10.00)
ompA9	10/12/14/15/ 18/19	BH06280	Q8VQ24	Outer membrane protein	UNA	Outer membrane	88985.9	9.01	-0.255	OM (10.00)
ompA3	246/248/254	BH12500	Q9L7A9	Omp43 precursor	Membrane	Porin activity	44224.4	8.53	-0.254	OM (9.93)
UNA	281	BH10650	Q6G2V1	Outer membrane protein	Outer membrane	Transporter activity	48299.3	9.71	-0.075	OM (10.00)
plp	321/322	BH02000	Q6G5U1	Peptidyl-prolyl cis-trans isomerase	UNA	Isomerase, rotamase	35596.8	5.82	-0.442	Outer membrane integral membrane protein
pap31	349/367/394	JC6528	UPI0000 17CA3D	31 K major protein, Pap31 - *Bartonella henselae* phage	UNA	UNA	30074.7	5.37	-0.207	OM (9.93)
pap31	363	AAK97307	Q94M28	Pap31	Cell outer membrane, integral to membrane	Porin activity	25663.7	4.90	-0.125	OM (9.94)
hbpA	339/357/390/ 344/361/ 338/363	BH02560	Q6G4S7	Hemin-binding protein a	Cell outer membrane, integral to membrane	Porin activity	29916.6	5.37	-0.205	OM (9.93)
hbpD	373/375/379/ 389/577/578	BH04810	Q6G487	Hemin-binding protein d	Cell outer membrane, integral to membrane	Porin activity	30269.7	9.06	-0.158	OM (9.45)
UNA	579	BH07600	Q6G3K7	Putative uncharacterized protein	Outer membrane	UNA	17635.4	9.12	-0.025	UNK

UNA = unannotated; UNK = unknown.

Appendix Table 4. Analysis of carbonate-insoluble *Bartonella henselae* Houston-1 proteins with subcellular location unknown identified in this study by PMF using MALDI-TOF-MS.

Gene	Spot ID	Locus	UniProtKB annotations			Predicted		GRAVY	PA-SUB (P)	Pfam model	Pfam E-value	SignalP (P)	HMMTOP
			Accession No.	Protein names	Molecular function	Mass	pI						
mutL	3/48	BH02690	Q6G4R4	DNA mismatch repair protein mutL	ATP binding, mismatched DNA binding	66925.5	6.21	-0.137	No prediction	HATPase_c (PF02518)	3.20E-08	No	0
UNA	160	BH02520	Q6G4T1	Putative uncharacterized protein	Metallopeptidase activity, protein dimerization activity	50692.6	5.39	-0.193	Extracellular (92.5%)	Peptidase_M20 (PF01546)	2.60E-42	No	0
UNA	84/87	BH08800	Q6G3A3	Putative uncharacterized protein	Hydrolase activity	61351.5	6.56	-0.087	No prediction	Lactamase_B (PF00753)	3.40E-14	No	1
guaB	127/128/126/124/125/121/119	BH01800	Q6G4W9	Inosine-5'-monophosphate dehydrogenase	IMP dehydrogenase activity	52783.6	6.29	0.015	No prediction	IMP dehydrogenase/GMP reductase domain (PF00478)	2.30E-225	No	0
AmpS	204	BH02500	Q6G4T3	Aminopeptidase	Aminopeptidase activity	45279.4	5.96	-0.081	Extracellular (63.8%)	Peptidase_M29 (PF02073)	3.80E-200	No	0
ctpA	212/213/217	BH01630	Q6G4Y5	Carboxy-terminal processing protease ctpA	Protein binding, serine-type peptidase activity	47046.2	8.91	-0.200	Extracellular (100%)	PDZ (PF00595)	1.30E-10	Yes (1)	1
tyrC	308	BH16210	Q6G1Q3	Cyclohexadienyl dehydrogenase	Binding, prephenate dehydrogenase ($NADP^+$) activity	33696.5	5.69	0.074	Cytoplasm (99.5%)	Prephenate dehydrogenase (PF02153)	1.30E-95	No	2
pyrC2	190	BH08190	Q6G3F4	Dihydroorotase	Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	46866.5	5.51	-0.062	Cytoplasm (98.9%)	Amidohydrolase (PF01979)	2.70E-06	No	0
etfA	353	BH12300	Q6G2H6	Electron transfer flavoprotein alpha-subunit	FAD binding, electron carrier activity	32464.2	5.57	0.138	No prediction	Electron transfer flavoprotein domain (PF01012)	2.70E-33	No	0
UNA	234/240	BH14010	Q6G265	Expressed protein	UNA	41031.7	6.98	-0.258	No prediction	Protein of unknown function (PF06866)		No	0
UNA	335/337	BH16250	Q6G1P9	Hypothetical protein BH16250	UNA	34390.0	9.36	0.204	No prediction	ABC transporter substrate binding protein (PF04392)	2.10E-97	Yes (1)	2
UNA	326	BH02430	Q6G4T9	Periplasmic phosphate binding protein	UNA	37605.1	6.91	0.000	Periplasm (84.9%)	Sorbitol phosphotransferase enzyme II C-terminus (PF07663)	0.8	Yes (0.894)	1
UNA	309/310	BH04920	Q45197	Phage related protein	UNA	36163.1	5.01	-0.524	No prediction	Phage Tail Collar Domain (PF07484)	5.90E-22	No	0
UNA	350/351	BH15840	Q6G1R7	sapC-related protein	UNA	31208.6	6.51	-0.467	No prediction	SapC (PF07277)	1.00E-42	No	0
sucD	306/307	BH16550	Q6G1M2	Succinyl-CoA ligase [ADP-forming] subunit alpha	Ligase	30991.7	6.47	0.001	Cytoplasm (100%)	CoA_binding (PF02629)	4.10E-43	No	1
bcp	483	BH08680	Q6G3B5	Bacterioferritin co-migratory protein	Antioxidant activity, oxidoreductase activity	18228.0	6.83	-0.269	No prediction	AhpC-TSA (PF00578)	2.60E-48	No	0
lemA	544	BH07870	Q6G3I1	Cytoplasmic membrane protein	UNA	24505.2	8.66	-0.198	No prediction	LemA (PF04011)	4.10E-98	Yes (0.625)	1
UNA	561/421/467/470/469	BH13580	Q6G291	Expressed protein	Oxidoreductase activity	19696.3	6.13	-0.388	No prediction	Redoxin (PF08534)	1.10E-37	No	0
UNA	494	BH06190	Q6G5D3	Hit-like protein involved in cell-cycle regulation	Catalytic activity	15792.3	6.51	-0.185	Cytoplasm (95%)	HIT (PF01230)	6.50E-39	No	0
IalB	466/514	BH08730	Q6G3B0	Putative uncharacterized protein	UNA	22444.8	8.99	-0.267	No prediction	IalB (PF06776)	2.80E-36	Yes (1)	1
UNA	388	BH11610	Q6G5L9	Putative uncharacterized protein	UNA	31840.4	6.91	-0.292	No prediction	DUF1460 (PF07313)	2.10E-149	No	0

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Appendix Table 4. Continued.

Gene	Spot ID	Locus	UniProtKB annotations			Predicted		GRAVY	PA-SUB (P)	Pfam model	Pfam E-value	SignalP (P)	HMMTOP
			Accession No.	Protein names	Molecular function	Mass	pI						
map	372	BH08350	Q6G3D8	Methionine aminopeptidase	Aminopeptidase, hydrolase, protease	30807.1	5.99	-0.202	No prediction	Metallopeptidase family M24 (PF00557)	3.70E-87	No	0
parA1	426	BH13200	Q6G2C5	parA protein	Cobyrinic acid a, c-diamide synthase activity	22269.6	5.81	0.222	Cytoplasm (60.9%)	CbiA (PF01656)	1.10E-32	No	0
hutB	406	BH04950	Q6G473	Periplasmic hemin-binding protein	Iron ion transmembrane transporter activity	32546.3	9.5	0.237	Periplasm (100%)	Peripla_BP_2 (PF01497)	5.30E-33	Yes (1)	1
ssb4	456/533/534	BH10130	Q6G302	Single-stranded DNA-binding protein	Single-stranded DNA binding	19312.2	5.75	-1.233	Cytoplasm (99.8%)	SSB (PF00436)	7.20E-30	No	0
UNA	504	BH13950	Q6G271	Putative uncharacterized protein	UNA	10012.6	6.97	-0.399	No prediction	PB002926	3.40E-41	No	0
UNA	332	BH04240	Q6G4E2	Putative uncharacterized protein	Contains 1 YrdC-like domain	34604.0	6.73	0.131	Cytoplasm (80.1%)	Sua5_yciO_yrdC (PF01300)	1.00E-45	No	1
UNA	381	BH14810	Q6G5P2	UPF0082 protein BH14810	UNA	27010.3	5.1	-0.401	No prediction	DUF28 (PF01709)	3.60E-146	No	0
SodA	448	BH04630	Q6G4A3	Superoxide dismutase	Metal ion binding, superoxide dismutase activity	23119.0	5.77	-0.484	No prediction	Iron/manganese superoxide dismutases (PF00081)	6.30E-35	No	1
ftsY	175/182	BH16620	Q6G1L5	Cell division protein ftsY	7S RNA binding, GTP binding, nucleoside-triphosphatase activity	45785.4	8.32	-0.345	Cytoplasm	SRP54-type protein, helical bundle domain (PF02881)	1.20E-05	No	1
htrA3	158/159	BH10940	Q6G2T2	Serine protease	Protein binding, serine-type peptidase activity	54892.7	6.31	-0.150	Extracellular (100%), periplasm (100%)	Trypsin (PF00089)	3.90E-31	Yes (1)	3
sdhA	62	BH15780	Q6G1S3	Succinate dehydrogenase flavoprotein subunit	FAD binding, electron carrier activity, oxidoreductase activity, acting on the CH-CH group of donors	66972.7	6.04	-0.236	Inner membrane (100%)	FAD_binding_2 (PF00890)	1.60E-174	No	1
UNA	402	BH08630	Q6G3C0	ATP-dependent transporter	ATP binding, ATPase activity	27853.3	5.61	-0.071	Inner membrane (100%)	ABC_tran (PF00005)	5.30E-28	No	1
UNA	98/99/92	BH12180	Q6G2I6	ABC transporter, periplasmic oligopeptide-binding	Transporter activity	61340.4	8.84	-0.276	Inner membrane (100%)	Extracellular solute-binding proteins (PF00496)	1.60E-70	Yes (0.998)	1
UNA	81/82/83/86	BH11380	Q6G2P3	Putative ABC transporter ATP-binding protein	ATP binding, ATPase activity	60988.0	5.36	-0.405	No predictions	ABC transporter (PF00005)	3.50E-57	No	0

UNA = unannotated.