

In Vitro Protein Expression Profile of *Campylobacter jejuni* Strain NCTC11168 by Two-dimensional Gel Electrophoresis and Mass Spectrometry*

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Abstract

Objective To investigate the protein expression profiles of the major food-borne pathogen *Campylobacter jejuni* NCTC11168.

Methods Membrane and soluble cellular proteins were extracted from the genome-sequenced *C. jejuni* strain NCTC11168. Protein expression profiles were determined using two-dimensional gel electrophoresis (2-DE). All the detected spots on the 2-DE map were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) analysis.

Results A total of 537 and 333 spots were detected from the whole cell and membrane-associated proteins of *C. jejuni* NCTC11168 cultured on Columbia agar medium at 42 °C by 2-DE and Coomassie Brilliant Blue staining, respectively. Analyses of whole cell and membrane-associated proteins included 399 and 133 spots, respectively, which included 182 and 53 functional proteins identified by MALDI-TOF/TOF analysis.

Conclusion The comprehensive expression protein profiles of *C. jejuni* NCTC11168 obtained in this study will be useful for elucidating the roles of these proteins in further pathogenesis investigation.

Key words: *Campylobacter jejuni*; Two-dimensional gel electrophoresis; MALDI-TOF; Soluble cellular protein; Membrane protein

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INTRODUCTION

Campylobacter jejuni causes human gastroenteritis worldwide and is a common cause of traveler's diarrhea. In addition, *C. jejuni* infection has been associated with the development of Guillain-Barre syndrome, the leading cause of acute flaccid paralysis worldwide^[1-3]. Using the genome sequence of *C. jejuni*, an extensive proteome expression database of the pathogen has been useful for the investigation of microbial physiology and pathogenesis. Currently, proteomics analysis is a key analytical method for investigating bacterial proteomes^[4-5]. Techniques such as

two-dimensional gel electrophoresis (2-DE) and two-dimensional difference gel electrophoresis (2-DE DIGE) are widely used to determine bacterial gene regulation and the stress response under different conditions. However, most studies on *C. jejuni* have a focus on the regulation of specific proteins^[6-7]. In the present study, 2-DE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) protein analyses were used to determine a comprehensive protein expression profile and protein identification for the genome-sequenced *C. jejuni* strain NCTC11168. The data obtained in this study will be helpful for the further investigation of *C. jejuni*.

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MATERIALS AND METHODS

Bacterial Culture and Sample Preparation

C. jejuni NCTC11168 was grown on *Campylobacter* Columbia agar (Oxoid, Basingstoke, UK) containing 5% defibrinated sheep blood under microaerophilic conditions (10% CO₂, 5% O₂ and 85% N₂) at 42 °C for 24 h. Whole cell proteins were extracted using a standard protocol modified in our laboratory^[8-9]. Briefly, bacterial cells were harvested from the agar plates into normal saline [0.9% (w/v) NaCl] and washed three times in saline by centrifugation at 1500 g at 4 °C for 10 min. The bacterial pellet was suspended in lysate buffer [6 mol/L urea, 2 mol/L thiourea, 4.0% (w/v) CHAPS, 40 mmol/L DL-dithiothreitol (DTT) and 0.5% (v/v) immobilized pH gradient (IPG) pH 3-10 buffer] and sonicated on ice three times for 30 s at 30 s intervals until the suspension became clear. Cell debris was collected by centrifugation at 134 000 ×g at 4 °C for 15 min.

Outer membrane proteins were extracted as previously described with some modifications^[10-11]. Briefly, bacterial pellets were re-suspended in 50 mmol/L Tris-buffer (pH 7.5) and sonicated on ice several times for 30 s at 30 s intervals. Lysates were centrifuged at 5000 ×g for 10 min to remove particulate matter, then the supernatants were collected and mixed with 5 mL of 0.1 mol/L Na₂CO₃ (pH 11) at 4 °C for 1 h with shaking. The mixed supernatant was further centrifuged at 100 000 ×g for 1 h and the pellets washed with 50 mmol/L Tris-buffer. Supernatants were then centrifuged at 100 000 ×g for 1 h and re-suspended in lysate buffer [6 mol/L urea, 2 mol/L thiourea, 4.0% (w/v) 3-3-Cholamidopropyl dimethylammonio propanesulfonic acid (CHAPS), 40 mmol/L dithiothreitol (DTT) and 0.5% (v/v) immobilized pH gradient (IPG) buffer, pH 3-10]. Both the membrane and cellular protein supernatants were cleaned using the 2-D clean-up kit (Amersham Biosciences, Piscataway, NJ, USA) and proteins were quantified using the 2-D quant kit (GE Healthcare Bio-Science Corp. Piscataway NJ08855, USA) according to the manufacturer's instructions. Proteins were then subjected to 2-DE. Sample preparations and 2-DE were repeated at least three times using the same standard protocols.

Two-Dimensional Polyacrylamide Gel Electrophoresis

One-dimensional isoelectric focusing (IEF) and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were

performed according to the manufacturer's instructions (Amersham Biosciences). Equal amounts of protein (450 µg in 450 µL rehydration buffer for membrane protein and 800 µg in 450 µL rehydration buffer for cellular proteins) were loaded onto dry 24 cm IPG strips (pH 3-10 NL; Amersham Biosciences). IEF was carried out for 90 000 Vh over a 24 h period. Thereafter, the strips were equilibrated twice in equilibration buffer [50 mmol/L Tris-HCl, pH 6.8, 6 mol/L urea, 30% glycerol, 2% SDS, with 0.25% DTT for the first time, and 4.8% indole-3-acetic acid (IAA) for the second time]. Strips were then subjected to SDS-PAGE using 12.5% gels at 2.5 watts per gel for 30 min followed by 18 watts per gel until the bromophenol blue tracking dye reached the bottom of the gel.

Detection of Protein Spots on 2-DE Gels

Gels subjected to 2-DE were stained with Coomassie Brilliant Blue (CBB) G-250 (BioRad, Hercules, CA, USA). Individual gels were imaged using an ImageScanner (Amersham Biosciences) with an 8-bit grayscale 400 dots-per-inch (dpi) transparency adapter. Spot detection, quantification (% volume) and pattern matching were completed using the ImageMaster 2D Platinum software (Amersham Biosciences).

Identification of Proteins by MALDI-TOF-TOF Analysis

The entire visualized spots on 2-DE gels of *C. jejuni* cellular and membrane protein fractions were excised from the gel. In-gel digestion was performed as previously described^[10]. Protein identification was carried out using tandem matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) mass spectrometry (4700 MALDI-TOF/TOF mass spectrometer; Applied Biosystems, Foster City, CA, USA). Briefly, the protein samples were mixed (1:1) with a saturated matrix solution (α -cyano-4-hydroxycinnamic acid prepared in 50% acetonitrile/0.1% trifluoroacetic acid) on a sample plate. The mass spectra were obtained by 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 tandem mass spectrometer 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 800-4000 Da using 1500 laser shots. MS/MS spectra were acquired using 2000 laser shots with air as the

collision gas. The singly-charged peaks were analyzed using an interpretation method provided in the 4000 Series Explorer software that selected the five most intense peaks and automatically generated the MS/MS spectra by excluding the peaks associated with the matrix and those that were formed because of trypsin autolysis. Spectra were processed and analyzed by the Global Protein Server Workstation (Applied Biosystems), which uses internal Mascot software (version 2.1; Matrix Science, London, UK) for searching the peptide mass fingerprints and MS/MS data. One missed tryptic cleavage and variable modified carboxymethyl (C), oxidation (M) was selected and 0.3 Da peptide fragment tolerance and 0.3 Da MS/MS ion tolerances were setting as the parameters. The searches were performed by using the NCBI non-redundant protein database. Identification was performed using the Global Protein Server Workstation (GPS) and confidence intervals greater

than 95% were accepted.

RESULTS

Expression Profiles and Identification of the Cellular and Membrane Proteins

A total of 537 spots were detected from the whole cell soluble proteins of *C. jejuni* NCTC11168 cultured on Columbia agar at 42 °C by 2-DE and CBB staining (Figure 1). All 537 spots were used for the protein identification by MALDI-TOF-TOF analysis. A total of 399 spots that belonged to 182 *C. jejuni* proteins were identified according to the database. The locations of the protein spots on 2-DE map were marked in Figure 1. The encoded gene names and the characterizations of the identified proteins were summarized in Supplementary file 1 (found in the manuscript at website of BES: www.besjournal.com).

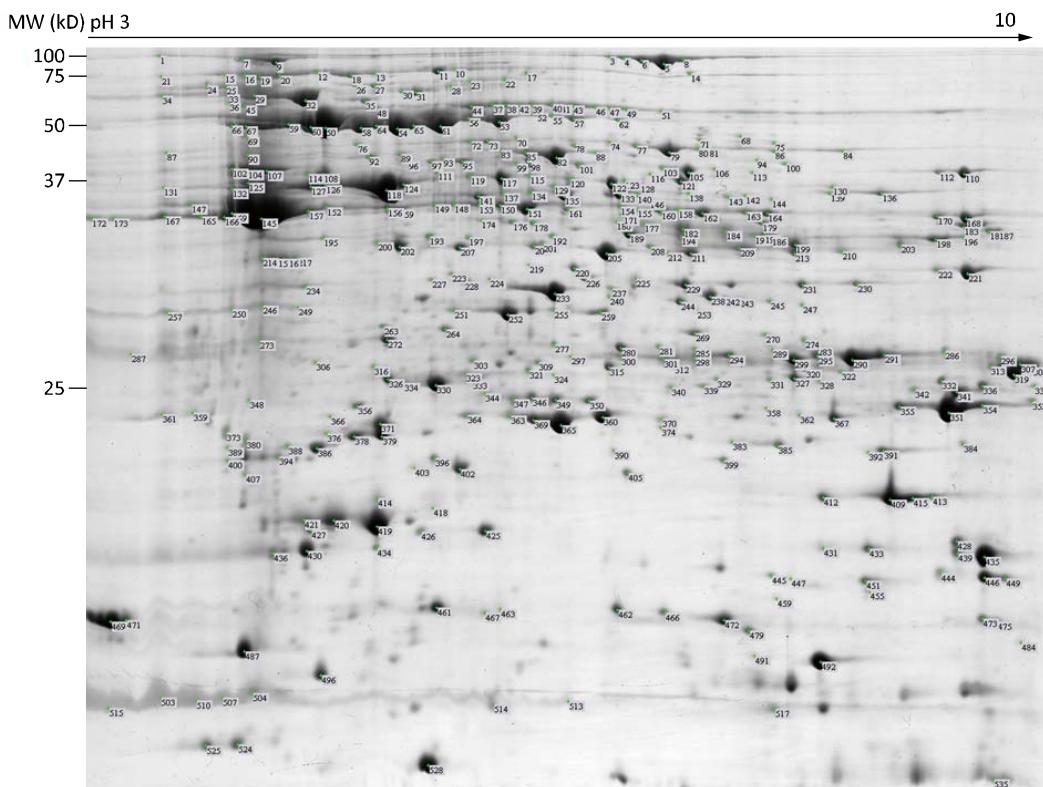


Figure 1. Whole cellular proteins of *C. jejuni* NCTC11168 cultured on agar at 42 °C identified by 2-DE. The locations of the 399 identifiable spots representing 182 proteins are marked. All 182 identified proteins are listed in Supplementary file 1 (found in the manuscript at website of BES: www.besjournal.com).

A total of 333 spots were detected from the membrane and membrane-associated proteins. All detected spots were subjected to protein identification and 53 functional proteins were identified. The membrane and membrane-associated protein expression profile and the

location of the identified proteins are presented in Figure 2. The encoded gene names and the characterizations of the identified membrane and membrane-associated proteins are summarized in Supplementary file 2 (found in the manuscript at website of BES: www.besjournal.com).

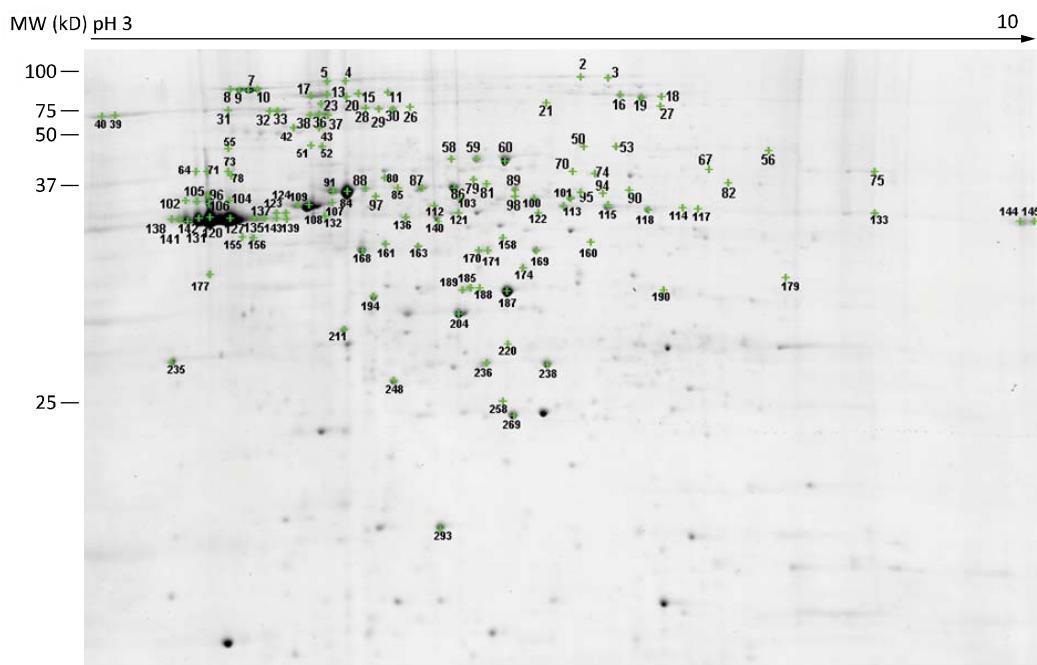


Figure 2. Membrane and membrane-associated proteins of *C. jejuni* NCTC11168 on the 2-DE map. *C. jejuni* NCTC11168 was cultured on agar at 42 °C. The membrane and membrane-associated proteins were extracted by sodium carbonate precipitation and ultracentrifugation, and subjected to 2-DE and MALDI-TOF/TOF analysis. The identified expressed proteins are indicated by spot ID on gels and a summary of the identified proteins is given in Supplementary file 2 (found in the manuscript at website of BES: www.besjournal.com).

Functional Classification of Identified Proteins

Thirteen proteins identified from the membrane and membrane-associated fractions were not detected in the whole cell fraction and 142 proteins were detected only from the whole cell fraction, while 40 proteins were identified both from the whole cell extraction and the membrane fraction. In total, 195 proteins of *C. jejuni* were identified in this study. The functional categories of all identified expressed proteins were assigned according to the gene ontology (GO) function description using WEGO (Web Gene Ontology Annotation Plot, <http://wego.genomics.org.cn/cgi-bin/wego/index.pl>)^[12].

The percentage of genes in each GO category was clustered into three groups and 14 classes (Figure 3). Overall, 182 proteins from cellular

fraction were classified into cellular components, molecular function and biological process groups, while 53 membrane and membrane-associated proteins were only classified into the molecular function group. Most of the membrane-associated proteins had functions related to catalytic and cell binding.

DISCUSSION

Characterizing a microbe's whole proteome profile coupled with the protein identification by mass spectrometry is useful for deciphering the function of proteins^[13-14]. In this study, the profiles of the expressed cellular and membrane proteins of *C. jejuni* NCTC11168 *in vitro* were characterized. *C. jejuni* NCTC11168 was originally isolated from a case

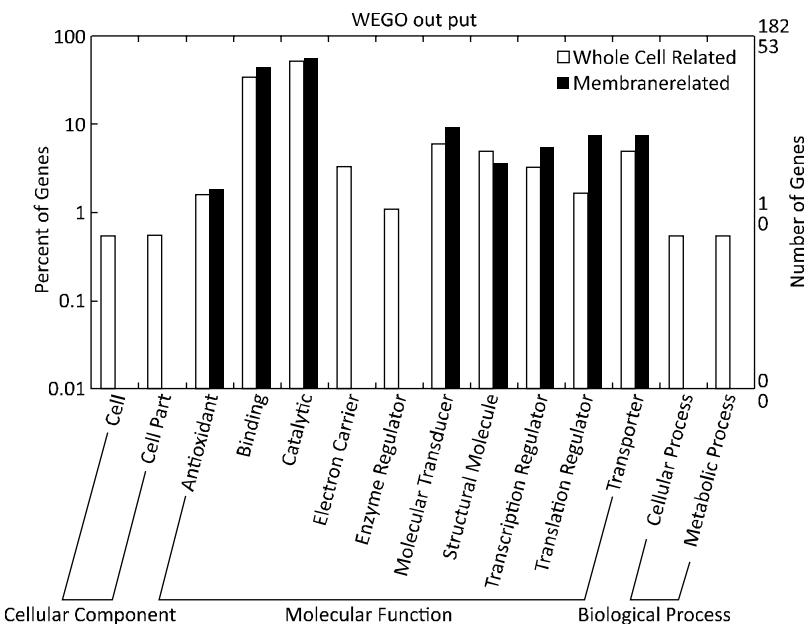


Figure 3. Gene ontology (GO) categories of the identified cellular and membrane-associated proteins. The functional categories of all identified expressed proteins were assigned according to GO function. The percentage of genes in each GO category was determined using WEGO.

of human enteritis and was the first sequenced *Campylobacter* genome^[15]. This isolate has been typically used to investigate *Campylobacter* physiology and pathogenesis^[16-17]. The localization and the expression of the identified proteins *in vitro* would be useful for further immuno-proteomic, comparative proteomic analysis and the functional study of each protein in *C. jejuni*. In a previous study, we used comparative analysis to determine differentially expressed proteins at 37 and 42 °C *in vitro*^[9]. The current study was the first to comprehensively analyze differentially proteins for this *C. jejuni* reference strain. In addition, the identification of membrane and membrane-associated proteins will further assist the analysis of pathogenesis of *C. jejuni*.

Using the genomic sequence of *C. jejuni* NCTC11168, 1634 theoretical proteins were predicted^[15]. So far, 1132 individual proteins of *C. jejuni* have been described, and 267 membrane and membrane-associated proteins are currently listed in the UniPro database. In our study, only 195 cellular proteins were identified. Thus, the coverage of the identification in this study, especially for the membrane protein, was not adequate. Although it is unknown why this coverage was so low, the method of protein sampling or the protein identification was not likely to be the cause since other research

obtained 27.4% of the *C. jejuni* theoretical proteome using 2-DE/MS combined with 2-DLC/MS/MS methods^[18].

In conclusion, the present study provided comprehensive proteomic profiles of *C. jejuni* NCTC11168. The identification of locations and the characteristics of these expressed proteins will provide the basis for further investigation of the functional roles of these proteins in the pathogenesis of *C. jejuni*.

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Supplementary file 1. Characteristics of identified proteins in the cellular protein fraction extracted from *C. jejuni* NCTC11168.

Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score	Protein C. I.%*
7,36,45,59,66,67,69,90,102,104 ,107,125,132,145,148,149,152, 153,156,157,165,166,167,169,1 72,173,373,380,389,400,407,15 5,436	major outer membrane protein aconitase hydratase	<i>Cj1259(porA)</i> <i>Cj0835c(acnB)</i>	gi 85036659 gi 15792173	45560.6 92734.8	4.8 6.04	521 698	100 100
1,3,4,5,6,8,52,68, 94,106,113,123,138,142,143,14 4,146	71,80, oxireductase, putative	<i>Cj0415</i>	gi 121612363	63618.2	8.76	297	100
37,38,39,40,41,42,43,44,46,47, 48,49,51, 12,13,15,16,18,19,20,21	translation elongation factor G outer membrane fibronectin-binding protein	<i>Cj0493(fusA)</i> <i>Cj1478c(cadF)</i>	gi 86151758 gi 86152850	766398.8 36000.9	5.07 5.8	229 238	100 100
118, 124, 126, 127, 131, 306, 316, 347, 390	translation elongation factor Tu flagellin	<i>Cj0470(tuf)</i> <i>Cj1339c(flaA)</i>	gi 57504721 gi 15792662	43580.2	5.11	627	100
53,54,55,56,57,58,61 223, 224, 240, 253, 340 161, 363, 364, 365, 369, 370, 177,180, 182, 184, 186, 190, 194,	60 kDa chaperonin Cpn60 alkyl hydroperoxide reductase putative cytochrome c	<i>Cj0758(grpE)</i> <i>Cj0334(ahpC)</i> <i>Cj0037c</i>	gi 18308148 gi 15791702 gi 15791436	43156.9 21933.2 38818.7	5.22 5.66 8.08	189 455 248	100 100 100
503, 504, 507, 510, 513, 515 414, 418, 419, 420, 421 50,60, 76, 89, 93, 214, 215, 216, 217	DNA-binding protein HU probable thiol peroxidase heat shock protein nifU protein like protein	<i>Cj0913c(hupB)</i> <i>Cj0779(tpx)</i> <i>Cj1221</i> <i>Cj0239c</i>	gi 58979367 gi 15792117 gi 3063761 gi 145344779	10297.7 18428.4 57990.5 34843.3	10.29 5.13 5.02 4.88	93 124 465 90	99,753 100 100 99,484

(Continued)

Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW ^s	Protein PI ^f	Protein Score	Protein Score C.I.%*
22, 23,24, 25 110, 112, 168, 170	fumarate reductase serine protease (protease D0)	<i>Cj0409(frdA)</i> <i>Cj1228c(htrA)</i>	gi 57237464 gi 15792552	73767.1 50975.9	6.33 8.86	90 454	99.541 100
82, 83, 85, 88 74,77, 79, 81, 289,291, 290, 295 344,346, 349, 350 297, 308, 300, 352	trigger factor (peptidyl-prolyl cis /trans isomerase,chaperone) ATP synthase subunit A succinyl-CoA synthetase alpha chain superoxide dismutase (Fe) major antigenic peptide PEB3	<i>Cj0193c(trg)</i> <i>Cj0105</i> <i>Cj0534(sucD)</i> <i>Cj0169(sodB)</i> <i>Cj0289c(peb3)</i>	gi 145344750 gi 15791493 gi 15791895 gi 15791556 gi 121613001	50903.9 54767.1 30001.8 24797.1 27478.4	6.06 6.03 7.64 5.83 9.42	524 324 115 119 101	100 100 99.998 100 99.96
Chain A, Campylobacter Jejuni Ycei Crystal Structure Of Protein, Cj0420			gi 88193003	20019.2	7.29	294	100
409, 412, 413, 415 115, 119, 117 122,128, 120 341, 342, 354 150, 151, 159 209, 210, 213 330, 333,334 296, 313, 319 249, 250, 246, 257 428, 435, 439 10, 11, 99 348, 359, 361	Structural Genomics S-adenosylmethionine synthetase argininosuccinate synthase conserved hypothetical protein putative MCP-type signal transduction protein cytochrome c551 peroxidase two-component regulator peptidyl-prolyl cis-trans isomerase bacterial extracellular solute-binding proteins, family 3 putative periplasmic protein major outer membrane protein 50S ribosomal protein L3	<i>Cj1096c(metK)</i> <i>Cj0665c(argG)</i> <i>Cj1380</i> <i>Cj0448c</i> <i>Cj0358</i> <i>Cj0355c</i> <i>Cj0598c(peb4)</i> <i>Cj0982c(cjaA)</i> <i>Cj0998c</i> <i>Cj0129c</i> <i>Cj1707c(rplC)</i>	gi 15792421 gi 88597140 gi 86150025 gi 15791812 gi 57237413 gi 15791723 gi 15791956 gi 86151024 gi 15792325 gi 15791517 gi 15793010	43626.9 45579.4 26551.8 40490.6 36793.1 25526.4 30499.3 30917.2 20535.9 83146.7 20723.8	5.45 5.76 8.84 5.58 8.64 5.28 9.23 6.14 9.11 5.5 9.91	143 518 105 121 239 238 170 114 90 262 92	100 100 99.984 100 99.998 100 100 100 99.551 100 99.651

(Continued)

Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score	Protein Score C. 1.%*
201, 205, 204	oxidoreductase branched-chain amino-acid ABC transport system periplasmic binding protein	Cj0559 Cj1018c(livK)	gi 15791920 gi 15792345	33656.3 39690.6	5.73 8.94	118 378	100 100
183, 196, 198	putative MCP-type signal transduction protein	Cj1110c	gi 15792435	48320.4	6	141	100
137, 147	major antigenic peptide PEB2	Cj0778(peb2)	gi 15792116	27303.8	9.46	137	100
323, 338	flagellin subunit protein FlaB	Cj1338c(FlaB)	gi 88596729	59163.8	5.4	336	100
64, 65	ATP synthase subunit B	Cj0107	gi 15791495	50818.1	4.97	631	100
108, 114	thioredoxin reductase	Cj0146c(trxB)	gi 15791534	33096.8	5.6	84	98.171
251, 252	acetyl-CoA carboxylase	Cj1290c(accC)	gi 86149992	49101.9	6.01	202	100
116, 121	acetyl-CoA carboxylase alpha subunit	Cj0443(accA)	gi 15791810	34231.8	5.44	138	100
227, 228,	amino acid ABC transporter, periplasmic amino acid-binding protein PEB1	Cj0921c(peb1A)	gi 121613532	28146.1	8.81	197	100
351, 355	GTP-binding protein typA homolog	Cj0039c(typA)	gi 15791438	66440.5	5.2	393	100
30, 31	methyl-accepting chemotaxis protein fumurate hydratase	Cj0144 Cj1364c(fumC)	gi 88596730 gi 15792687	19266.7 50677.9	7.71 6.12	158 388	100 100
447, 451	fumarylidinol dehydrogenase	Cj1598(hisD)	gi 86149477	46446.6	5.62	127	100
105, 103	phosphoglycerate kinase	Cj1402c(pgk)	gi 15792720	43563.8	6.07	106	99.987
96, 98	UDP-glucose 4-epimerase	Cj1131c(galE)	gi 86153044	36722.4	5.59	121	100
154, 160	carboxyl-terminal protease	Cj0511	gi 86149018	48246.8	8.7	313	100
219, 220	dihydrodipicolinate synthase	Cj0806(dapA)	gi 86152125	32675.9	6.02	147	100
136, 139	NADP-dependent malic enzyme, truncation	Cj1287c	gi 86149968	43925.1	5.88	501	100
229, 225	3-oxoacyl-(acyl carrier protein) synthase	Cj0442(fabF)	gi 15791809	42673.8	5.65	211	100
133, 129	inorganic pyrophosphatase	Cj0638c(ppa)	gi 15791998	19322.1	4.87	96	99.879
134, 135	transthyretin-like periplasmic protein	Cj0715	gi 15792064	15910.2	8.65	119	100

(Continued)

Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score	Protein Score C. I.%*
383	putative protein disulphide isomerase	Cj0872(<i>dsbA</i>)	gi 15792204	24541.2	7.68	159	100
281, 294	putative methyltransferase	Cj1419c	gi 37719599	29628.9	5.92	100	100
426, 425	chemotaxis protein	Cj0283c(<i>cheW</i>)	gi 15791653	19504.3	5.48	120	100
221, 222	iron ABC transporter, periplasmic	Cj0175c(<i>AfuA</i>)	gi 60594117	36046.7	8.69	315	100
26, 27	iron-binding protein	Cj0755c(<i>cfrA</i>)	gi 15792094	77506.5	5.29	534	100
28, 34	putative iron uptake protein	Cj0262c	gi 15791633	72786.7	4.94	236	100
97, 95	putative methyl-accepting chemotaxis signaltransduction protein	Cj0092	gi 15791480	49210.1	5.66	220	100
75, 86,	putative periplasmic protein	Cj1345c	gi 15792668	52462.2	6.74	355	100
187, 188	putative periplasmic protein	Cj1643	gi 15792948	41349.5	9.04	587	100
371, 379	putative oxidoreductase subunit	Cj0414	gi 145844667	26909.2	5.5	138	100
327, 328	putative oxidoreductase	Cj0807	gi 145845053	28146.3	6.77	223	100
242, 245	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	Cj0132(<i>pxC</i>)	gi 15791520	32862.1	6.34	151	100
202, 200	hypothetical protein Cj0069	Cj0069	gi 15791461	38767.4	5.23	157	100
158, 162	2-oxoglutarate ferredoxin oxidoreductase	Cj0536(<i>oorA</i>)	gi 15791897	41026.2	6.12	133	100
362, 367	hypothetical protein Cj0073c	Cj0073c	gi 15791463	24581.1	7.06	86	98.819
402, 403	ferritin	Cj0612c(<i>cft</i>)	gi 15791972	19473.7	5.34	84	98.085
431, 433	putative ubiquinol-cytochrome C reductase iron-sulfursubunit	Cj1186c(<i>petA</i>)	gi 15792510	18104.2	7.63	92	99.71
449, 446	50S ribosomal protein L10	Cj0476(<i>rplJ</i>)	gi 15791840	17704.4	8.91	133	100
174, 176	bifunctional 3,4-dihydroxy-2-butanone 4-phosphate	Cj0572(<i>ribA</i>)	gi 15791932	37900.2	5.61	393	100

(Continued)						
Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score C. I.%*
321, 524	thiazole synthase	Cj1045c(<i>thzG</i>)	gi 15792372	27629.5	5.67	174
163, 164	hypothetical protein Cjejd_02001989	Cj1682c(<i>gltA</i>)	gi 145560290	47981.3	6.47	329
301, 307	extracellular tungstate binding protein	Cj1540	gi 86152754	29812.7	9.3	81
29, 33	methyl-accepting chemotaxis protein	Cj1564	gi 86149417	71723.6	5.15	134
247, 231	periplasmic solute binding protein for ABC transport system	Cj0143c(<i>ZntC</i>)	gi 15791531	34556	6.71	83
9	flagellar hook protein FlgE	Cj0043f(<i>flgE</i>)	gi 88597739	91891.1	4.86	445
14	putative secreted serine protease	Cj1365c	gi 15792688	116232.2	7.03	128
17, 70	pyruvate ferredoxin/flavodoxin oxidoreductase	Cj1476c	gi 57238499	131313	5.96	148
32	molecular chaperone DnaK	Cj0759(<i>dnaK</i>)	gi 15792097	67377	4.98	644
35	heat shock protein 90	Cj0518(<i>hspG</i>)	gi 15791880	69610.9	5.09	166
62	phosphoenolpyruvate carboxykinase bifunctional phosphoribosylaminoimidazole carboxamideformyltransferase/IMP cyclohydrolase	Cj0932c(<i>pckA</i>)	gi 15792261	59016.7	5.76	254
73	putative phosphate acetyltransferase	Cj0953c(<i>purH</i>)	gi 15792282	56366.8	5.49	418
78	iron-sulfur cluster binding protein	Cj0688(<i>ftsA</i>)	gi 15792037	56142.1	5.75	323
87	cell division protein ftsA	Cj0074c	gi 57237082	54157.5	6.51	228
92	putative transcription termination factor	Cj0695(<i>ftsA</i>)	gi 145845179	50788	5.12	639
100	glutamyl-tRNA amidotransferase subunit A	Cj1156(<i>rho</i>)	gi 40217918	47151.1	7.68	454
101	hypothetical protein Cj0172c	Cj1059c(<i>gatA</i>)	gi 15792386	49253.1	5.85	174
111	tyrosyl-tRNA synthetase	Cj1271c(<i>tryS</i>)	gi 15792595	45391.6	5.32	317
130	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Cj0858c(<i>muraA</i>)	gi 15792196	45158	5.93	248
140					310	100

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Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score	Protein Score C.I.%*
171	histidinol-phosphate aminotransferase	<i>Cj0317(hisc)</i>	gi 15791685	41342.1	5.83	250	100
178	co-chaperone protein Dnal	<i>Cj1260c(dnal)</i>	gi 86150999	41580.7	6.12	127	100
179	phosphoserine aminotransferase	<i>Cj0326(serC)</i>	gi 15791694	40460.2	6.41	448	100
189	aspartate-semialdehyde dehydrogenase	<i>Cj1023c</i>	gi 15792350	38350.9	5.89	327	100
191	chorismate mutase\prophenate dehydratase	<i>Cj0316(pheA)</i>	gi 15791684	40451.9	6.34	127	100
192	N-acetylneuraminic acid synthetase	<i>Cj1141(neuB1)</i>	gi 15792640	38622.9	5.63	242	100
193	RecA	<i>Cj1673c(RecA)</i>	gi 437276	36992.2	5.23	263	100
195	DNA-directed RNA polymerase alpha subunit	<i>Cj1595(rpoA)</i>	gi 15792900	37662.6	4.97	160	100
197	flagellar P-ring protein precursor	<i>Cj1462(fgl)</i>	gi 15792779	37017.4	5.59	182	100
199	putative NADP-dependent alcohol dehydrogenase	<i>Cj1548c</i>	gi 15792856	38936.4	6.71	99.844	100
203	tolB protein, putative	<i>Cj0112(tolB)</i>	gi 86149640	44707.9	8.6	100	100
207	fructose-bisphosphate aldolase	<i>Cj0597(fba)</i>	gi 86151770	38739.7	5.38	174	100
208	hypothetical protein Cj1543	<i>Cj1543</i>	gi 15792851	35534.3	5.93	148	100
211	ketol-acid reductoisomerase	<i>Cj0632(lvcC)</i>	gi 15791992	36911	6.1	121	100
212	transaldolase	<i>Cj0281c(tal)</i>	gi 86153790	36838.2	6.85	171	100
226	possible periplasmic phosphate binding protein	<i>Cj0613(pstS)</i>	gi 145844955	36056.7	6.67	202	100
230	ribose-phosphate pyrophosphokinase	<i>Cj0918c(prsA)</i>	gi 15792247	33677	7.68	216	100
234	chemotaxis protein	<i>Cj0285c(cheV)</i>	gi 15791655	35835.6	4.92	167	100
238	cysteine synthase A	<i>Cj0912c(cysM)</i>	gi 57237740	32390	6.25	268	100
243	tryptophanyl-tRNA synthetase	<i>Cj0388(trpS)</i>	gi 15791755	36061.6	6.28	195	100

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Spot ID	Protein Name	Gene Code	No.	Protein Accession	Protein MW [§]	Protein PI [§]	Score	Protein Score	
								C. I.%*	
255	hypothetical protein Cj0021c	Cj0021c	gi 15791420	33077.9	5.66	94	99.775		
259	enoyl-(acyl carrier protein) reductase	Cj1400c(fabI)	gi 57238437	29847.4	5.67	173	100		
263	hypothetical protein CJE0666	Cj0561c	gi 57238240	35004.3	5.53	191	100		
264	hypothetical protein Cj0706	Cj0706	gi 15792055	27794.5	5.47	101	99.96		
269	3-methyl-2-oxobutanoate hydroxymethyltransferase	Cj0298c(panB)	gi 15791666	30182.6	6.09	118	100		
270	pantoate--beta-alanine ligase	Cj0297c(panC)	gi 15791665	32066.6	6.6	97	99.89		
272	elongation factor P	Cj0551(efp)	gi 15791912	21084.7	5.18	145	100		
273	histidine-binding protein precursor	Cj0734c(hisJ)	gi 15792083	27781.5	6.48	111	99.996		
274	PDZ domain protein	Cj1642	gi 121612648	40438.9	8.89	89	99.394		
280	putative periplasmic ATP/GTP-binding protein	Cj1041c	gi 15792368	31719	6.85	212	100		
283	2-dehydro-3-deoxyphosphooctonate aldolase	Cj0384c(kdsA)	gi 15791751	29761.4	6.84	155	100		
285	phosphomethylpyrimidine kinase	Cj1082c(thiD)	gi 15792407	29229.8	6.18	87	99.04		
286	putative membrane fusion component of efflux system	Cj1032	gi 15792359	27760.8	8.82	100	100		
287	50S ribosomal protein L1	Cj0475(rplA)	gi 15791839	25031.4	9.61	104	99.98		
298	hypothetical protein Cj1420c	Cj1420c	gi 15792738	29551.7	6.26	102	99.968		
299	putative periplasmic protein	Cj0772c	gi 15792110	28620.5	7.77	145	100		
303	formate dehydrogenase accessory protein	Cj1508c(fdhD)	gi 57238531	28571.6	5.69	83	97.643		
309	3-deoxy-D-manno-octulosonate cytidylyltransferase	Cj0813(kdsB)	gi 86152189	27121.9	5.64	95	99.844		
312	oxidoreductase	Cj0833c	gi 145845229	27985.5	6.04	107	99.99		
315	phosphoribosylaminoimidazole-succinocarboxamide synthase	Cj0512(purC)	gi 15791874	26921.2	5.8	146	100		

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Spot ID	Protein Name	Gene Code	No.	Protein Accession	Protein MW [§]	Protein PI [§]	Protein Score	Protein Score C. I.%*
322	pyridoxal phosphate biosynthetic protein	<i>Cj1238(pdxJ)</i>	gi 15792562	28992.6	7.18	100	99.95	
324	metallo-beta-lactamase family protein	<i>Cj0809c</i>	gi 186152045	23050.4	5.76	84	97.9	
326	non-haem iron protein	<i>Cj0012c</i>	gi 15791411	24509.9	5.49	97	99.902	
329	phosphoribosylformylglycinamide synthase subunit I	<i>Cj0514(purQ)</i>	gi 15791876	24915.8	6.22	114	99.998	
331	cytotoxic lethal distending toxin	<i>Cj0078c(cdtB)</i>	gi 15791468	28954.9	7.93	106	99.987	
332	lipoprotein, NLPA family	<i>Cj0771c</i>	gi 186152148	28883.7	8.37	196	100	
336	putative periplasmic protein	<i>Cj0770c</i>	gi 15792108	28629.6	9.07	147	100	
339	putative periplasmic protein	<i>Cj0151c</i>	gi 15791539	30545.2	7.66	165	100	
356	hypothetical protein <i>CjE1348</i>	<i>Cj1214c</i>	gi 57238085	26658.8	5.11	158	100	
358	two-component regulator	<i>Cj1261(racR)</i>	gi 15792585	25382.3	6.45	98	99.912	
360	ferredoxin oxidoreductase, gamma subunit	<i>Cj0538</i>	gi 57167583	20146.4	5.47	118	100	
374	putative hydrolase	<i>Cj1233</i>	gi 15792557	23692.1	6.08	150	100	
378	adenylyl kinase	<i>Cj0639c(adk)</i>	gi 15791999	21394.2	5.11	182	100	
384	possible outer membrane protein	<i>Cj1724c</i>	gi 15793024	24014.3	8.99	139	100	
385	hypothetical protein <i>Cjejd_02001846</i>	<i>Cj1545c</i>	gi 145960156	22210.2	6.5	225	100	
391	ribosome releasing factor	<i>Cj0234c(frr)</i>	gi 57237294	20741.9	7.79	99	99.934	
392	GTP cyclohydrolase II protein	<i>Cj0996(RibaA)</i>	gi 15792223	21481.1	7.66	190	100	
394	peptidyl-prolyl cis-trans isomerase	<i>Cj0115(slyD)</i>	gi 15791503	20119.6	4.75	81	96.35	
396	single-strand DNA-binding protein	<i>Cj1071(ssb)</i>	gi 15792396	20670.8	5.27	124	100	
399	phosphoheptose isomerase	<i>Cj1149c(gmhA)</i>	gi 15792473	20072.4	6.21	81	95.712	
405	transcription antitermination protein NusG	<i>Cj0473(nusG)</i>	gi 15791837	20182.7	6	172	100	
427	transcription elongation factor GreA	<i>Cj0287c(greA)</i>	gi 86149591	18000.2	4.9	91	99.627	

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Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW [§]	Protein PI [§]	Protein Score	Protein C.I.%*
444	putative signal-transduction sensor protein outer-membrane lipoprotein carrier	<i>Cj1189c</i>	gi 15792513	19300.7	7.71	202	100
445	bacterioferritin homolog	<i>Cj0943(lvA)</i>	gi 121612737	19214	6.29	94	99.795
455	acetolactate synthase III small subunit hypothetical protein CJE0476	<i>Cj0271</i>	gi 15791642	16700.8	7.71	163	100
459	3-dehydroquinate dehydratase	<i>Cj0575(lvH)</i>	gi 15791935	17330.2	6.75	161	100
461	possible bacterioferritin (3R)-hydroxymyristoyl ACP dehydratase	<i>Cj0427</i>	gi 57237482	13239.3	5.31	98	99.918
462	hypothetical protein Cj0573	<i>Cj0066c(aroQ)</i>	gi 15791458	17584	5.78	83	97.475
463	nucleoside diphosphate kinase	<i>Cj1534c</i>	gi 15792842	17193.5	5.55	124	100
466	riboflavin synthase subunit beta	<i>Cj0273(fabZ)</i>	gi 15791644	16429.5	6.07	89	99.38
467	putative periplasmic protein	<i>Cj0573</i>	gi 15791933	17062.3	5.47	91	99.59
472	50S ribosomal protein L7/L12	<i>Cj0332c(ndk)</i>	gi 15791700	15131.9	6.15	126	100
479	chemotaxis regulatory protein	<i>Cj0383c(ribH)</i>	gi 15791750	16675.8	6.42	124	100
484	hypothetical protein Cj1164c	<i>Cj0909</i>	gi 15792238	15732.6	9.5	103	99.975
487	hypothetical protein CJE0509	<i>Cj0477(rplI)</i>	gi 14559749	13037	4.7	89	99.394
496	thioredoxin chaperonin, 10 kDa	<i>Cj1118c(cheY)</i>	gi 15792443	14427.5	5.02	146	100
514	hypothetical protein Cj164c	<i>Cj1164c</i>	gi 15792488	10255.9	6.07	182	100
517	thioredoxin	<i>Cj0459c</i>	gi 57237513	9551	6.41	122	100
525	chaperonin, 10 kDa	<i>Cj0147c(trxA)</i>	gi 15791535	11345.7	4.54	94	99.795
528	carbon storage regulator homolog	<i>Cj1220(cpn10)</i>	gi 57168105	9425.1	5.38	88	99.237
535		<i>Cj1103(csrA)</i>	gi 15792428	8435.8	8.96	83	97.294

※ Identifications with a GPS confidence interval >95% were accepted. § Theoretical value

Supplementary file 2. Characteristics of identified proteins in the membrane protein fraction extracted from *C. jejuni* NCTC11168.

Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW	Protein PI ^s	Protein Score ^s	Protein Score C. I.%*
27	CTP synthetase	<i>Cj0027(pyrg)</i>	gi 15791426	60362.7	6.32	84	98.122
168	hypothetical protein	<i>Cj0069</i>	gi 15791461	38767.4	5.23	223	100
50, 53	ATP synthase subunit A	<i>Cj0105(atpA)</i>	gi 15791493	54767.1	6.03	213	100
11, 20, 43, 15	outer membrane protein	<i>Cj0129c</i>	gi 15791517	83146.7	5.5	455	100
190	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	<i>Cj0132(pxC)</i>	gi 57237139	32904.2	6.34	80	95.891
235	methyl-accepting chemotaxis protein thioredoxin-disulfide reductase	<i>Cj0144</i>	gi 121612938	25400.9	4.57	99	99.943
204	trigger factor	<i>Cj0146c(trxB)</i>	gi 86153593	33167.8	5.6	163	100
97, 58, 59, 60, 79, 194	cysteine desulfurase	<i>Cj0193c(tig)</i>	gi 15791580	50935.9	5.69	222	100
122	putative methyl-accepting chemotaxis protein transduction protein	<i>Cj0240c</i>	gi 15791612	43026	5.79	124	100
31, 39, 40, 177	chemotaxis protein CheA	<i>Cj0262c</i>	gi 15791633	72786.7	4.94	207	100
4, 5, 293	alkyl hydroperoxide reductase hypothetical protein	<i>Cj0284c</i>	gi 57237339	85222.5	4.89	118	100
269	fumurate reductase	<i>Cj0334(ahpC)</i>	gi 15791702	21933.2	5.66	238	100
248	3-oxoacyl-(acyl carrier protein) synthase	<i>Cj0355c</i>	gi 109691725	25493.5	5.39	143	100
18, 19, 16	acetyl-CoA carboxylase alpha subunit	<i>Cj0409(frdA)</i>	gi 57237464	73767.1	6.33	88	99.349
98	transcription elongation factor NusA	<i>Cj0442(fabF)</i>	gi 15791809	42673.8	5.65	107	99.991
186	elongation factor Tu	<i>Cj0443(accA)</i>	gi 15791810	34231.8	5.44	187	100
103	hypothetical protein	<i>Cj0460(nusA)</i>	gi 57237514	40616.5	5.51	91	99.666
108, 91, 107, 124, 123, 155, 156, 109, 84, 88	phosphoribosylaminoimidazole-succinocarbamate synthase	<i>Cj0470(tuf)</i>	gi 15791834	43566.2	5.11	405	100
17, 13	oxamide synthase	<i>Cj0493(fusA)</i>	gi 109691583	76639.7	5.09	199	100
236, 238		<i>Cj0512(purC)</i>	gi 86153356	26937.2	5.8	88	99.364

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Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW	Protein PI ^f	Protein Score ^g	Protein Score ^h	Protein Score ⁱ
							C. I.%*	C. I.%*
115, 118	2-oxoglutarate:acceptor oxidoreductase, alpha subunit	<i>Cj0536(orA)</i>	gi 86151524	40996.2	6.12	204	100	100
258	OrcC subunit of 2-oxoglutarate:acceptor oxidoreductase	<i>Cj0538(orC)</i>	gi 86153309	20070.4	5.67	156	100	100
171, 170, 169	oxidoreductase	<i>Cj0559</i>	gi 15791920	33656.3	5.73	132	100	100
211	hypothetical protein	<i>Cj0561c</i>	gi 57238240	35004.3	5.53	129	100	100
21	aspartyl-tRNA synthetase	<i>Cj0640c(aspS)</i>	gi 15792000	66124.9	5.8	234	100	100
23	putative iron uptake protein	<i>Cj0755(cfrA)</i>	gi 15792094	77506.5	5.29	124	100	100
33, 32	molecular chaperone DnaK	<i>Cj0759(dnaK)</i>	gi 15792097	67377	4.98	136	100	100
90, 3, 70	aconitate hydratase	<i>Cj0835c(acnB)</i>	gi 15792173	92734.8	6.04	139	100	100
95	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	<i>Cj0858c(murA)</i>	gi 15792196	45158	5.93	260	100	100
26, 28, 29, 30,	30S ribosomal protein S1	<i>Cj0893c(rpsA)</i>	gi 15792223	62757.2	5.34	87	99.18	99.873
179	ribose-phosphate pyrophosphokinase	<i>Cj0918c(prsA)</i>	gi 86153102	33648.9	7.68	96	100	100
89	putative outer membrane component of efflux system	<i>Cj1031(cmeD)</i>	gi 15792358	48940.5	6.04	451	99.794	99.794
56	inositol-5-monophosphate dehydrogenase	<i>Cj1058c(guaB)</i>	gi 57237943	52162.4	7.15	211	100	100
81, 85, 86, 87,	S-adenosylmethionine synthetase	<i>Cj1096c(metK)</i>	gi 15792421	43626.9	5.45	93	99.794	99.794
100, 101	putative MCP-type signal transduction protein	<i>Cj110c</i>	gi 15792435	48320.4	6	258	100	100
174	UDP-glucose 4-epimerase	<i>Cj1131c(gnE)</i>	gi 86150760	36722.4	5.59	270	100	100
136, 140	elongation factor Ts	<i>Cj1181c(tsf)</i>	gi 15792505	39530.2	5.24	87	99.121	99.121
67	putative glycolate oxidase subunit D	<i>Cj1213c(glcD)</i>	gi 15792537	50619.3	6.5	150	100	100
42, 51, 52, 80	heat shock protein	<i>Cj1221(groEL)</i>	gi 3063761	57990.5	5.02	114	99.998	99.998
133, 75	protease DO	<i>Cj1228c(htrA)</i>	gi 121613042	50984.9	8.97	139	100	100

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Spot ID	Protein Name	Gene Code	Protein Accession No.	Protein MW	Protein PI [§]	Protein Score [§]	Protein Score C.I.%*
82	tyrosyl-tRNA synthetase	<i>Cj1271c(tyrS)</i>	gi 15792595	45391.6	7.03	83	97.742
74	acetyl-CoA carboxylase	<i>Cj1290c(accc)</i>	gi 57238340	49129.9	6.01	105	99.986
158	N-acetyleneuraminic acid synthetase	<i>Cj1317(neuB3)</i>	gi 15792640	38622.9	5.63	164	100
94, 113	phosphoglycerate kinase	<i>Cj1402c(pgk)</i>	gi 15792720	43563.8	6.07	171	100
163	flagellar P-ring protein FlgI	<i>Cj1462(FlgI)</i>	gi 86149502	36987.4	5.59	145	100
188, 189, 185, 187, 220	outer membrane fibronectin-binding protein	<i>Cj1478c(cadF)</i>	gi 86152850	36000.9	5.8	194	100
160	hypothetical protein	<i>Cj1543</i>	gi 15792851	35534.3	5.93	97	99.91
112	peptide chain release factor 1	<i>Cj1612(prfA)</i>	gi 57504876	39794.7	5.28	123	100
161	RecA	<i>Cj1675c(recA)</i>	gi 437276	36997.2	5.23	184	100
114, 117	hypothetical protein	<i>Cj1682c(gltA)</i>	gi 109692142	47981.3	6.47	89	99.392
10, 8, 2, 9, 7	flagellar hook protein	<i>Cj1729c(flgE)</i>	gi 15793032	91863.1	4.86	480	100

※ Identifications with a GPS confidence interval >95% were accepted. § Theoretical value