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Research Article

PARTIAL PROTEOME MAP OF *CAMPYLOBACTER JEJUNI* STRAIN NCTC11168
BY GEL-FREE PROTEOMICS ANALYSIS

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Abstract

A proteome map of the foodborne pathogen *Campylobacter jejuni* NCTC11168 was analyzed using a state-of-the-art gel-free proteomic approach for the first time. A whole cell protein extract was prepared from the *C. jejuni* strain NCTC11168 grown in brain heart infusion (BHI) broth at 42°C under microaerobic conditions. A gel-free technique using isobaric tags for relative and absolute quantitation (iTRAQ) was employed to create a protein expression profile of the strain. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to identify the proteins. Protein functionalities were searched to classify them. A total of 235 proteins were identified in the whole cell protein fraction of *C. jejuni* NCTC11168 cells using iTRAQ analysis. Functional grouping of the identified proteins showed that forty percent of these proteins were associated with energy metabolism, protein synthesis and genetic information processing. iTRAQ was faster, easier and proved more sensitive than two-dimensional gel-based proteomics approaches previously applied to *C. jejuni*, making it an attractive tool for further studies of cellular physiological response.

Keywords: foodborne pathogen; *Campylobacter jejuni*; iTRAQ analysis; protein functions; proteomics; proteome

Introduction

Campylobacter jejuni has emerged as one of the most important foodborne pathogen associated with human gastroenteritis worldwide. In most developed countries, *C. jejuni* causes more cases of foodborne gastroenteritis each year than any other identified bacterial pathogen (Sliva *et al.*, 2011). Generally, the infection caused by *C. jejuni* is self-limiting, however, some patients may suffer serious complications (Young *et al.*, 2007), which include septicaemia, meningitis and polyneuropathic disorders such as Guillain-Barre syndrome (Skirrow, 1994). The high incidence and serious complications of campylobacteriosis makes *C. jejuni* a pathogen of considerable clinical and economic importance (On *et al.*, 2006).

C. jejuni NCTC11168 was originally isolated from a human diarrhoeal faeces sample in 1977 (Skirrow, 1977) and became the first fully sequenced genome in the *C. jejuni* species in 2000 (Parkhill *et al.*, 2000). The genome sequence of *C. jejuni* NCTC11168 has provided a valuable resource for *Campylobacter* post-genomic study. Many genomic, transcriptomic and proteomic studies have used *C. jejuni* NCTC11168 as a reference strain for comparison analysis (Birk *et al.*, 2012; On *et al.*, 2006; Stintzi and Whitworth, 2003; Zhang *et al.*, 2009). However, most of the proteomic studies on *C. jejuni* focused on alternation of

specific protein expression and had not constructed a protein expression profile for *C. jejuni* NCTC11168 until Zhang *et al.* (2013) attempted to develop a protein profile of *C. jejuni* NCTC11168 using two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF) protein analyses.

With higher resolution power and ability to quantify and identify proteins at the same time, gel-free proteomics is gaining popularity to study microbial proteomic study and making classical 2-DE proteomics redundant. We used isobaric tags for relative and absolute quantization (iTRAQ) proteomic analysis to construct a partial proteome of *C. jejuni* NCTC11168. To our knowledge, this is the first report on *C. jejuni* NCTC11168 proteome employing a gel-free technique to determine its partial proteome.

Material and Methods

Bacterial culture and sample preparation

C. jejuni NCTC11168 was stored in glycerol broths under frozen condition (-80°C). Prior to the experiment, the frozen culture were inoculated onto Columbia base agar containing 5% defibrinated sheep blood (BA; Oxoid) and incubated at 42°C for 48 h in a microaerobic environment (10% CO₂, 5% O₂ and 85% N₂) to recover from frozen storage. After 48h incubation for recovery, cells from a single colony were

inoculated in 100 ml BHI broth and incubated for 24 h with shaking 120 rpm at 42°C under microaerobic condition to get log phase cultured cells. Whole-cell proteins were extracted from the liquid culture using a protocol modified from the protocol reported by Kalmokoff et al. (2006). Briefly, bacterial cells in the broth were centrifuged at 4,000 x g at 4°C for 10 min and washed 2 times in the PBS (phosphate buffered saline) buffer (pH 7.4) by being centrifuged at 4,000 x g at 4°C for 10 min. Then the bacterial pellet was suspended in 1 ml lysis buffer (Protease inhibitor cocktail of aprotinin, 7 M urea, 2 M thiourea, 4.0% w/v CHAPS, 1% DTT and 0.2% Biolytes) and sonicated on ice six times for 20 s with 20 s intervals. Following sonication, the protein sample was solubilised by incubating the lysate on ice for 1 h. Then, cell debris was discarded by centrifuge at 12,000 g at 4°C for 15 min. The supernatants contain the total protein were collected by centrifugation as above. The concentration of whole-cell protein was determined with 2D Quant kit (GE healthcare) according to manufacturer's protocol.

***i*TRAQ labeling**

80 µg proteins from the whole-cell protein extract were precipitated by addition of 4 volumes of MeOH/CHCl₃. The precipitated pellet was resuspended in 60 µl of 0.5M TEAB (Triethylammonium bicarbonate buffer), then reduced with 20 µl of 100 mM TCEP (Tris 2-carboxyethyl phosphine hydrochloride) in 0.5M TEAB. After reduction, the protein sample was alkylated with 20 µl of 150 mM iodoacetamide) in 0.5 M TEAB. 10 µg trypsin was added into the alkylated sample for protein digestion. The mixture was incubated at 37°C for overnight. After the incubation, dried down the digest and resuspended it in 20 µl 0.5 M TEAB. *i*TRAQ labelling was done according to the *i*TRAQ labelling protocol provided with the *i*TRAQ reagents-8plex kit (AB Sciex Pvt. Ltd).

Strong cationic exchange chromatographic fraction

*i*TRAQ labeled sample was subjected to LC-MS/MS and fractionated using strong cation exchange (SCX) chromatography. The *i*TRAQ-labeled and dried digest was transferred into a new Eppendorf tube and resuspended in 0.1% TFA (Trifluoroethanoic acid). The Eppendorf tube was then put into a conditioned C18 empore disc for shaking 3 h. After 3 h shaking, the empore disc which contain eppies was washed with 0.1% TFA containing 5% ACN (Acetonitrile). After washing, 50% ACN in 0.1% TFA was used to elute the peptides from empore disc. Then, dried down the empore disc and prepared 40 µl of 0.1% formic acid for SCX fractionation. The SCX fractionation was performed using High-pressure liquid chromatography. Empore-purified each fraction from the SCX fractionation step (flow through, 1%, 5%, 10%, 20%, 30%, 40%, 60%, 80% and 100%) was as mentioned above.

LC-MS/MS (Liquid Chromatography-Mass Spectrometry) analysis

LC-MS/MS was performed on a nanoAdvance UPLC (ultra performance liquid chromatography) coupled to a maXis impact mass spectrometer equipped with a CaptiveSpray source (BrukerDaltonik, Bremen, Germany). 2 µl of sample was loaded on a C18AQ nano trap (Bruker, 75 µm × 2 cm, C18AQ, 3 µm particles, 200 Å pore size). The trap column was then switched in line with the analytical column (Bruker Magic C18AQ, 100 µm × 15 cm C18AQ, 3 µm particles, 200 Å pore size). The column oven temperature was 50°C. Elution was with a gradient from 0% to 40% B in 90 min at a flow rate of 800 nl/min. Solvent A was LCMS-grade water with 0.1% FA and 1% ACN; solvent B was LCMS-grade ACN with 0.1% FA and 1% water.

Samples were measured in auto MS/MS mode, with a mass range of m/z 50-2200. One MS was followed by 10 MS/MS of the most intense ions. Acquisition speed was 2 Hz in MS and 10 or 5 Hz in MS/MS mode depending on precursor intensity. Precursors were selected in the m/z 400-1400 range, with charge states 2-5 (singly charge ions were excluded). Active exclusion was activated after 1 spectrum for 0.3 min. Peptides with a score below 20, and proteins with fewer than 2 peptides were discarded. Only unique peptides were used for quantitation. Normalisation was based on division by channel sum.

Data processing

Data file processing, protein identification, and relative abundance quantification were performed using DataAnalysis software (Bruker), concatenated and submitted to an in-house Mascot server (v2.4) (Matrix Science, UK). The following search parameters were used: Taxonomy *Campylobacter jejuni*; Enzyme semitrypsin; Cysteine modification carbamidomethyl; MS tolerance 0.02 Da; MS/MS tolerance 0.1 Da; 1 missed cleavage; instrument specificity ESI-QUAD-TOF. Mascot *i*TRAQ parameters included variable *i*TRAQ8plex (N-term, K, Y), with reporter ions defined as appropriate for the experiment

Results and Discussion

A total of 235 proteins were identified in the whole cell protein extract of *C. jejuni* NCTC11168 grown in BHI broth at 42°C using *i*TRAQ analysis in this study. The characterization of identified proteins is summarized in Table 1. The genome of *C. jejuni* NCTC11168 contains approximate 1699 predicted genes, which is predicted to encode 1654 proteins (Parkhill *et al.*, 2000). In our study, 235 proteins were identified and the coverage of the identification is 14.2%. A previous investigation to obtain an *in vitro* protein profile of *C. jejuni* NCTC11168 using 2-DE combined with LC/MS/MS had identified 195 proteins and achieved 11.8% coverage of protein identification (Zhang *et al.*, 2013). A more recent study used in-gel digestion combined with nanoflow LC-MS/MS and achieved 86% coverage of identification (Liu *et al.*, 2012).

The method of protein sample preparation and the protein identification techniques may have contributed to this result; however, the number of LC-MS/MS replicate analyses undertaken by Liu et al. (2012) is also an important factor. Of the 1428 proteins identified by these authors, 253 were identified only in experiments where the NCTC11168 culture was used to infect the COS-1 cell line. A further 341 proteins were only detected under *in vitro* conditions analogous to ours and those of Zhang et al. (2013) where analyses were replicated (a total of 126 replicate analyses were undertaken in the study). The proteins most susceptible to non-detection were found to be expressed at low abundance. Given that, we believe the iTRAQ method described here is a potentially valuable tool for identifying the major expressed proteins and that its ease of use makes it an attractive and cost-effective tool for proteomic studies of microorganisms.

The functional categories of all identified expressed proteins were assigned according to protein functional description using UniProt (Universal Protein Resource) and NCBI (National Center for Biotechnology Information) protein database. Overall, 235 identified proteins from whole-cell protein extract were clustered into 15 groups basing on their functionality (Fig. 1).

Energy metabolism, ribosomal protein and genetic information processing are the three largest protein groups. The three groups of proteins account 44% of 235 identified proteins in this study. This *C. jejuni* NCTC 11168 proteome dataset coupled with functional classification will be useful for further comparative proteomic analysis and physiology investigation of this pathogen. The information presented in this study will be useful to investigate patho-physiology of *C. jejuni* and future research

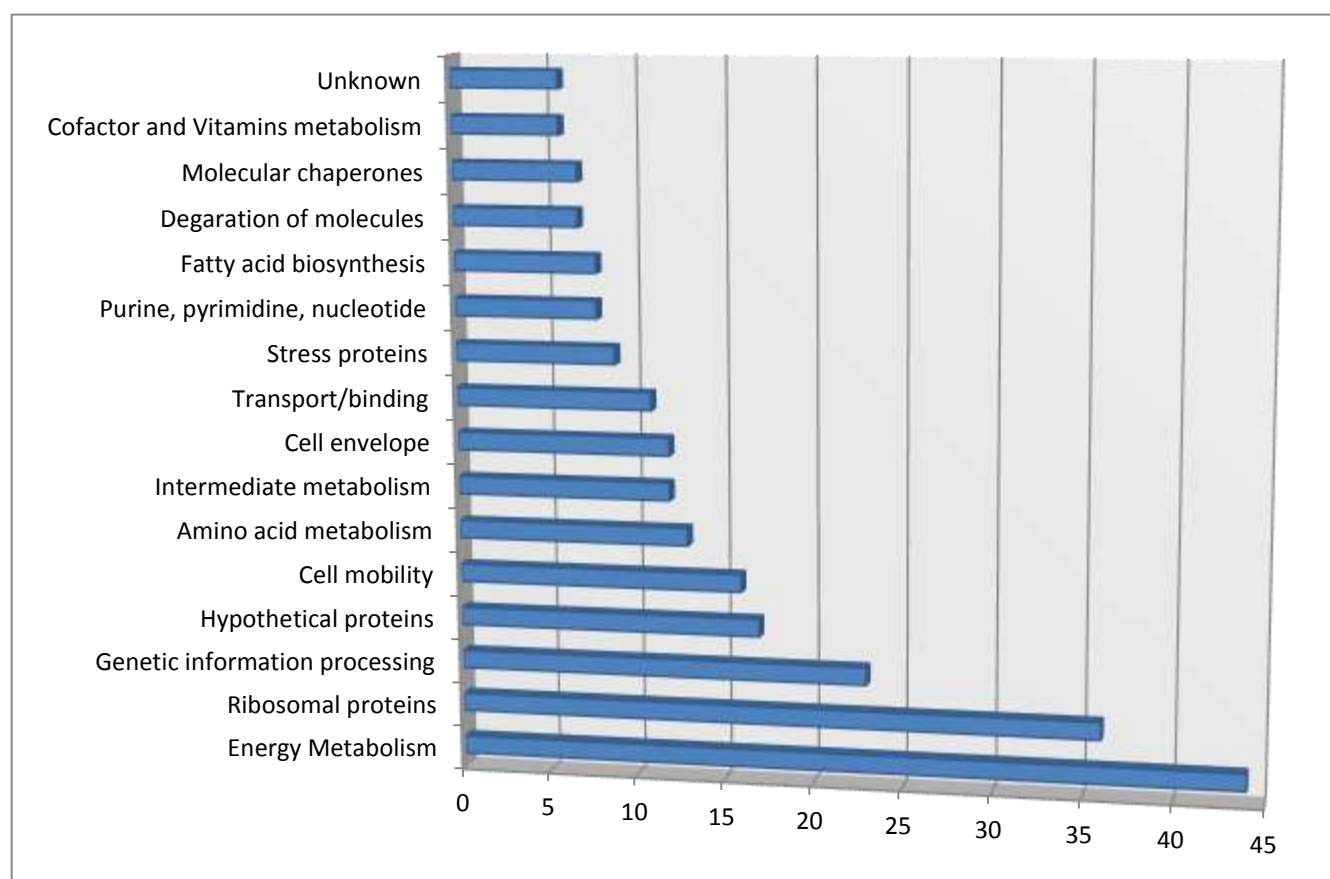


Fig. 1. The functional grouping of all identified proteins of *C. jejuni* NCTC11168 grown in BHI broth at 42°C under microaerophilic conditions.

Table 1: List of identified proteins in *C. jejuni* NCTC11168 using iTRAQ analysis

Accession	Protein name	Homology match	Score	Mass	Biological function
gi 88596234	co-chaperonin GroEL	<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	4881	57991	Helps protein folding
gi 86150126	flagellin subunit protein FlaA	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	2486	59075	Flagellar assembly
gi 3290034	flagellin B	<i>C. jejuni</i>	1568	59220	Flagellar assembly
gi 455428	flagellin protein	<i>C. jejuni</i>	706	59652	Major component of flagellum
gi 56806980	flagellin A	<i>C. jejuni</i>	312	11704	Flagellar assembly. Partial flagellin A
gi 57237524	elongation factor Tu	<i>C. jejuni</i> RM1221	2171	43623	Translation factor
gi 419643309	nitrate reductase catalytic subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> ATCC 33560	1779	105531	Electron transport. growth
gi 419682579	alkyl hydroperoxide reductase	<i>C. jejuni</i> subsp. <i>jejuni</i> 1213	1610	22037	Antioxidant activity
gi 86149534	chemotaxis protein CheA	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	1328	85349	Chemotaxis and Signal transduction
gi 86150607	protease DO	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	1316	50976	serine-type endopeptidase activity
gi 86150500	conserved hypothetical protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	1197	20593	unknown
gi 121613277	methyl-accepting chemotaxis protein	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	1196	72901	Chemotaxis and Signal transduction
gi 218563153	methyl-accepting chemotaxis signal transduction protein	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168	1137	73191	Chemotaxis and Signal transduction
gi 121613238	methyl-accepting chemotaxis protein	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	1131	72546	Chemotaxis and Signal transduction
gi 121613017	methyl-accepting chemotaxis protein	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	560	77220	Chemotaxis and Signal transduction
gi 419638103	putative MCP-type signal transduction protein	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9879	550	48561	Chemotaxis and Signal transduction
gi 419589392	putative MCP-type signal transduction protein	<i>C. coli</i> 317/04	505	48552	Chemotaxis and Signaltransduction
gi 419619379	putative MCP-type signal transduction protein	<i>C. jejuni</i> subsp. <i>jejuni</i> 51494	478	40704	Chemotaxis and Signaltransduction
gi 86150964	quinone-reactive Ni/Fe-hydrogenase, large subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> 260.94	1144	64194	Eletron transport
gi 419640337	pyruvate ferredoxin/ flavodoxin oxidoreductase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23357	1118	132355	Generates acetyl-CoA for TCA cycle
gi 205355941	fumarate reductase flavoprotein subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8421	1065	75125	Eletron transport
gi 148926924	heat shock protein dnaK	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	1061	67432	heat shock response
gi 86150249	thiol peroxidase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	928	18771	Reduce H ₂ O ₂ and organic hydroperoxides
gi 86150091	flavodoxin	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	802	17224	Electron transport

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Accession	Protein name	Homology match	Score	Mass	Biological function
gi 218563111	formate dehydrogenase large subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168	724	104573	Anaerobic respiration
gi 284055744	Chain A, Crystal Structure Of Putative Bacterioferritin	<i>C. jejuni</i>	709	17574	Ferric iron binding
gi 85036689	major outer membrane protein	<i>C. jejuni</i>	704	45632	porin activity, ion transport
gi 86153725	chemotaxis protein CheV	<i>C. jejuni</i> subsp. <i>jejuni</i> HB93-13	634	35822	Chemotaxis and Signal transduction
gi 283955551	ATP synthase F1 sector beta subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> 1336	623	50885	ATP-Proton motive force
gi 419623938	F0F1 ATP synthase subunit alpha	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23218	620	54851	ATP-Proton motive force
gi 384441131	Elongation factor G	<i>C. jejuni</i> subsp. <i>jejuni</i> M1	606	76983	Translation elongation factor
gi 57237741	DNA-binding protein HU	<i>C. jejuni</i> RM1221	595	10268	Prevent DNA denaturation
gi 419637637	2-methylisocitrate dehydratase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9879	563	93523	TCA cycle
gi 88597680	aspartate ammonia-lyase	<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	533	52166	Amino acid degradation
gi 148926719	fumarate hydratase	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	115	50947	TCA cycle
gi 57237475	hypothetical protein CJE0469	<i>C. jejuni</i> RM1221	524	21025	unknown
gi 330689730	Chain A, The Virulence Factor Peb4 And The Periplasmic Protein	<i>C. jejuni</i>	504	28359	
gi 86150070	conserved hypothetical protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	463	29837	unknown
gi 57237362	50S ribosomal protein L25/general stress protein Ctc	<i>C. jejuni</i> RM1221	462	19506	rRNA binding, stress respnse
gi 57238028	cytochrome C553	<i>C. jejuni</i> RM1221	459	11002	Electron transport
gi 419629625	DNA-directed RNA polymerase subunit beta'	<i>C. jejuni</i> subsp. <i>jejuni</i> 60004	425	169643	Catalyzes the transcription of DNA into RNA
gi 57237529	50S ribosomal protein L1	<i>C. jejuni</i> RM1221	423	25031	Protein expression regulation
gi 419658906	trigger factor	<i>C. jejuni</i> subsp. <i>jejuni</i> 2008-979	411	50970	Protein folding chaperones
gi 218562793	elongation factor Ts	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168	377	39758	Translational accuracy
gi 57238608	DNA-directed RNA polymerase subunit alpha	<i>C. jejuni</i> RM1221	377	37734	Catalyzes the transcription of DNA into RNA
gi 86149002	ribosomal protein L7/L12	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	366	13065	Translational accuracy
gi 57237465	fumarate reductase iron-sulfur subunit	<i>C. jejuni</i> RM1221	365	28282	TCA cycle
gi 57238267	succinyl-CoA synthetase subunit beta	<i>C. jejuni</i> RM1221	342	41897	TCA cycle
gi 384447762	DNA-directed RNA polymerase subunit beta	<i>C. jejuni</i> subsp. <i>jejuni</i> IA3902	336	156229	Catalyzes the transcription of DNA into RNA

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Accession	Protein name	Homology match	Score	Mass	Biological function
gi 415729844	DNA polymerase III beta subunit, central domain protein	<i>C. jejuni</i> subsp. <i>jejuni</i> DFVF1099	327	28735	DNA replication
gi 283953694	50S ribosomal protein L5	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	296	20083	Part of 50S ribosomal subunit
gi 57168680	thioredoxin	<i>C. jejuni</i> RM2228	284	11501	Antioxidants
gi 57237002	glutamine synthetase, type I	<i>C. jejuni</i> RM1221	281	54367	Glutamine synthesis
gi 57237749	ABC transporter aspartate/glutamate-binding protein	<i>C. jejuni</i> RM1221	278	28243	Amino acid transport
gi 86149479	enolase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	276	45253	Glycolysis, carbohydrate degradation
gi 419641626	periplasmic nitrate reductase, small subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23357]	268	19251	Anaerobic respiration
gi 218562328	acetate kinase	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168	268	44429	Convert acetate to acetyl-CoA
gi 86149326	formate dehydrogenase, iron-sulfur subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	267	24717	Respiration - Anaerobic
gi 57236893	30S ribosomal protein S10	<i>C. jejuni</i> RM1221	253	11665	Binding of tRNA
gi 86150638	oxaloacetate decarboxylase, alpha subunit, putative	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	252	66161	ATP-Proton motive force
gi 57237305	hypothetical protein CJE0298	<i>C. jejuni</i> RM1221	248	32085	unknown
gi 415730403	multi-sensor signal transduction histidine kinase	<i>C. jejuni</i> subsp. <i>jejuni</i> DFVF1099	247	25368	signal transduction
gi 57238000	chemotaxis protein CheY	<i>C. jejuni</i> RM1221	245	14428	Chemotaxis and signal transduction
gi 57238092	molecular chaperone GroES	<i>C. jejuni</i> RM1221	240	9452	Folding chaperones
gi 57238690	citrate synthase	<i>C. jejuni</i> RM1221	240	48109	TCA cycle
gi 86149847	argininosuccinate synthase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	236	45778	Argininosuccinate synthase activity
gi 57238707	50S ribosomal protein L16	<i>C. jejuni</i> RM1221	235	16365	Peptidyl transferase activity
gi 57238440	glyceraldehyde 3-phosphate dehydrogenase A	<i>C. jejuni</i> RM1221	233	36691	Glycolysis, carbohydrate degradation
gi 57237544	30S ribosomal protein S7	<i>C. jejuni</i> RM1221	224	17681	rRNA binding proteins
gi 57238704	50S ribosomal protein L14 [<i>C. jejuni</i> RM1221	215	13354	Part of 50S ribosomal subunit
gi 86150520	rubrerythrin	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	210	25023	H ₂ O ₂ decomposition
gi 57237338	chemotaxis protein CheW	<i>C. jejuni</i> RM1221	205	19504	signal transduction, chemotaxis

Table 1: List of identified proteins in *C. jejuni* NCTC11168 using iTRAQ analysis

Accession	Protein name	Homology match	Score	Mass	Biological function
gi 419641974	serine hydroxymethyltransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> ATCC 33560	205	46079	Provide one-carbon source
gi 57237300	cysteine desulfurase	<i>C. jejuni</i> RM1221	204	43191	cysteine desulfurase activity
gi 317510573	methionine adenosyltransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> 305	202	40926	Methionine adenosyltransferase activity
gi 57237017	transthyretin-like protein	<i>C. jejuni</i> RM1221	198	15910	unknown
gi 57237413	cytochrome C551 peroxidase	<i>C. jejuni</i> RM1221	193	37021	Cytochrome-c peroxidase activity
gi 57238192	nonheme iron-containing ferritin	<i>C. jejuni</i> RM1221	189	19531	Iron-storage, DNA-binding/protection
gi 419621644	inosine 5'-monophosphate dehydrogenase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23216	186	52391	Regulation of cell growth
gi 57237122	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD	<i>C. jejuni</i> RM1221	181	20547	Folding helper with chaperones
gi 86149608	conserved hypothetical protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	171	39053	unknown
gi 57237410	DNA-binding response regulator	<i>C. jejuni</i> RM1221	166	25614	Signal transduction, transcription regulation
gi 88596859	ketol-acid reductoisomerase	<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	158	37139	Branched-chain amino acid metabolism
gi 317509581	isocitrate dehydrogenase, NADP-dependent	<i>C. jejuni</i> subsp. <i>jejuni</i> 305	156	86588	Isocitrate dehydrogenase activity for TCA cycle
gi 57238730	50S ribosomal protein L3	<i>C. jejuni</i> RM1221	154	20824	Part of 50S ribosomal subunit
gi 57236972	ATP-dependent protease ATP-binding subunit HslU	<i>C. jejuni</i> RM1221	154	49747	Peptidase activity, acting on L-amino acid peptides
gi 384447418	F0F1 ATP synthase subunit epsilon	<i>C. jejuni</i> subsp. <i>jejuni</i> IA3902	146	13825	ATP synthesis coupled proton transport
gi 57238512	cbb3-type cytochrome C oxidase subunit II	<i>C. jejuni</i> RM1221	145	25078	Electron transport
gi 57238069	aspartyl/glutamyl-tRNA amidotransferase subunit B	<i>C. jejuni</i> RM1221	144	53256	Carbon-nitrogen ligase activity for translation
gi 57238619	ATP/GTP-binding protein	<i>C. jejuni</i> RM1221	141	40355	Cations antiporter
gi 419622743	cytochrome c552	<i>C. jejuni</i> subsp. <i>jejuni</i> IA3902	138	69795	Nitrite reductase use for anaerobic respiration
gi 57237746	ribose-phosphate pyrophosphokinase	<i>C. jejuni</i> RM1221	137	33848	Nucleotide biosynthesis
gi 86150182	oxidoreductase, short chain dehydrogenase/reductase family	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	136	28233	
gi 86148986	acyl carrier protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	130	8592	Important component in fatty acid synthesis
gi 148926276	malate oxidoreductase	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	129	44170	Malate metabolism, TCA cycle
gi 153952659	adenylate kinase	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	129	21432	Adenine nucleotide metabolism
gi 13509099	aspartate-semialdehyde dehydrogenase	<i>C. jejuni</i>	127	21405	Oxidoreductase

Table 1: List of identified proteins in *C. jejuni* NCTC11168 using iTRAQ analysis

Accession	Protein name	Homology match	Score	Mass	Biological function
gi 57238058	ubiquinol--cytochrome C reductase, iron-sulfur subunit	<i>C. jejuni</i> RM1221	127	18332	Electron transport
gi 57237954	30S ribosomal protein S18	<i>C. jejuni</i> RM1221	127	10322	Helps stabilize the platform of the 30S subunit
gi 419694122	thioredoxin reductase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9872	126	34000	Oxidoreductase for defense against oxidative damage
gi 86149254	ribosomal protein L4	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	126	22273	Important for 50S assembly
gi 57238437	enoyl-ACP reductase	<i>C. jejuni</i> RM1221	125	29961	Catalyze a key regulatory step in fatty acid synthesis
gi 57237528	50S ribosomal protein L11	<i>C. jejuni</i> RM1221	123	15127	Part of 50s ribosomal subunit
gi 57238502	30S ribosomal protein S9	<i>C. jejuni</i> RM1221	121	14128	Structural constituent of ribosome
gi 57237722	30S ribosomal protein S1	<i>C. jejuni</i> RM1221	120	62827	Binds mRNA to initiate translation
gi 153952052	30S ribosomal protein S2	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	119	30449	Structural constituent of 30S subunit
gi 57237197	ATP-dependent Clp protease proteolytic subunit	<i>C. jejuni</i> RM1221	119	21807	hydrolyzes proteins to small peptides
gi 86149225	3-methyl-2-oxobutanoate hydroxymethyltransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	116	30354	Pantothenate biosynthesis
gi 57237694	glutamate-1-semialdehyde aminotransferase	<i>C. jejuni</i> RM1221	111	46517	Porphyrin biosynthesis
gi 86148961	heat shock protein HtpG	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	111	69640	heat shock response
gi 205355989	putative phospho-sugar mutase	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8421	110	48938	Amino sugar metabolism
gi 68248462	ribosomal protein L22	<i>C. jejuni</i>	110	15885	Important for 50S assembly
gi 146386525	Chain A, Crystal Structure Of Peb3	<i>C. jejuni</i>	109	26351	ABC transporter
gi 57237728	4-methyl-5(B-hydroxyethyl)-thiazole monophosphate biosynthesis enzyme	<i>C. jejuni</i> RM1221	108	20427	Thiamine biosynthesis
gi 121613200	glucosamine--fructose-6-phosphate aminotransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	103	67687	Amino sugar metabolism
gi 57169062	ribosomal protein L13	<i>C. coli</i> RM2228	105	15864	A early assembly protein of 50S ribosomal subunit
gi 3413445	galE	<i>C. jejuni</i>	103	37224	lipopolysaccharide synthesis
gi 37719580	putative UDP-glucose 4-epimerase	<i>C. jejuni</i>	102	35556	Gluconeogenesis
gi 57238703	50S ribosomal protein L24	<i>C. jejuni</i> RM1221	101	8335	One of two assembly initiator proteins for 50S subunit
gi 57238607	30S ribosomal protein S4	<i>C. jejuni</i> RM1221	100	23895	Important for translational accuracy
gi 86152514	50 kDa outer membrane protein	<i>C. jejuni</i> subsp. <i>jejuni</i> HB93-13	99	53832	Membrane protein

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gi 317511439	L-Serine ammonia-lyase, partial	<i>C. jejuni</i> subsp. <i>jejuni</i> 305]	99	36633	Amino acid degradation
gi 57237048	hypothetical protein CJE0033	<i>C. jejuni</i> RM1221	99	26440	unknown
gi 57237923	acetyl-CoA carboxylase subunit A	<i>C. jejuni</i> RM1221	98	54834	acetyl-CoA carboxylase activity
gi 86149797	cjaC protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	98	27835	Transfer substances through membrane
gi 86150082	conserved hypothetical protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	93	43511	unknown
gi 317511289	highly acidic protein, partial	<i>C. jejuni</i> subsp. <i>jejuni</i> 305	93	45096	unknown
gi 205356548	translation initiation factor IF3	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8421	93	14527	Translation initiation factor
gi 57237076	3-dehydroquinate dehydratase	<i>C. jejuni</i> RM1221	92	17755	phenylalanine, tyrosine and tryptophan synthesis
gi 153951531	2-oxoglutarate-acceptor oxidoreductase subunit OorC	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	91	20141	TCA cycle
gi 218561850	hypothetical protein Cj0170	<i>C. jejuni</i> subsp. <i>jejuni</i> NCTC 11168	90	28767	unknown
gi 148926946	major antigenic peptide PEB2	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	87	24485	Major antigenic peptide
gi 283954138	aspartate kinase, monofunctional class	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	87	42771	Catalyses biosynthesis of amino acid
gi 419694729	phenylalanyl-tRNA synthetase subunit beta	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9872	87	87969	Aminocayl-tRNA synthetase activity
gi 57238606	30S ribosomal protein S11	<i>C. jejuni</i> RM1221	87	13942	Structural constituent of ribosome 30S subunit
gi 57238712	50S ribosomal protein L23	<i>C. jejuni</i> RM1221	87	10561	rRNA binding, structural constituent of 50S subunit
gi 419627400	carboxyl-terminal protease	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23263	85	48990	Hydrolase and protease
gi 419641319	biotin carboxylase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23357	84	49485	Participates in fatty acid synthesis
gi 475890	fur	<i>C. jejuni</i>	84	18175	Transcription regulation. Repressor
gi 121612631	saccharopine dehydrogenase	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	83	45561	Lysine metabolism
gi 86150038	dTDP-4-dehydrorhamnose 3,5-epimerase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	81	21245	Lipopolysaccharide biosynthesis
gi 86150084	methyltransferase, FkbM family protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	81	33582	transfer of a methyl group
gi 153951193	50S ribosomal protein L10	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	80	17775	rRNA binding, structural constituent of 50S subunit
gi 283955815	pyruvate kinase	<i>C. jejuni</i> subsp. <i>jejuni</i> 1336	80	53954	Glycolysis, carbohydrate degradation
gi 88597146	phosphate acetyltransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	79	56356	transferring acyl groups
gi 283955175	hypothetical protein C414_000420088	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	78	16753	unknown

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Accession	Protein name	Homology match	Score	Mass	Biological function
gi 57238139	quinone-reactive Ni/Fe-hydrogenase, small subunit	<i>C. jejuni</i> RM1221	78	39846	Eletron transport
gi 57238700	30S ribosomal protein S8	<i>C. jejuni</i> RM1221	77	14795	helps assembly of 30S subunit
gi 419633975	branched-chain amino acid aminotransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23269	77	34036	Branched-chain amino acid metabolism
gi 86151404	trimethylamine-N-oxide reductase 2 precursor	<i>C. jejuni</i> subsp. <i>doylei</i> 260.94	76	93857	Catalyzes an anaerobic reaction
gi 57237495	succinate dehydrogenase, C subunit	<i>C. jejuni</i> RM1221	75	31983	TCA cycle
gi 419648255	succinate dehydrogenase, flavoprotein subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9217	72	67163	Electron transport
gi 57237820	delta-aminolevulinic acid dehydratase	<i>C. jejuni</i> RM1221	71	36733	Catalyzes biosynthesis of tetrapyrroles
gi 57238056	ubiquinol--cytochrome C reductase, cytochrome C1 subunit	<i>C. jejuni</i> RM1221	71	41617	Electron transport
gi 419641014	ATP-dependent chaperone protein ClpB	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23357	70	95538	Protein folding chaperones
gi 57238266	succinyl-CoA synthase, alpha subunit	<i>C. jejuni</i> RM1221	69	30216	TCA cycle
gi 4704599	fibronectin binding protein	<i>C. jejuni</i>	69	32429	Helps adhere to infected cell
gi 57168772	ribosomal protein L21	<i>C. coli</i> RM2228	68	11602	rRNA binding protein
gi 86150649	conserved hypothetical protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	68	16323	unknown
gi 57236953	aspartyl-tRNA synthetase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23269	66	66676	Helps tRNA bind to amino acid aspartate
gi 419632940	polynucleotide phosphorylase/polyadenylase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23269	65	79322	mRNA processing and degradation
gi 57238263	2-oxoglutarate-acceptor oxidoreductase subunit OorB	<i>C. jejuni</i> RM1221	65	31758	TCA cycle
gi 57237500	acetyl-CoA carboxylase carboxyltransferase subunit alpha	<i>C. jejuni</i> RM1221	65	34473	ATP binding, acety-CoA carboxylase activity
gi 86149076	malate:quinone oxidoreductase, putative	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	64	50953	TCA cycle
gi 57237388	flagellar motor protein MotA	<i>C. jejuni</i> RM1221	64	28279	Rotary flagellar machine
gi 57237383	nucleoside diphosphate kinase	<i>C. jejuni</i> RM1221	62	15161	Synthesis of nucleoside triphosphates
gi 148925618	putative capsule polysaccharide export system periplasmic protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	62	58916	Polysaccharide transmembrane transporter
gi 57237527	transcription antitermination protein NusG	<i>C. jejuni</i> RM1221	61	20183	Transcription elongation
gi 283954636	ATP-dependent protease La	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	61	90364	Intercellular selective proteolysis
gi 57238618	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	<i>C. jejuni</i> RM1221	60	42635	Acyltransferase, transferase
gi 86150017	putative sugar transferase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	60	89945	Hydrolase and transferase activity

Table 1: List of identified proteins in *C. jejuni* NCTC11168 using iTRAQ analysis

Accession	Protein name	Homology match	Score	Mass	Biological function
gi 57238609	50S ribosomal protein L17	<i>C. jejuni</i> RM1221	60	13240	Structural constituent of ribosome
gi 121612363	oxidoreductase, putative	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	51	64074	Catalyzes oxidoreduction
gi 57238698	50S ribosomal protein L18	<i>C. jejuni</i> RM1221	58	13288	Part of subunit of 50S,contacts 5S and 23S rRNA
gi 57237913	lipoprotein	<i>C. jejuni</i> RM1221	58	18559	Lipoprotein
gi 153951934	2-oxoglutarate-acceptor oxidoreductase subunit OorA	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	58	41211	TCA cycle
gi 57237952	30S ribosomal protein S6	<i>C. jejuni</i> RM1221	57	14689	Structural constituent of 30S subunit
gi 57238706	50S ribosomal protein L29	<i>C. jejuni</i> RM1221	57	7029	Structural constituent of ribosome 50S subunit
gi 317455537	Chain A, Crystal Structure Of Phosphoglycerate Kinase	<i>C. jejuni</i> RM	57	43921	Glycolysis, carbohydrate degradation
gi 57238142	2-nitropropane dioxygenase family oxidoreductase	<i>C. jejuni</i> RM1221	56	39995	
gi 57237459	hypothetical protein CJE0453	<i>C. jejuni</i> RM1221	56	30726	unknown
gi 86149461	ATP-sulfurylase family protein	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	56	44829	Sulfate adenyltransferase activity
gi 57236924	competence protein ComEA	<i>C. jejuni</i> RM1221	56	8847	DNA repair, take up exogenous DNA
gi 633730	RpsO	<i>C. jejuni</i> subsp. <i>jejuni</i> 81-176	56	10206	A binding protein
gi 57237099	hypothetical protein CJE0087	<i>C. jejuni</i> RM1221	56	49254	unknown
gi 40217918	putative transcription termination factor	<i>C. jejuni</i>	55	47265	Transcription termination factor
gi 57236939	CTP synthetase	<i>C. jejuni</i> RM1221	55	60765	Pyrimidine, nucleotide metabolism
gi 57237008	hypothetical protein CJE0806	<i>C. jejuni</i> RM1221	54	27951	unknown
gi 57236997	peptidyl-prolyl cis-trans isomerase D,-like protein	<i>C. jejuni</i> RM1221	54	57475	Folding chaperones
gi 57238696	50S ribosomal protein L15	<i>C. jejuni</i> RM1221	53	14019	Structural constituent of 50S subunit
gi 419645158	preprotein translocase subunit SecA	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9081	53	98244	Tansport protein
gi 57236974	50S ribosomal protein L9	<i>C. jejuni</i> RM1221	52	16272	rRNA binding
gi 57237003	hypothetical protein CJE0800	<i>C. jejuni</i> RM1221	52	25980	unknown
gi 407941670	threonyl-tRNA ligase	<i>C. jejuni</i> subsp. <i>jejuni</i> PT14	51	69916	Aminoacyl-tRNA synthetase
gi 419635264	superoxide dismutase	<i>C. jejuni</i> subsp. <i>jejuni</i> 55037	51	25056	superoxide dismutase activity
gi 57238518	hypothetical protein CJE1668	<i>C. jejuni</i> RM1221	51	22100	unknown

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gi 86150584	GMP synthase	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	48	57397	Purine metabolism
gi 283955572	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	<i>C. jejuni</i> subsp. <i>jejuni</i> 1336	48	31300	acetyl-CoA carboxylase activity
gi 283954942	hypothetical protein C414_000290003	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	47	44381	unknown
gi 57237342	transcription elongation factor GreA	<i>C. jejuni</i> RM1221	46	18056	transcriptional regulation
gi 57238656	transketolase	<i>C. jejuni</i> RM1221	44	70001	Gluconeogenesis
gi 419645546	NADH dehydrogenase subunit G	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9081	44	94588	Electron transport
gi 57238554	UTP-glucose-1-phosphate uridylyltransferase	<i>C. jejuni</i> RM1221	43	30831	Nucleotide sugar metabolism
gi 57238705	30S ribosomal protein S17	<i>C. jejuni</i> RM1221	43	9600	a rRNA binding protein
gi 153951811	molybdenum cofactor biosynthesis protein MogA	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	35	20371	biosynthesis of the molybdenum cofactor
gi 86150511	thiamine biosynthesis protein ThiF	<i>C. jejuni</i> subsp. <i>jejuni</i> CF93-6	42	30047	Thiamin biosynthesis
gi 419622789	aspartate aminotransferase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23216	42	43692	Aminotransferase activity
gi 57237180	iron ABC transporter periplasmic iron-binding protein	<i>C. jejuni</i> RM1221	42	37406	ABC transporter. Iron ion transport
gi 317511751	isoleucyl-tRNA synthetase	<i>C. jejuni</i> subsp. <i>jejuni</i> 305	42	106086	Amino-acyl tRNA synthetases and modification
gi 88596055	twin-arginine translocation pathway signal	<i>C. jejuni</i> subsp. <i>jejuni</i> 84-25	40	72004	Translocate folded proteins
gi 419648839	histidyl-tRNA synthetase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 9217	40	47897	Amino-acyl tRNA synthetases and modification
gi 57238697	30S ribosomal protein S5	<i>C. jejuni</i> RM1221	40	15787	Translational accuracy
gi 57238257	prolyl-tRNA synthetase	<i>C. jejuni</i> RM1221	40	65053	Deliver amino acid to ribosome
gi 329666276	Chain A, Crystal Structure Of Adenylosuccinate Synthetase	<i>C. jejuni</i>	38	46529	Purine biosynthesis
gi 148926982	carbamoyl-phosphate synthase large chain	<i>C. jejuni</i> subsp. <i>jejuni</i> CG8486	37	122342	arginine and pyrimidine biosynthesis
gi 57237499	3-oxoacyl-ACP synthase II	<i>C. jejuni</i> RM1221	37	43103	Acyltransferase, transferase
gi 153951812	hypothetical protein JJD26997_0724	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	36	41387	unknown
gi 57237957	flagellar assembly protein FliW [<i>Campylobacter jejuni</i> RM1221]	<i>C. jejuni</i> RM1221	36	14892	Stabilize flagellin during assembly
gi 283955143	3-deoxy-8-phosphooctulonate synthase	<i>C. jejuni</i> subsp. <i>jejuni</i> 414	36	29826	Synthesis of lipopolysaccharides
gi 57237051	cytochrome C family protein	<i>C. jejuni</i> RM1221	35	39104	Electron transfer,heme binding
gi 419622287	UDP-GlcNAc-specific C4,6 dehydratase/C5 epimerase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23216	35	37683	nucleoside-diphosphate sugar epimerase activity

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Accession	Protein name	Homology match	Score	Mass	Biological function
gi 57238611	histidinol dehydrogenase	<i>C. jejuni</i> RM1221	34	46687	Histidine biosynthesis
gi 57238510	cytochrome C oxidase, cbb3-type, subunit III	<i>C. jejuni</i> RM1221	33	31370	Electron transport
gi 57238249	elongation factor P	<i>C. jejuni</i> RM1221	32	21199	Stimulates efficient translation and peptide-bond synthesis
gi 57237163	50S ribosomal protein L31	<i>C. jejuni</i> RM1221	32	7757	rRNA binding
gi 57237111	F0F1 ATP synthase subunit delta	<i>C. jejuni</i> RM1221	32	20497	ATP-Proton motive force
gi 57238711	50S ribosomal protein L2	<i>C. jejuni</i> RM1221	31	30515	rRNA binding, transferase activity
gi 419641488	short chain dehydrogenase/reductase family oxidoreductase	<i>C. jejuni</i> subsp. <i>jejuni</i> LMG 23357	30	28161	Interconversion between alcohols and aldehydes or ketones
gi 153952664	glucosamine-1-phosphate acetyltransferase	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	30	48372	Cell wall synthesis
gi 881376	OmpR protein, partial	<i>C. jejuni</i>	29	22640	Major outer membrane protein synthesis regulator
gi 57237336	transaldolase	<i>C. jejuni</i> RM1221	29	37122	pentose-phosphate pathway
gi 153952472	30S ribosomal protein S3	<i>C. jejuni</i> subsp. <i>doylei</i> 269.97	28	26067	mRNA binding, rRNA binding
gi 57237149	cation ABC transporter ATP-binding protein	<i>C. jejuni</i> RM1221	28	33050	ABC transporter
gi 57238563	flavodoxin	<i>C. jejuni</i> RM1221	24	22239	Plays important role in electron transport
gi 57237119	translocation protein TolB	<i>C. jejuni</i> RM1221	24	44711	Cell envelope integrity

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