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RESEARCH ARTICLE

Isolation and 2-D-DIGE proteomic analysis of intracellular and extracellular forms of *Listeria monocytogenes*

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The pathogenicity of *Listeria monocytogenes* is related to its ability of invading and multiplying in eukaryotic cells. Its main virulence factors are now well characterized, but limited proteomic data is available concerning its adaptation to the intracellular environment. In this study, *L. monocytogenes* EGD (serotype 1/2a) grown in human THP-1 monocytes (24 h) were successfully separated from host organelles and cytosolic proteins by differential and isopycnic centrifugation. For control, we used cell homogenates spiked with bacteria grown in broth. Proteomes from both forms of bacteria were compared using a 2-D-DIGE approach followed by MALDI-TOF analysis to identify proteins. From 1684 distinct spots, 448 were identified corresponding to 245 distinct proteins with no apparent contamination of host proteins. Amongst them, 61 show underexpression (stress defense; transport systems, carbon metabolism, pyrimidines synthesis, D-Ala-D-Ala ligase) and 22 an overexpression (enzymes involved in the synthesis of cell envelope lipids, glyceraldehyde-3-phosphate, pyruvate and fatty acids). Our proteomic analysis of intracellular *L. monocytogenes* (i) suggests that bacteria thrive in a more favorable environment than extracellularly, (ii) supports the concept of metabolic adaptation of bacteria to intracellular environment and (iii) may be at the basis of improved anti-*Listeria* therapy.

Keywords:
2-D-DIGE / Intracellular / *Listeria monocytogenes* / Microbiology / THP-1 monocytes

1 Introduction

Listeria monocytogenes is a Gram-positive intracellular food-borne pathogen causing rare but severe diseases in immunocompromised patients [1, 2]. *L. monocytogenes* invades eukaryotic cells, crossing epithelial barriers and disseminating in the body, while avoiding humoral defenses [3–5]. Virulence proteins related to eukaryotic cells invasion have now been extensively described [6–8], and increasing data is being obtained about its metabolic adaptation to the intracellular environment [9, 10]. Growth of *L. monocytogenes* in eukaryotic cells was reported to be dependent on the availability of aromatic amino acids, threonine and adenine [11, 12]. Intracellular *L. monocytogenes* also overexpresses the hexose phosphate (hpt) [13, 14] and the ABC arginine transporters (ArpJ) [15, 16], and the lipoate protein ligase (LpIA1), allowing bacteria to use the corresponding molecules as source of carbon and nitrogen and to promote pyruvate dehydrogenase activity [17, 18]. Thiamine and biosynthesis of thiamine precursors are also required for intracellular replication of

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L. monocytogenes [19] as well as the expression of the oligopeptide permease OppA [20]. This triggered several authors to analyze the adaptation of intracellular *L. monocytogenes* in a more global way.

Whole genome microarray, reverse transcriptase PCR analyses and the construction of deletion mutants have been successfully used to investigate the transcriptional expression profile of intracellular *L. monocytogenes* [21, 22]. We elected to use gel-based proteomic analysis, since it allows revealing in a more direct fashion global differences in the phenotype of bacteria when exposed to different environments [23, 24]. For bacteria growing in eukaryotic cells, these studies require, however, adequate separation from the host-cell proteins to avoid difficulties in identification and quantification of the specific changes occurring in the bacteria themselves. In the present study, we have developed a method to obtain a suitable bacterial load while minimizing contamination by the host-cell proteins, allowing the protein expression profiling of intracellular bacteria *versus* their extracellular counterpart, using the 2-D-DIGE technique. This led us to identify 83 proteins displaying significant changes in abundance, which allowed us to present an overview of the potential metabolic adaptation of *L. monocytogenes* to its intracellular environment.

2 Materials and methods

2.1 Bacterial strain, culture conditions

L. monocytogenes EGD (serotype 1/2a) was obtained from P. Berche (*Laboratoire de Microbiologie, Faculté de Médecine Necker*, Paris, France) [25]. Bacteria were grown for 24 h at 37°C in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD, USA) under gentle agitation using Innova 44 shaker (New Brunswick Scientific, Edison, NJ, USA) operated at 130 rpm. Colony counting to enumerate bacteria and adjust inocula for phagocytosis or amount of extracellular bacteria for collection was performed on tryptic soy agar (Difco, Becton Dickinson).

2.2 Monocyte culture and intracellular infection

THP-1 cells (ATCC TIB-202) were cultivated, and used as described [26], with the following changes: batches of $2–4 \times 10^9$ THP-1 cells were exposed to $3–6 \times 10^9$ bacteria during 1 h at 37°C in RPMI-1640 medium supplemented with 200 mM glutamine and 10% heat-inactivated FCS (culture medium). Non-internalized bacteria were removed by four successive low-speed centrifugations (Eppendorf centrifuge 5810R; 1000 rpm with rotor A-4-81; 10 min). Cells (with about 0.3 bacteria *per* cell) were resuspended in culture medium supplemented with gentamicin (1 mg/L [1 × MIC]) [27]). Incubation was carried out for 24 h at 37°C in a 5% CO_2 – 95% air atmosphere, yielding an approximately 700-fold increase in CFU *per* mg cell protein. No cell lysis or significant change in pH was noticed. Cells were collected by low-speed centrifugation, washed twice with ice-cold PBS and processed for cell fractionation.

2.3 Purification of intracytosolic bacteria by cell fractionation (differential and isopycnic centrifugation)

Infected cells were homogenized with an all-glass Dounce tissue grinder (10 and 5 passages with the loose and tight-fitting pestle, respectively). A cytoplasmic extract was prepared through three successive low-speed centrifugations (700, 600 and $500 \times g$; 10 min), and further subjected to centrifugation at $3200 \times g$ for 40 min to separate the so-called ''large granules fraction'' (containing the bulk of mitochondria and lysosomes as assessed by assay of the corresponding marker enzymes (see Section 2.5)) from microsomes (endoplasmic reticulum, Golgi apparatus and pericellular membrane) and soluble proteins [28]). This fraction, which also contained the bulk of the cell-associated viable bacteria (as assessed by CFU counting) was then gently resuspended in 0.25 M sucrose – 1 mM EGTA – 3 mM imidazole (pH 7.4) and applied by 4 mL aliquots on top of 30 mL of 1.10–1.30 g/cm^3 linear sucrose gradients resting on a cushion of 6 mL of 1.34 g/cm^{3}sucrose. After centrifugation (VTi 50 rotor; Beckman Instruments, Fullerton, CA, USA) at $200\,000 \times g$ for 3 h, fractions were collected, weighed, their densities determined by refractometry and assayed for total proteins [29], marker enzymes and viable bacteria (CFU counting). Fractions with densities of 1.22–1.28 g/cm^3 were pooled for proteomic analysis after 30-fold dilution in sterile cold water and collection of bacteria centrifugation at $3200 \times g$ to remove eukaryotic soluble proteins.

2.4 Preparation of extracellular bacteria

Bacteria grown in broth for 24 h at 37°C (approximately 2×10^9 CFU/mL (stationary phase)) were collected by centrifugation and washed with ice-cold PBS. They were mixed with uninfected THP-1 cells (collected by lowspeed centrifugation as described in Section 2.2) at a bacteria:cell ratio similar to that observed for infected THP-1 monocytes after 24 h of intracellular growth. This preparation was then handled exactly as described above for infected cells.

2.5 Biochemical assays

The mitochondrial enzyme cytochrome *c*-oxidase (E.C. 1.9.3.1.) and the lysosomal enzyme *N*-acetyl-β-hexosaminidase (E.C. 3.2.1.30.) were assayed as described earlier [30].

2.6 Sample preparation for 2-D-DIGE analysis

Samples were resuspended in a buffer (30 mM Tris, 5 mM EDTA, 5 mM $MgCl_2$, pH 9) containing protease inhibitors (1 mM PMSF, 10 μg/mL pepstatin A and 10 μg/mL leupeptin). They were then subjected to sonication (three cycles of 30 s at 50 watts at 15 s intervals; Labsonic L, Braun Biotech International, Melsungen, Germany) while maintained on ice, boiled (5 min) and stored at −80°C. After thawing, samples were exposed to DNase I (5 μg/mL; 30 min; 4°C), proteins were solubilized in 30 mM Tris buffer pH 8.5 containing 7 M urea, 2 M thiourea and 4% CHAPS (30 min with periodical vortex mixing). After centrifugation (20 000 × *g*, 10 min), supernatants were precipitated by the addition of 3 volumes of cold acetone and stored overnight at −20°C. Precipitated proteins were collected by centrifugation for 20 min at 20 000 × *g*, and solubilized in 30 mM Tris buffer pH 8.5 containing 7 M urea, 2 M thiourea and 4% CHAPS at room temperature. After centrifugation at 20 000 × *g* for 10 min at room temperature, samples were used for protein assay (Quick Start Bradford protein assay, BioRad Laboratories, Hercules, CA, USA) and stored at −80°C until use for Cy-Dye labeling.

2.7 Cy-Dye labeling, IEF and SDS-PAGE

Twenty-five microgram of proteins from eight independent samples (four from extracellular and four from intracellular *L. monocytogenes*) were cross-labeled with 200 pmol of Cy3 (two samples for each condition) or Cy5 (two samples for each condition) dyes. An internal standard was then prepared by mixing equal aliquots of all eight samples and the resulting mixture was labeled with Cy2 dye. Reaction was carried out for 30 min and stopped by the addition of an excess of lysine (10 mM in water). An equal volume of 2-D-sample buffer (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG 4–7 buffer and 0.4% DTT) was added to each sample. Four mixtures were prepared, each made of the following: (i) a sample from extracellular *L. monocytogenes* proteins labeled with one dye (Cy3 or Cy5); (ii) a sample from intracellular *L. monocytogenes* proteins labeled with the other dye (Cy5 or Cy3) and (iii) the internal standard. These mixtures were then used for running independent 2-D gels. First-dimension isoelectric focusing was carried out on Immobiline dry-strips (Amersham Biosciences, Amersham/G.E. Healthcare) providing a continuous 24 cm pH 4–7 gradient. Strips were first rehydrated overnight with a solution of 8 M urea, 1% CHAPS, 0.4% DTT, 0.5% IPG buffer (Amersham Biosciences) and 0.002% bromophenol blue. After adding samples, a current voltage of subsequently 300 V (3 h), 1000 V (8 h in gradient) and 8000 V (3 h), in total 8000 up to 50 000 V × h, was applied to separate proteins. Strip equilibration was performed in two steps of 15 min, first in the presence of 1.5 M Tris-HCl, 6 M urea, 30% glycerol, 2% SDS, 1% DTT and second with 1.5 M Tris-HCl, 6 M urea, 30% glycerol, 2% SDS and 2.5% iodoacetamide. SDS-PAGE was then performed on 10–13% polyacrylamide gradient gels using a 2-D Optimizer (Nextgen Sciences, Letchworth Garden City, UK).

2.8 Gel analysis

Gels were scanned with a Typhoon 9400 imager (Amersham Biosciences) in the fluorescence mode to visualize cyanin-labeled proteins and were analyzed using the DeCyder 2D Software v6.5 (GE Healthcare). A cut-off value was set at a 1.5-fold increase or decrease and differences in spot intensities were analyzed by Student's *t*-test with $p < 0.05$ (with visual inspection of the results).

2.9 Protein identification and determination of metabolic function

Preparative 200 μg of unlabeled proteins 2-D gels were used, and stained with Krypton (Pierce Biotechnologies). The spots of interest were excised (Ettan Spot Picker (GE Healthcare)), gel plugs dried with ACN and digested with trypsin (10 ng/μL; Promega, Madison, WI, USA). Peptides were extracted with 5% formic acid, desalted on C-18 stage tips (Proxeon A/S, Odense, Denmark) and directly eluted onto the MALDI target with matrix solution (DHB and CHCA 1:1). For analysis of tryptic peptides, MALDI-TOF MS was carried out using a MALDI micro MX (Waters, Milford, MA, USA). Spectra were acquired in a positive reflectron mode (5200 V) and collected within the mass range of 900–3000 m/z. The autolytic fragments of trypsin acted as internal lockmass. Mass spectra were acquired using the MassLynx 4.0 software (Waters) and data were used to identify protein candidates by spectral similarity against available *L. monocytogenes* protein database (33 500 sequences) and global database (containing the human proteins, MSDB, 3 239 079 sequences) using an in-house MASCOT search server (version 2.2, Matrix Sciences, Boston, MA, USA) and a ProteinLynx Global Server (version 2.2.5, Waters). For MALDI-MS analysis, peptide mass fingerprinting search parameters were as follows: 50 ppm for peptide mass tolerance, single tryptic miss-cleavage allowed, oxidation of methionine residues as partial modification and carbamidomethyl as fixed modifications. Only significant hits ($p < 0.05$), as defined by the MASCOT probability analyses (protein scores greater than 60 [*Listeria* database] or 80 [global database]), were accepted but always verified manually. Protein analysis was performed using both the *Kyoto encyclopedia of genes and genomes* (http://www.genome.jp/kegg) and the *database of prokaryotic operons* (http://csbl1.bmb.uga.edu/OperonDB/index.php).

3 Results and discussion

3.1 Isolation of intracellular *L. monocytogenes*

A prerequisite in our study was a satisfactory separation of intracellular bacteria to discriminate unambiguously between bacterial and host-cell proteins [23]. This was made possible here because the majority of intracellular *L. monocytogenes* sojourns in the cytosol, making it relatively simple to separate the bulk of them from host cell organelles by simple isopycnic centrifugation.

Figure 1 shows the density distribution of bacteria, of total protein and of marker enzymes (mitochondria and lysosomes) after isopycnic centrifugation of large granules fractions isolated from (i) uninfected monocytes to which bacteria have been added at the time of collection (spiked cells) or (ii) cells infected with bacteria (24 h incubation post-phagocytosis). For spiked cells, bacteria were completely separated from mitochondria, as evidenced by the distribution of cytochrome *c*-oxidase. In contrast, about 20% of *N*-acetyl-β-hexosaminidase was found to co-migrate with the bacteria (the remaining of this enzyme was found co-migrating with cytochrome *c*-oxidase, denoting the difficulty of separating lysosomes from mitochondria in THP-1 cells by centrifugation in sucrose gradients [Van Bambeke *et al.*, unpublished]). For infected cells, separation of bacteria from mitochondria was also observed. In contrast to spiked cells, the distribution profile of *N*-acetyl-β-hexosaminidase was slightly dissociated from that of cytochrome *c*-oxidase, and little, if any co-migration with bacteria was detected. This allowed us to select four fractions (in the density range from 1.22 to 1.28 g/cm^3) as starting material for proteomic analysis of bacteria from infected cells. Fractions with similar density limits were used for spiked cells. These two sets of fractions contained 91 and 79% of the bacteria layered on the gradient, and 59 and 64% of the total number of bacteria present in the corresponding starting homogenates.

The trailing profile of bacteria distribution towards lighter densities for intracellular *L. monocytogenes*, compared to that of bacteria added to cell homogenates, probably denotes that part of these bacteria may still be located in phagosomes. These were not used in our analysis, which is about half of whole cell content in bacteria. Surprisingly, actin, which surrounds *L. monocytogenes* after its transfer to cytosol ([31]), was not detected in our samples. This may result from desorption during the separation procedures and/or binding of actin to host-cell membrane proteins [31]. Conversely, the presence of part of the lysosomal enzyme *N*-acetyl-β-hexosaminidase in fractions containing bacteria added to cell homogenates (controls) could be due to binding of lysosomes to the bacterial envelope in the low-ionic

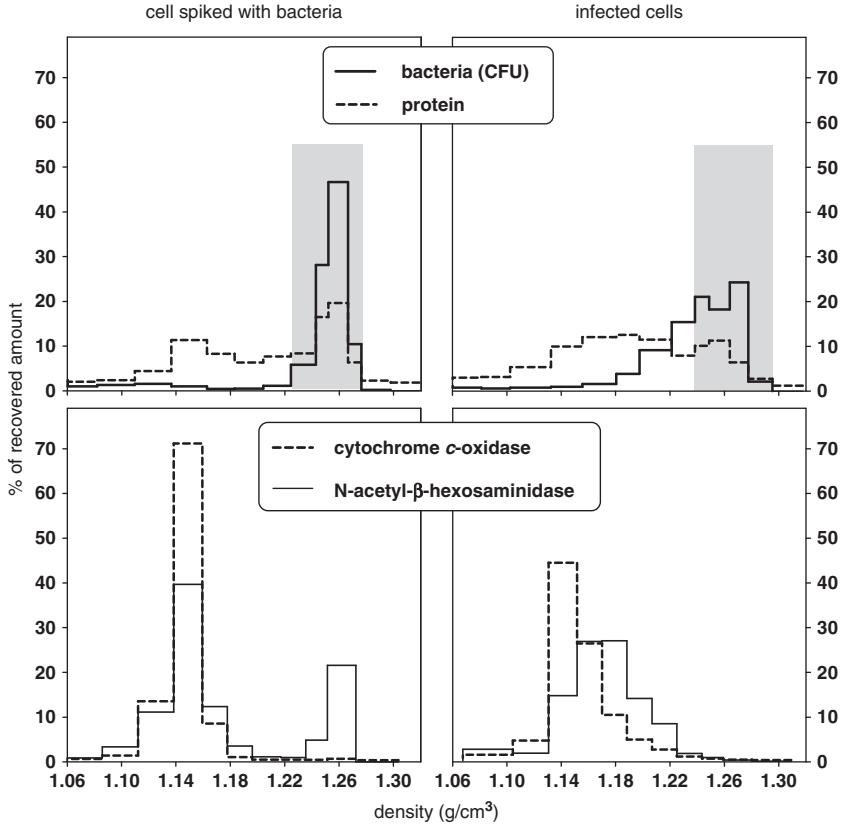

Figure 1. Density distribution profiles of bacteria and total protein (top) and of cytochrome *c*-oxidase and *N*-acetyl-β-hexosaminidase (bottom) after isopycnic centrifugation of the ''large granules fraction'' prepared from uninfected cells spiked with bacteria grown in broth prior to homogenization (left) or from cells infected with bacteria for 24 h (right). The shaded fractions are those used for proteomic analysis.

strength medium used for cell homogenization and fractionation.

3.2 Proteomic analysis

3.2.1 Global results and analysis

Using a pH gradient of 4–7, a total of 1684 different protein spots could be observed on our gels, out of which 448 could reliably be analyzed by MS, yielding a total of 245 different identified proteins (see Table A in the Supporting Information and Figs. 2 and 3), none of which belonged to the eukaryotic host. Table 1 shows the list of proteins (83) grouped by function in the main known metabolic pathways and/or function, for which the abundance was considered as significantly different ($\geq \pm 1.5$-fold) between samples prepared from cells spiked with bacteria (corresponding to *L. monocytogenes* grown in broth) and samples prepared from cells infected with bacteria (corresponding to intracellular *L. monocytogenes*). Globally, more proteins showed underexpression ($n = 61$) than overexpression ($n = 22$).

Grouping significantly differentially expressed proteins according to their putative functions revealed striking general trends, which have been schematically depicted in Fig. 4 and are analyzed hereunder.

3.2.2 Proteins involved in stress defense

The host cell cytosol does not appear as a particularly stressful environment, based on the decreased abundance of several stress proteins. This is consistent with the decreased abundance of (i) anti-oxidant defense proteins (Sod, TrxA, Lmo1583, Lmo0786 and Lmo1059), and (ii) of enzymes participating to the pentose phosphate pathway (Lmo2674 [transaldolase] and Lmo2743 [ribose 5-phosphate epimerase]). Actually, in contrast to stimulated THP-1 cells [32], untreated THP-1 cells are highly permissive for *L. monocytogenes* growth [25] because their production of oxygen and nitrogen reactive species is minimal [27]. Our data confirms the observation that *L. monocytogenes* can grow inside eukaryotic cell without the aid of stress-induced proteins [33]. Other studies, however, have shown an upregulation of stress response genes (summarized in [34]). The corresponding proteins could, however, not be detected here. Moreover, the genes encoding several stress-related proteins, for which a lower abundance is seen (Table 1), have been reported to be either unchanged or increased in their expression (except for Lmo1580, Sod and Lmo1583, for which discrepant reports on gene expression were presented) [21, 22]. One protein, Lmo1860 (a sulfoxide reductase) involved in defense against reactive oxygen and nitrogen species, showed an increased expression. However, data obtained with other intracellular bacteria [35, 36] suggests that this protein mainly improves adherence of pathogens to eukaryotic cells.

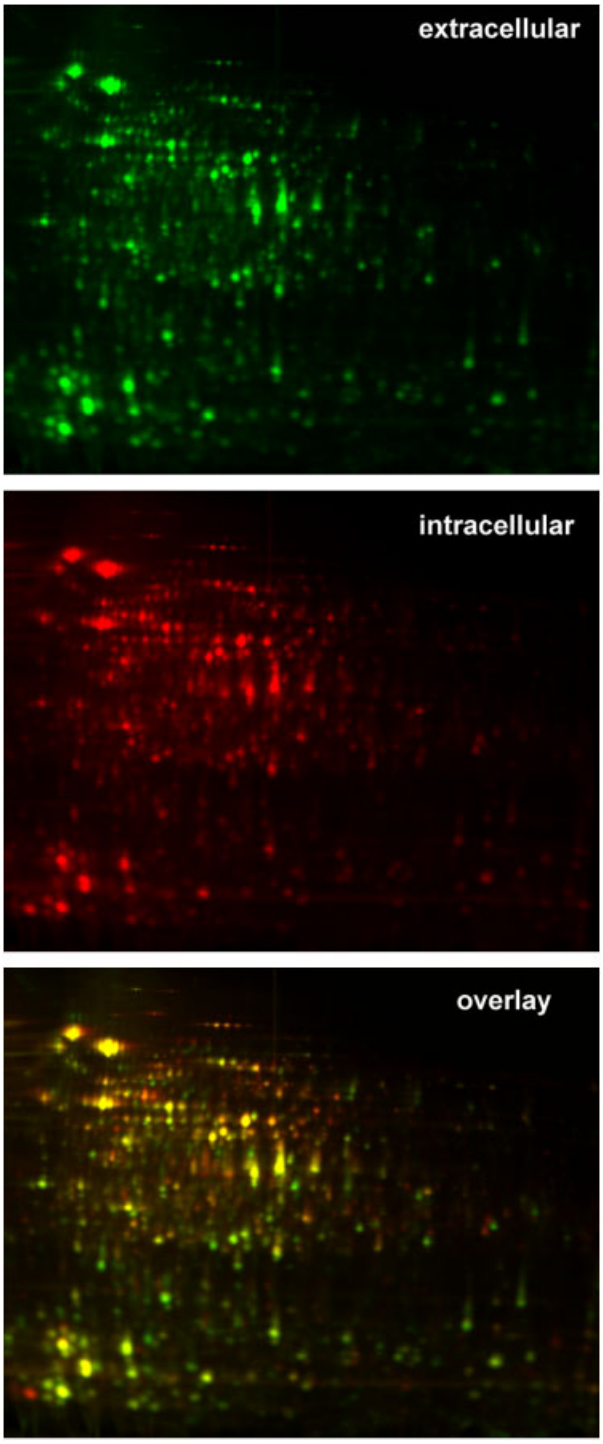

Figure 2. Cy3-labeling of extracellular (top) and Cy5-labeling of intracellular (middle) proteomes of *L. monocytogenes*. Bottom: overlay of the two above pictures.

Table 1. Proteins with significant changes in abundance between bacteria grown in broth (extracellular) and bacteria grown in THP-1 monocytes (intracellular)

Spot ID[a)]	Protein name[b)]	E.C. no.[c)]	Description[b)]	Fold change	*p*-Value
1. Cell envelope and transport processes					
1.1 Cell wall synthesis					
11	Lmo1078	2.7.7.9	Similar[d)] to putative UDP-glucose pyrophosphorylases	3.49	4.80E−04
14	Lmo1084	1.1.1.133	Similar to DTDP-L-rhamnose reductase	3.07	8.60E−04
16	Mbl	n/a	Similar to MreB-like protein	1.58	2.80E−03
9	DdlA	6.3.2.4	D-alanyl-D-alanine ligase	−1.51	1.00E−02
					4.80E−04
1.2 Transport/binding proteins and lipoproteins					
25	TcsA	n/a	$CD4^+$ T cell-stimulating antigen, lipoprotein	3.92	7.5E−06
24	Lmo1223	n/a	Similar to ABC transporter, ATP-binding proteins	−1.6	2.00E−04
28	Lmo2372	n/a	Similar to ABC-transporter ATP binding proteins	−2.68	3.90E−04
17	Lmo0096	2.7.1.69	Similar to PTS system mannose-specific, factor IIAB	−2.8	3.60E−03
21	Lmo0783	2.7.1.69	Similar to mannose-specific phosphotransferase system (PTS) component IIB	−2.95	3.80E−05
2. Intermediary metabolism					
2.1. $C0_2$ utilization					
53	Lmo0811	4.2.1.1	Similar to carbonic anhydrase	1.63	1.90E−02
2.2. Glyceraldehyde-3-P-synthesis					
130	Dra	4.1.2.4	Similar to deoxyribose-phosphate aldolase	2.31	2.20E−03
47	Lmo0521	3.2.1.86	Similar to 6-phospho-β-glucosidase	2.16	4.90E−02
2.3. Carbon metabolism					
74	Lmo2695	2.7.1.-	Similar to dihydroxyacetone kinase	−1.51	7.50E−03
70	FruB	2.7.1.56	Similar to fructose-1-phosphate kinase	−1.64	4.90E−03
56	Lmo0957	3.5.99.6	Similar to glucosamine-6-phosphate isomerase	−1.66	2.40E−03
48	Lmo0539	4.1.2.40	Similar to tagatose-1,6-diphosphate aldolase	−1.78	6.60E−03
63	Lmo1906	4.2.3.3	Similar to methylglyoxal synthase	−1.94	4.80E−04
2.4. Hexose monophosphate shunt					
76	Lmo2743	2.2.1.2	Similar to transaldolase	−1.56	2.50E−03
73	Lmo2674	5.3.1.6	Similar to ribose 5-phosphate epimerase	−3.12	2.50E−05
2.5. Pyruvate formation					
105	DaaA	2.6.1.21	D-amino acid aminotransferase	3.1	2.50E−04
2.6. Pyruvate utilization					
66	Pta	2.3.1.8	Similar to phosphotransacetylase	−1.77	1.60E−03
102	Lmo1579	1.4.1.1	Similar to alanine dehydrogenase	−1.77	1.90E−03
137	Lmo1414	2.3.1.9	Similar to acetyl-CoA:acetyltransferase	−1.82	1.80E−03
52	Lmo0722	1.2.3.3	Similar to pyruvate oxidase	−2.51	2.60E−02
2.7. Glutamate utilization					
92	Lmo0560	1.4.1.4	Similar to NADP-specific glutamate dehydrogenase	2.89	1.40E−03
2.8. Threonine synthesis					
114	ThrC	4.2.3.1	Highly similar to threonine synthase	1.52	3.20E−03
2.9. Peptide and aminoacid catabolism					
113	Lmo2363	4.1.1.15	Similar to glutamate decarboxylase	−1.55	2.80E−02
135	Lmo1372	1.2.4.4	Similar to branched-chain α-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase α subunit)	−1.62	4.90E−03
103	Lmo1603	n/a	Similar to aminopeptidase	−1.64	5.00E−04
104	Lmo1611	n/a	Similar to aminopeptidase	−1.64	2.10E−03
94	Lmo1011	2.3.1.89	Similar to tetrahydrodipicolinate succinylase	−1.76	2.50E−03
95	Lmo1217	n/a	Similar to endo-1,4-β-glucanase and to aminopeptidase	−1.83	4.80E−03
101	Lmo1578	3.4.13.9	Similar to X-Pro dipeptidase	−1.84	7.00E−03

Table 1. Continued

Spot ID[a)]	Protein name[b)]	E.C. no.[c)]	Description[b)]	Fold change	*p*-Value
2.10. Formation of purines and thiamine precursor					
120	PurM	6.3.3.1	Phosphoribosylaminoimidazole synthetase	2.29	1.40E−03
121	PurQ	6.3.5.3	Phosphoribosylformylglycinamidine synthetase I	1.99	7.20E−03
127	Ndk	2.7.4.6	Similar to nucleoside diphosphate kinase	−1.71	2.20E−03
2.11. Pyrimydine formation					
124	PyrD	1.3.3.1	Highly similar to dihydroorotase dehydrogenase	−1.94	1.60E−03
119	Lmo1691	3.6.1.23	Similar to deoxyuridine triphosphate nucleotidohydrolases	−2.08	2.50E−03
123	PyrF	4.1.1.23	Highly similar to orotidine 5′-phosphate decarboxylases	−2.48	2.60E−02
131	Upp	2.4.2.9	Highly similar to uracil phosphoribosyltransferase	−2.81	3.20E−03
126	PyrAB	6.3.5.5	higHly similar to carbamoyl-phosphate synthetase (catalytic subunit)	−4.8	7.40E−03
2.12. Fatty acid synthesis					
139	FabD	2.3.1.39	Similar to malonyl CoA-acyl carrier protein transacylase	1.92	1.80E−02
138	FabG	1.1.1.100	Similar to 3-ketoacyl-acyl carrier protein reductase	1.65	7.10E−03
2.13. Metabolism of coenzymes and prosthetic groups					
151	Lmo2101	4.-.-.-	Similar to a protein required for pyridoxine synthesis	3.18	4.70E−03
145	Lmo0786	1.7.-.-	Similar to acyl-carrier protein phosphodiesterase and to NAD(P)H dehydrogenase	−1.78	2.30E−04
2.14. Other function					
51	Lmo0640	n/a	Similar to oxidoreductase	−1.65	1.30E−02
49	Lmo0554	1.1.-.-	Similar to NADH-dependent butanol dehydrogenase	−2.06	1.30E−03
50	Lmo0613	n/a	Similar to oxidoreductase	−2.16	7.20E−03
3. Information pathways					
3.1. Regulation					
156	Lmo0287	n/a	Similar to two-component response regulator	1.82	2.90E−03
3.2 RNA modification					
167	Lmo0935	2.1.1.-	Similar to *B. subtilis* CspR protein, rRNA methylase homolog	−2.09	2.30E−03
3.3. Ribosomal proteins					
171	Lmo1938	n/a	Similar to similar to ribosomal protein S1 like protein	1.79	6.40E−04
169	RpsF	n/a	Ribosomal protein S6	−1.61	5.60E−03
3.4. Aminoacyl-tRNA synthetases					
172	AspS	6.1.1.12	Aspartyl-tRNA synthetase	6.62	8.70E−05
3.5. Protein modification					
179	Lmo1860	1.8.4.11	Similar to peptidyl methionine sulfoxide reductases	5.41	4.30E−03
4. Stress induced proteins					
187	Ctc	n/a	Similar to *B. subtilis* general stress protein	−1.57	2.80E−03
3	Lmo1059	n/a	Family of thioredoxins	−1.74	3.40E−02
192	Lmo2369	n/a	Similar to *B. subtilis* general stress protein 13 containing a ribosomal S1 protein domain	−1.76	2.70E−02
210	Lmo0796	n/a	Conserved protein with unknown function	−2.29	2.40E−03
225	Lmo1580	n/a	Similar to unknown protein	−2.33	5.90E−03
195	sod	1.15.1.1	Superoxide dismutase	−2.36	3.50E−04
33	TrxA	n/a	Thioredoxin	−2.8	7.60E−03
198	Lmo2067	3.5.1.24	Similar to conjugated bile acid hydrolase	−2.89	4.10E−06
196	Lmo1583	1.11.1.15	Similar to thiol peroxidases	−3.98	4.70E−03
230	Lmo1830	n/a	Similar to conserved hypothetical proteins	−4.42	6.30E−07
238	Lmo2391	n/a	Conserved protein with unknown function similar to *B. subtilis* YhfK protein	−9.58	2.80E−05
5. Detoxification					
197	Lmo1967	n/a	Similar to toxic ion resistance proteins	1.62	2.40E−03

Table 1. Continued

Spot ID[a)]	Protein name[b)]	E.C. no.[c)]	Description[b)]	Fold change	*p*-Value
6. Unknown metabolic function					
215	Lmo1001	n/a	Similar to *B. subtilis* protein YkvS	9.14	1.20E−04
242	Lmo2487	n/a	Similar to *B. subtilis* YvlB protein	2.12	2.30E−03
234	Lmo2072	n/a	Similar to a putative DNA binding proteins	1.72	1.10E−02
202	Lmo0256	n/a	Conserved protein with unknown function	1.57	9.10E−03
239	Lmo2401	n/a	Similar to conserved protein with unknown function and to *B. subtilis* YutF protein	−1.61	1.00E−04
224	Lmo1577	n/a	Similar to unknown proteins	−1.62	1.10E−03
218	Lmo1029	n/a	Similar to conserved protein with unknown function	−1.67	1.80E−03
212	Lmo0930	n/a	Conserved protein with unknown function, similar to *B. subtilis* YhfI protein	−1.97	7.30E−05
4	Lmo1257	n/a	n/a	−1.99	1.10E−02
241	Lmo2426	n/a	Conserved protein with unknown function	−2.14	4.30E−03
44	Lmo2724	n/a	Similar to unknown proteins	−2.32	1.10E−03
222	Lmo1468	n/a	Similar to unknown proteins	−2.53	1.60E−03
213	Lmo0965	n/a	Similar to *B. subtilis* YjbK protein	−2.6	4.00E−03
1	Lmo0418	n/a	Glutamine-amido-transferase	−3.91	9.40E−06
207	Lmo0515	n/a	Conserved protein with unknown function	−2.87	4.30E−03
2	Lmo0953	n/a	Putative lipoprotein	−6.25	1.50E−04
237	Lmo2340	n/a	Similar to *Erwinia chrysanthemi* IndA protein	−9.97	4.00E−06

Spot ID numbers in **bold** (with the function of the corresponding protein) refer to the proteins with increased abundance, and those in *italics* (also with the corresponding function) proteins with decreased abundance.

a) See annotated spots on the 2-D proteome map shown in Fig. 3.

b) Protein name and description from ListiList, a database dedicated to the analysis of the genomes of the food-borne pathogen, *Listeria monocytogenes* (available on http://genolist.pasteur.fr/ListiList/).

c) Enzyme class number as described on KEGG website (http://www.genome.jp/kegg/).

d) Similar refers to genomic similarity (based on ListiList) and does not imply similarity of functions.

3.2.3 Proteins involved in the biosynthesis of nucleotides and thiamine

The decreased abundance of several of these proteins suggests an effect at the level of their common promoter, since PyrD, PyrF and PyrAB are part of a single operon (http://csbl1.bmb.uga.edu/OperonDB/index.php). The large supply of nucleotides from the host cells may explain this effect, which is consistent with the decreased abundance of ribose-phosphate epimerase (an important enzyme in the pathway leading to purines and pyrimidines). PurQ and PurM, also encoded by the same operon, were more abundant while Ndk, needed in the final phosphorylation step of both purine and pyrimide pathway, was less abundant. This could be an indication that 1(5′-phosphoribosyl)-5-amino-imidazole, instead of being used in the purine pathway, is hijacked for the formation of thiamine, a vitamin known to be required for intracellular replication of *L. monocytogenes* [19]. Glutamate, which can form ribosylamine-5-phosphate, could also supply the precursors required for thiamine synthesis. It can be made available from glutamine present in the cell culture medium or produced from 2-oxoglutarate derived from interrupted citrate cycle by overexpression of glutamate dehydrogenase (Lmo0560), as detected here (glutamate will not be converted to aminobutanoate because of the decreased expression of glutamate decarboxylase).

3.2.4 Proteins involved in transport and metabolism of carbohydrates

Intracellular *L. monocytogenes* seems to rely less on the uptake of mannose, fructose, or galactose, as the corresponding transporters (Lmo0096 and Lmo0783) and catabolic enzymes (FruB and Lmo0539) are less abundant. It has been proposed that the intracellular form of *L. monocytogenes* takes advantage of the ample supply of hexose phosphates present in the host cytosol by (i) overexpressing its hexose phosphate transporter [13] and (ii) increasing the expression of genes encoding for enzymes involved in the pentose phosphate pathway [34]. However, we found here a decreased abundance of two enzymes of the pentose phosphate pathway (see above). More downstream in the carbohydrate metabolism, we see an overexpression of proteins leading to the formation of glyceraldehyde-3-phosphate, a precursor of pyruvate. Consistent with the increased transcription of glycerol kinase and glycerol-phosphate dehydrogenase reported in the genomic studies, this may indicate that glycerol plays a major role in carbon metabolism of intracellular *L. monocytogenes* [34]. Metabolic studies using deleted mutants have identified the dihydroxyacetone kinase as being also a key enzyme in carbon metabolism [9]. We found a decreased expression of one form of this enzyme (Lmo2695), but two others

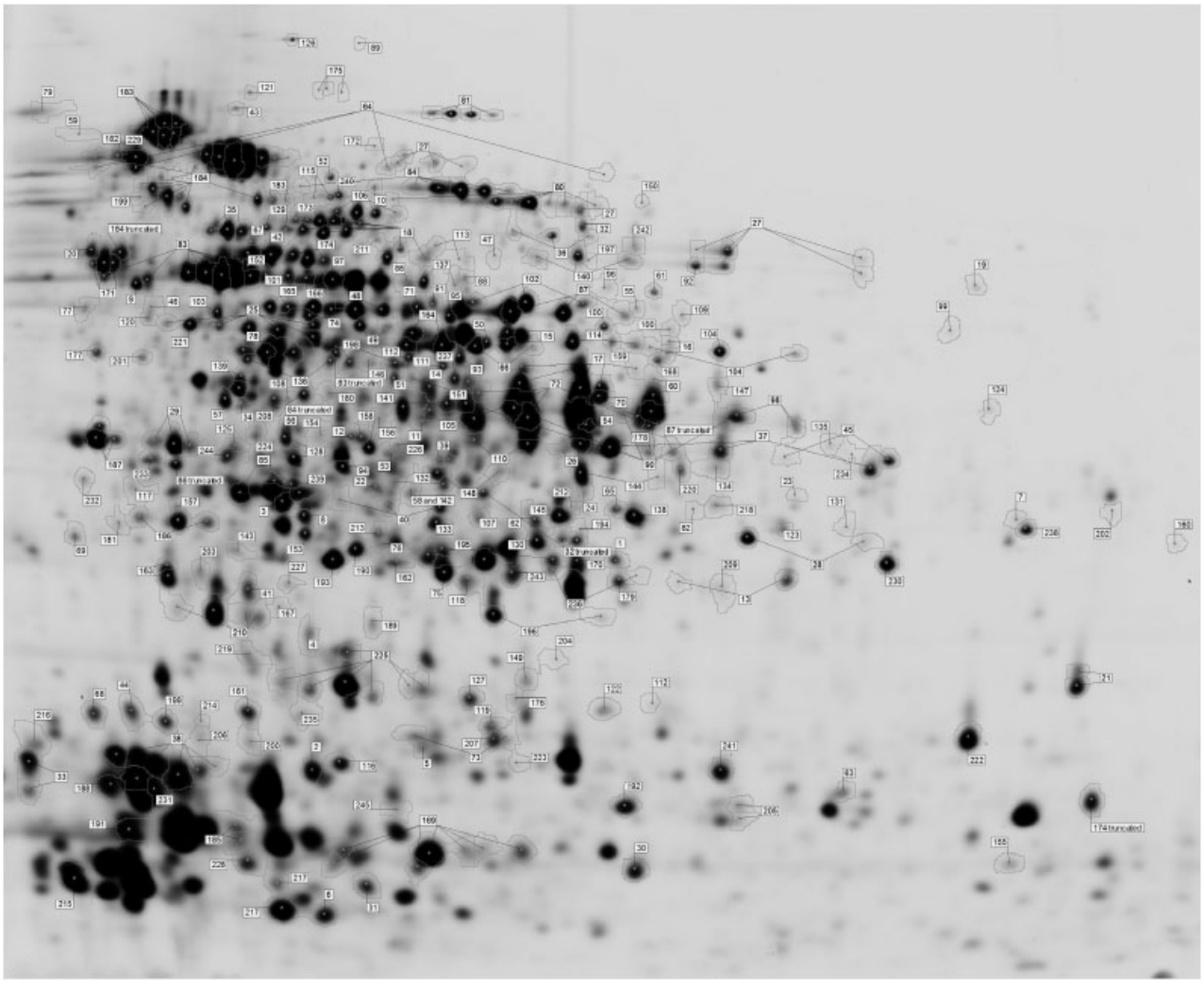

Figure 3. Master gel in the p*I* 4–7 of *L. monocytogenes* from both intracellular and extracellular environment. Annotated numbers refer to the identification of the proteins available in the Supporting Information and Table 1.

(Lmo0343 and Lmo2695) were unaffected. Altogether, this confirms the suggestion that glycerol and hexose monophosphate rather than glucose are dominant in carbon metabolism [22].

3.2.5 Decreased D-alanyl-D-alanine ligase content and impact on pyruvate and lipoic acid metabolism

The decreased abundance of the D-alanyl-D-alanine ligase will result in an increased pyruvate formation through the overexpression of D-alanine transaminase (DaaA), an enzyme essential for virulence [37]). Pyruvate formed in that way will actually be spared by the decreased abundance of Lmo1579 (an L-alanine decarboxylase working both ways) and of other proteins susceptible to use it also, such as Pta, Lmo0722 and Lmo1414. The excess of pyruvate may then be channeled toward the formation of fatty acids, which is consistent with (i) the increased abundance of FabD and FabG, both involved in the formation of type-II fatty acids (the corresponding genes are located on the same operon) and (ii) the decreased abundance of Lmo1414 acetyl-CoA:acetyltransferase and Lmo1372 (branched-chain amino acids α-keto acid dehydrogenase) involved in the lipid catabolism. In this context, it is interesting to note that intracellular *L. monocytogenes* has a specific need for the uptake of lipoic acid [17, 38], a necessary cofactor of pyruvate dehydrogenase. This shows that the overall carbohydrate metabolism is actually modified with consequences extending to the metabolism of lipids. Yet, it remains to be seen whether the expression of proteins involved in the uptake and/or synthesis of lipoic acid is modified.

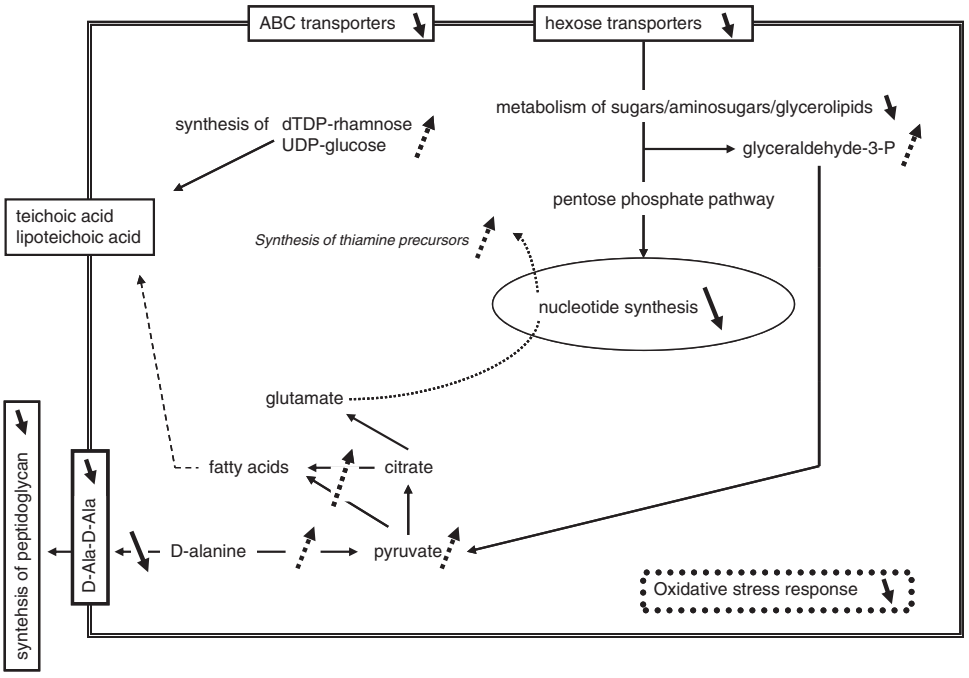

Figure 4. Overview of the potential modulations in metabolic pathways of intracellular *L. monocytogenes* compared to its extracellular form, as deduced from the changes in abundance of the proteins listed in Table 1. Downsided solid arrows and uppersided dashed arrows indicate a global decreased or increased expression of the enzymes or proteins involved in the corresponding pathway(s). Dashed links denote multi-steps pathways. Note that the citrate cycle in *L. monocytogenes* is interrupted at the level of the 2-oxoglutarate due to lack of 2-oxoglutaratedehydrogenase, making oxaloacetate production entirely dependent from the carboxylation of pyruvate and allowing citrate to be easily available for fatty acid synthesis and 2-oxoglutarate for conversion to glutamate.

3.2.6 Proteins involved in aminoacid metabolism

Beyond the changes potentially affecting glutamate production, which we discussed above (and that are supported by metabolic studies [34]), we see an increased abundance of ThrC. This should favor the biosynthesis of threonine, which is in line with the need of this amino acid for intracellular growth [11]. The pools of other amino acids, such as valine, leucine and isoleucine, or of aspartic acid, may be maintained at a sufficient level by a decrease of their degradation (caused by the decreased expression of Lmo1372, Lmo2363 and Lmo1011 proteins). In this context, a recent study showed that a significant fraction of amino acids used by intracellular *L. monocytogenes* may be provided by the host cell and that the biosynthesis of Thr proceeds *via* oxaloacetate by carboxylation of pyruvate [9]. The latter reaction will be favored (i) by the absence of phosphoenolpyruvate carboxylase in *L. monocytogenes* [9], (ii) the large concentration of CO_2 present in the culture atmosphere (5%) and important for intracellular growth of *L. monocytogenes* [39] and (iii) the overexpression of Lm0811, which can act as carbonic anhydrase. It is also important to note that the citric acid cycle of *L. monocytogenes* is interrupted by lack of 2-oxoglutarate dehydrogenase [40, 41], which should increase the availability of pyruvate for carboxylation to oxalacetate on the one hand, and for biosynthesis of fatty acids from acetyl CoA and/or citrate, on the other hand.

3.2.7 Proteins involved in the construction of the bacterial envelope

Among them, Mbl, an MreB-like protein involved in controlling the size of *L. monocytogenes*, is overexpressed. We know that it contributes to maintain the rod-shape aspect of the bacterium [42, 43], which makes it probably critical for intracellular *L. monocytogenes*, since it becomes animated of large and fast movements when reaching the cytosol [31]. Increased expression of Lmo1084 (present in *L. monocytogenes* but not in *L. innocua* or *L. welshimera* [44], two strains unable to reach the cytosol) and of Lmo1078 suggests an increased biosynthesis of dTDP-rhamnose and UDP-glucose. The latter are precursors of the teichoic acids and, with diglucosyl-diacylglycerol, of lipoteichoic acids [45]. Their synthesis may be favored by the increased abundance of Lmo2101, which produces pyridoxal 5-phosphate involved in transaminations leading to glucosamine formation.

A main point of interest is the decreased abundance of the D-alanyl-D-alanine ligase (DdlA), which was analyzed above with respect to pyruvate formation. This enzyme is critical for an early step in the biosynthesis of the pepti-

doglycan. Its decreased abundance may result in the formation of a thinner cell wall, which may explain the surprising observation that, in contrast to many other intracellular bacteria, *L. monocytogenes* becomes more susceptible to ampicillin when developing intracellularly as compared to broth [46, 47]. This phenomenon, unique for penicillins as opposed to other antibiotics [26, 47], fits well with their mode of action as inhibitors of the late stages of peptidoglycan biosynthesis [48, 49]). This rationalizes the clinical observation that ampicillin remains the most successful option for therapy of listeriosis in spite of its limited intracellular accumulation [50].

4 Concluding remarks

The present study, which is among the firsts to apply quantitative proteomics to intracellular infection by *L. monocytogenes*, complements previous efforts using genomic and metabolic approaches [10, 21, 22, 34]. Despite the lack of validated immunoassays in the present study (mainly because specific antibodies for the proteins detected are not commercially available), the present approach provides direct information on the abundance of proteins, making, in comparison with previous transcriptomic studies [21, 22], a more direct link with the potential phenotypic expression. These studies performed at the level of mRNA do not always match well with our data, which could be due to differences in experimental conditions (incubation time, strain of bacteria, host cell) but also from the effect of post-translational regulations that cannot be detected by micro-array techniques. As discussed above, we actually see a number of convergences between the present data and the results of metabolic flow analysis studies. Future studies focused on the activity of the proteins will be, in this context, necessary to assess their exact roles for the survival of intracellular *L. monocytogenes*. It will also be interesting to expand our work by including other serotypes (which are known to express different proteins [51]) and other eukaryotic host cells, as well as different cell states such as post-activation by cytokines known to modulate the intracellular fate of *L. monocytogenes* [25, 27, 32, 52]. As invasion of eukaryotic cells by bacteria remains a challenge in the management of infectious diseases [53–55], the type of investigation reported here, together with further metabolic studies and the use of defective mutants, may help in better understanding the pathogenesis of intracellular infection and in designing improved therapeutic approaches.

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Table A. List of all proteins identified by mass spectrometry

Spot no. [1]	Protein name [2]	Description [2]	Access no. [3]	Functional categories [2]	Mr [4]	pI [4]	Score [4]	Sequence coverage [4]	Peptides matched [4]
1	Lmo0418	Protein with unknown function	Q8Y9V2	6	20569	5.17	95	64	8
2	Lmo0953	Lmo0953 protein (lipoprotein, putative)	Q8Y8F1	6	8153	4.68	61	86	7
3	Lmo1059	Protein with unknown function	Q8Y859	6	20029	4.83	94	39	11
4	Lmo1257	Protein with unknown function	Q92CE7	6	18253	4.88	96	56	11
5	Lmo1643	Protein with unknown function	Q8Y6P1	6	15831	5.03	99	65	12
6	Lmo2707	Conserved protein with unknown functions	Q8Y3X2	6	8388	4.90	61	54	6
7	Lmo2792	DNA-binding protein	Q8Y3P3	6	29075	6.92	108	53	16
8	PheT	Phenylalanyl-tRNA synthetase beta subunit	Q8Y6S6	/	22520	4.89	88	64	14
9	DdlA	D-alanine--D-alanine ligase	Q8Y8P1	1.1	40701	4.49	68	24	10
10	DltA	D-alanine-activating enzyme (dae), D-alanine-D-alanyl carrier protein ligase (dcl)	Q8Y8D4	1.1	58125	5.04	102	45	25
11	Lmo1078	Similar * to putative UDP-glucose pyrophosphorylases	Q8Y840	1.1	32510	5.04	136	64	17
12	Lmo1081	Similar to glucose-1-phosphate thymidyl transferase	Q8Y837	1.1	32267	4.9	98	39	11
13	Lmo1082	Similar to dTDP-sugar epimerase	Q8Y836	1.1	21149	5.63	97	38	9
14	Lmo1084	Similar to DTDP-L-rhamnose reductase	Q8Y834	1.1	31265	5.05	69	52	11
15	MreB	Similar to cell-shape determining protein MreB	Q8Y6Y3	1.1	35595	5.16	137	46	18
	MreB	Similar to cell-shape determining protein MreB	Q8Y6Y3	1.1	35595	5.16	106	43	19
16	Mbl	Similar to MreB-like protein	Q8Y4C5	1.1	35785	5.45	142	57	21
17	Lmo0096	Similar to PTS system mannose-specific, factor IIAB	Q8YAM2	1.2	34972	5.33	75	38	9
	Lmo0096	Similar to PTS system mannose-specific, factor IIAB	Q8YAM2	1.2	34972	5.33	88	38	10
	Lmo0096	Similar to PTS system mannose-specific, factor IIAB	Q8YAM2	1.2	34972	5.33	61	45	14
18	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	119	52	23
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	150	70	28
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	153	64	26

	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	138	64	24
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	202	64	25
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	88	49	20
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	138	61	27
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	116	54	20
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	165	52	25
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	104	63	24
	Lmo0135	Similar to oligopeptide ABC transport system substrate-binding proteins	Q8YAJ0	1.2	58308	5.04	71	42	17
19	Lmo0152	Similar to oligopeptide ABC transporter-binding protein	Q8YAH4	1.2	62089	5.69	145	41	21
20	Lmo0181	Similar to sugar ABC transporter, sugar-binding protein	Q8YAE9	1.2	46662	4.53	75	40	16
21	Lmo0783	Similar to mannose-specific phosphotransferase system (PTS) component IIB	Q8Y8V9	1.2	17868	6.15	125	50	12
	Lmo0783	Similar to mannose-specific phosphotransferase system (PTS) component IIB	Q8Y8V9	1.2	17868	6.15	82	50	12
22	Lmo0848	Similar to amino acid ABC transporter, ATP-binding protein	Q8Y8P8	1.2	26772	4.97	65	49	18
23	Lmo0923	Similar to ABC transporter, ATP-binding protein (N-terminal part)	Q8Y8H9	1.2	26048	5.82	73	62	17
24	Lmo1223	Similar to ABC transporter, ATP-binding proteins	Q8Y7Q0	1.2	25518	5.32	91	81	14
25	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	171	59	21
	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	144	58	22
	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	177	62	23
	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	157	56	20
	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	142	59	20
	TcsA	CD4+ T cell-stimulating antigen, lipoprotein	Q48754	1.2	38448	5.02	147	63	22
26	Lmo2114	Similar to ABC transporter (ATP-binding protein)	Q8Y5F0	1.2	28353	5.32	127	58	18
	Lmo2114	Similar to ABC transporter (ATP-binding protein)	Q8Y5F0	1.2	28353	5.32	113	54	16
	Lmo2114	Similar to ABC transporter (ATP-binding protein)	Q8Y5F0	1.2	28353	5.32	111	61	18
27	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	61	31	13
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	126	49	23
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	109	48	22
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	75	36	16
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	65	23	14

	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	74	32	16
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	120	34	20
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	137	34	20
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	90	33	17
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	129	41	14
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	85	34	20
	Lmo2196	Similar to pheromone ABC transporter (binding protein)	Q9LAT7	1.2	62606	5.3	96	34	17
28	Lmo2372	Similar to ABC-transporter ATP binding proteins	Q8Y4R2	1.2	24509	5.78	126	64	12
	Lmo2372	Similar to ABC-transporter ATP binding proteins	Q8Y4R2	1.2	24509	5.78	101	59	15
29	Lmo2415	Similar to ABC transporter, ATP-binding protein	Q8Y4M2	1.2	29193	4.56	144	75	28
	Lmo2415	Similar to ABC transporter, ATP-binding protein	Q8Y4M2	1.2	29193	4.56	142	70	24
	Lmo2415	Similar to ABC transporter, ATP-binding protein	Q8Y4M2	1.2	29193	4.56	144	76	26
30	Lmo2683	Similar to cellobiose phosphotransferase enzyme IIB component	Q8Y3Z5	1.2	10998	5.39	61	54	7
31	Lmo2685	Similar to cellobiose phosphotransferase enzyme IIA component	Q927F6	1.2	10987	5.04	71	66	7
32	Lmo0355	Similar to flavocytochrome C fumarate reductase chain A	Q8YA11	1.4	54486	5.7	135	46	20
	Lmo0355	Similar to flavocytochrome C fumarate reductase chain A	Q8YA11	1.4	54486	5.7	68	35	18
	Lmo0355	Similar to flavocytochrome C fumarate reductase chain A	Q8YA11	1.4	54486	5.7	67	22	11
	Lmo0355	Similar to flavocytochrome C fumarate reductase chain A	Q8YA11	1.4	54486	5.7	70	29	14
33	TrxA	Thioredoxin	P0A4L3	1.4	11727	4.27	102	66	11
	TrxA	Thioredoxin	P0A4L3	1.4	11727	4.27	63	42	7
34	TrxB	Thioredoxin reductase	O32823	1.4	34266	4.79	148	63	19
35	AtpD	Highly similar to H+-transporting ATP synthase chain beta	Q8Y4C1	1.4	51576	4.73	158	67	29
	AtpD	Highly similar to H+-transporting ATP synthase chain beta	Q8Y4C1	1.4	51576	4.73	152	65	25
	AtpD	Highly similar to H+-transporting ATP synthase chain beta	Q8Y4C1	1.4	51576	4.73	141	69	28
	AtpD	Highly similar to H+-transporting ATP synthase chain beta	Q8Y4C1	1.4	51576	4.73	76	52	19
36	AtpA	Highly similar to H+-transporting ATP synthase chain alpha	Q8Y4C0	1.4	55110	5.33	92	27	12
	AtpA	Highly similar to H+-transporting ATP synthase chain alpha	Q8Y4C0	1.4	55110	5.33	73	29	13
	AtpA	Highly similar to H+-transporting ATP synthase chain alpha	Q8Y4C0	1.4	55110	5.33	84	29	13
37	Lmo2219	Similar to post-translocation molecular chaperone	Q8Y557	1.6	32698	5.59	85	39	11

	Lmo2219	Similar to post-translocation molecular chaperone	Q8Y557	1.6	32698	5.59	70	48	15
	Lmo2219	Similar to post-translocation molecular chaperone	Q8Y557	1.6	32698	5.59	73	45	16
38	Lmo0197	Putative septation protein spoVG 2	Q92F70	1.7	11397	4.56	68	55	8
	Lmo0197	Putative septation protein spoVG 2	Q92F70	1.7	11397	4.56	69	45	6
	Lmo0197	Putative septation protein spoVG 2	Q92F70	1.7	11397	4.56	68	53	8
	Lmo0197	Putative septation protein spoVG 2	Q92F70	1.7	11397	4.56	69	48	6
39	MinD	Highly similar to cell division inhibitor (septum placement) protein MinD	Q8Y6Y7	1.7	29219	5.03	124	54	18
	MinD	Highly similar to cell division inhibitor (septum placement) protein MinD	Q8Y6Y7	1.7	29219	5.03	117	52	19
40	MinC	Similar to cell-division inhibition (septum placement) protein MinC	Q8Y6Y6	1.7	25032	4.95	98	52	12
	MinC	Similar to cell-division inhibition (septum placement) protein MinC	Q8Y6Y6	1.7	25032	4.95	86	47	11
	MinC	Similar to cell-division inhibition (septum placement) protein MinC	Q8Y6Y6	1.7	25032	4.95	101	53	12
	MinC	Similar to cell-division inhibition (septum placement) protein MinC	Q8Y6Y6	1.7	25032	4.95	98	67	14
41	divIVA	Similar to cell-division initiation protein (septum placement)	Q8Y5N7	1.7	20336	4.77	72	53	14
42	FtsZ	Highly similar to cell-division initiation protein FtsZ	Q8Y5M5	1.7	41325	4.76	82	42	17
43	Lmo0130	Similar to 5'-nucleotidase, putative peptidoglycan bound protein (LPXTG motif)	Q8YAJ5	1.8	82459	4.96	121	46	32
44	Lmo0196	Similar to *B. subtilis* SpoVG protein	Q8YAD5	1.7	11250	4.46	61	60	9
45	Lmo1086	Similar to CDP-ribitol pyrophosphorylase	Q8Y832	2.1	26809	5.71	96	53	12
	Lmo1086	Similar to CDP-ribitol pyrophosphorylase	Q8Y832	2.1	26809	5.71	61	48	13
46	Lmo0348	Similar to dihydroxyacetone kinase	Q8YA18	2.1.1	36022	4.61	145	63	28
47	Lmo0521	Similar to 6-phospho-beta-glucosidase	Q8Y9K6	2.1.1	49245	5.05	215	65	31
48	Lmo0539	Similar to tagatose-1,6-diphosphate aldolase	Q8Y9I9	2.1.1	37831	4.94	164	65	16
	Lmo0539	Similar to tagatose-1,6-diphosphate aldolase	Q8Y9I9	2.1.1	37831	4.94	129	41	12
	Lmo0539	Similar to tagatose-1,6-diphosphate aldolase	Q8Y9I9	2.1.1	37831	4.94	183	68	19
49	Lmo0554	Similar to NADH-dependent butanol dehydrogenase	Q8Y9H4	2.1.1	43438	5.07	169	64	22
	Lmo0554	Similar to NADH-dependent butanol dehydrogenase	Q8Y9H4	2.1.1	43438	5.07	149	63	22
50	Lmo0613	Similar to oxidoreductase	Q8Y9B9	2.1.1	33919	5.14	89	52	14
51	Lmo0640	Similar to oxidoreductase	Q8Y993	2.1.1	34137	5.02	148	48	17
52	Lmo0722	Similar to pyruvate oxidase	Q8Y920	2.1.1	63013	4.89	209	56	31
53	Lmo0811	Similar to carbonic anhydrase	Q8Y8T3	2.1.1	27505	4.89	77	57	14

54	Lmo0814	Similar to oxidoreductases	Q8Y8T0	2.1.1	32527	5.34	61	47	16
55	Lmo0956	Similar to N-acetylglucosamine-6P-phosphate deacetylase	Q8Y8E8	2.1.1	41616	5.43	117	55	23
56	Lmo0957	Similar to glucosamine-6-phoasphate isomerase	Q8Y8E7	2.1.1	25609	4.83	79	37	9
	Lmo0957	Similar to glucosamine-6-phoasphate isomerase	Q8Y8E7	2.1.1	25609	4.83	68	47	19
57	Lmo1339	Similar to glucose kinase	Q8Y7E4	2.1.1	33862	4.71	96	59	13
58	PflC	Pyruvate-formate lyase activating enzyme	P0A442	2.1.1	28439	5.13	62	55	12
59	PykA	Highly similar to pyruvate kinases	Q8Y6W1	2.1.1	62673	5.39	112	56	26
60	Pfk	Highly similar to 6-phosphofructokinase	Q8Y6W0	2.1.1	34399	5.46	142	55	22
	Pfk	Highly similar to 6-phosphofructokinase	Q8Y6W0	2.1.1	34399	5.46	73	27	10
61	AckA	Highly similar to acetate kinase	Q8Y6V0	2.1.1	43879	5.32	208	68	33
62	Lmo1818	Similar to ribulose-5-phosphate 3-epimerase	Q8Y681	2.1.1	24002	5.23	66	44	7
63	Lmo1906	Similar to methylglyoxal synthase	Q8Y5Z7	2.1.1	14931	5.83	138	94	15
64	AlsS	Similar to alpha-acetolactate synthase protein, AlsS	Q8Y5Q0	2.1.1	61673	5.4	179	67	35
	AlsS	Similar to alpha-acetolactate synthase protein, AlsS	Q8Y5Q0	2.1.1	61673	5.4	172	59	30
	AlsS	Similar to alpha-acetolactate synthase protein, AlsS	Q8Y5Q0	2.1.1	61673	5.4	104	59	27
65	Lmo2094	Similar to L-fuculose-phosphate aldolase	Q8Y5G9	2.1.1	24303	5.4	96	77	17
66	Pta	Similar to phosphotransacetylase	Q8Y5G0	2.1.1	34449	5.19	88	39	7
	Pta	Similar to phosphotransacetylase	Q8Y5G0	2.1.1	34449	5.19	94	55	15
67	Lmo2118	Similar to phosphoglucomutase	Q8Y5E6	2.1.1	48628	4.7	79	35	16
68	Lmo2724	Similar to unknown proteins (3-demethylubiquinone-9 3-methyltransferase domain protein, putative)	Q8Y3V7	5.2	16366	4.47	105	73	11
69	Lmo2253	Similar to phosphoglucomutase	Q8Y524	2.1.1	23323	4.41	66	53	6
70	FruB	Fructose-1-phosphate kinase	Q8Y4U5	2.1.1	33276	5.38	96	47	12
71	Lmo2537	Similar to UDP-N-acetylglucosamine 2-epimerase	Q8Y4B4	2.1.1	42235	5.06	86	60	19
72	FbaA	Highly similar to fructose-1,6-bisphosphate aldolase type II	Q8Y498	2.1.1	30219	5.2	95	54	15
	FbaA	Highly similar to fructose-1,6-bisphosphate aldolase type II	Q8Y498	2.1.1	30219	5.2	67	55	13
	FbaA	Highly similar to fructose-1,6-bisphosphate aldolase type II	Q8Y498	2.1.1	30219	5.2	95	52	12
	FbaA	Highly similar to fructose-1,6-bisphosphate aldolase type II	Q8Y498	2.1.1	30219	5.2	97	55	14
	FbaA	Highly similar to fructose-1,6-bisphosphate aldolase type II	Q8Y498	2.1.1	30219	5.2	85	51	13

73	Lmo2674	Similar to ribose 5-phosphate epimerase	Q8Y404	2.1.1	16181	4.99	66	41	6
	Lmo2674	Similar to ribose 5-phosphate epimerase	Q8Y404	2.1.1	16181	4.99	61	56	8
74	Lmo2695	Similar to dihydroxyacetone kinase	Q8Y3Y4	2.1.1	34842	4.55	75	46	13
	Lmo2695	Similar to dihydroxyacetone kinase	Q8Y3Y4	2.1.1	34842	4.55	70	41	11
75	Lmo2696	Similar to hypothetical dihydroxyacetone kinase	Q8Y3Y3	2.1.1	21463	5.13	66	47	13
76	Lmo2743	Similar to transaldolase	Q8Y3T8	2.1.1	22738	4.99	86	59	12
77	PdhA	Highly similar to pyruvate dehydrogenase (E1 alpha subunit)	Q8Y865	2.1.2	41241	6.05	115	49	19
78	PdhB	Highly similar to pyruvate dehydrogenase (E1 beta subunit)	Q8Y864	2.1.2	35288	4.79	66	25	13
	PdhB	Highly similar to pyruvate dehydrogenase (E1 beta subunit)	Q8Y864	2.1.2	35288	4.79	100	45	22
	PdhB	Highly similar to pyruvate dehydrogenase (E1 beta subunit)	Q8Y864	2.1.2	35288	4.79	103	39	17
79	PdhC	Highly similar to pyruvate dehydrogenase (dihydrolipoamide acetyltransferase E2 subunit)	Q8Y863	2.1.2	58159	4.76	141	52	28
80	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	66	41	17
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	201	40	20
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	125	40	20
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	148	40	17
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	183	40	18
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	73	43	15
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	94	40	20
	PdhD	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	Q8Y862	2.1.2	49571	5.24	89	41	19
81	Tkt	Highly similar to transketolase	Q8Y7H4	2.1.2	71838	5.15	121	46	25
	Tkt	Highly similar to transketolase	Q8Y7H4	2.1.2	71838	5.15	63	26	18
	Tkt	Highly similar to transketolase	Q8Y7H4	2.1.2	71838	5.15	190	49	30
	Tkt	Highly similar to transketolase	Q8Y7H4	2.1.2	71838	5.15	135	49	28
	Tkt	Highly similar to transketolase	Q8Y7H4	2.1.2	71838	5.15	90	38	23
82	Lmo2205	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Q8Y571	2.1.2	26401	5.6	151	61	17
83	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	189	61	25

	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	189	61	25
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	211	60	23
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	176	59	23
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	230	61	24
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	197	52	15
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	118	64	24
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	153	57	26
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	143	60	23
	Eno	Highly similar to enolase	P64074	2.1.2	46444	4.7	83	48	17
84	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	81	52	20
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	165	55	28
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	208	50	23
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	135	42	20
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	188	55	27
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	132	56	26
	Pgm	Highly similar to phosphoglycerate mutase	Q8Y4I4	2.1.2	56139	4.89	66	31	12
85	Tpi	Highly similar to triose phosphate isomerase	Q8Y4I3	2.1.2	27090	4.73	99	50	12
	Tpi	Highly similar to triose phosphate isomerase	Q8Y4I3	2.1.2	27090	4.73	80	35	9
	Tpi	Highly similar to triose phosphate isomerase	Q8Y4I3	2.1.2	27090	4.73	61	43	10
86	Pgk	Highly similar to phosphoglycerate kinase	Q8Y4I2	2.1.2	42135	4.97	162	49	17
	Pgk	Highly similar to phosphoglycerate kinase	Q8Y4I2	2.1.2	42135	4.97	89	42	14
	Pgk	Highly similar to phosphoglycerate kinase	Q8Y4I2	2.1.2	42135	4.97	74	46	17
	Pgk	Highly similar to phosphoglycerate kinase	Q8Y4I2	2.1.2	42135	4.97	78	38	13
87	Gap	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	Q8Y4I1	2.1.2	36435	5.2	74	34	10
	Gap	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	Q8Y4I1	2.1.2	36435	5.2	100	40	14
	Gap	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	Q8Y4I1	2.1.2	36435	5.2	108	43	14
	Gap	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	Q8Y4I1	2.1.2	36435	5.2	111	38	12
88	CitC	Highly similar to isocitrate dehyrogenases	Q8Y6W5	2.1.3	46290	5.17	90	40	16
89	CitB	Highly similar to aconitate hydratases	Q8Y6P3	2.1.3	98473	4.89	214	43	36

90	CysK	Highly similar to cysteine synthase	Q8YAC3	2.2	32198	5.32	82	53	14
	CysK	Highly similar to cysteine synthase	Q8YAC3	2.2	32198	5.32	98	57	15
	CysK	Highly similar to cysteine synthase	Q8YAC3	2.2	32198	5.32	96	61	17
	CysK	Highly similar to cysteine synthase	Q8YAC3	2.2	32198	5.32	103	54	15
	CysK	Highly similar to cysteine synthase	Q8YAC3	2.2	32198	5.32	101	56	15
91	Lmo0265	Similar to succinyldiaminopimelate desuccinylase	Q9ZEY0	2.2	41943	5.16	74	38	17
92	Lmo0560	Similar to NADP-specific glutamate dehydrogenase	Q8Y9G8	2.2	49464	5.54	176	60	24
93	Lmo0978	Similar to branched-chain amino acid aminotransferase	Q8Y8D0	2.2	37752	5.11	259	64	23
94	Lmo1011	Similar to tetrahydrodipicolinate succinylase	Q8Y8A1	2.2	24876	4.95	81	46	12
95	Lmo1217	Similar to endo-1,4-beta-glucanase and to aminopeptidase	Q8Y7Q5	2.2	38933	5.14	72	55	15
96	ProA	Gamma-glutamyl phosphate reductase	Q93Q55	2.2	45709	5.33	64	49	16
97	Lmo1354	Similar to aminopeptidase P	Q8Y7C9	2.2	39163	4.96	88	40	11
98	Lmo1435	Similar to dihydrodipicolinate synthase	Q8Y766	2.2	31446	5.77	128	46	15
	Lmo1435	Similar to dihydrodipicolinate synthase	Q8Y766	2.2	31446	5.77	107	46	13
	Lmo1435	Similar to dihydrodipicolinate synthase	Q8Y766	2.2	31446	5.77	191	46	15
99	Lmo1436	Similar to aspartokinase I (alpha and beta subunits)	Q8Y765	2.2	43169	5.88	91	52	15
100	Lmo1437	Similar to aspartate-semialdehyde dehydrogenase	Q8Y764	2.2	37690	5.44	171	68	25
	Lmo1437	Similar to aspartate-semialdehyde dehydrogenase	Q8Y764	2.2	37690	5.44	68	51	18
101	Lmo1578	Similar to X-Pro dipeptidase	Q8Y6V3	2.2	40339	4.91	82	49	14
102	Lmo1579	Similar to alanine dehydrogenase	Q8Y6V2	2.2	39586	5.24	122	59	21
	Lmo1579	Similar to alanine dehydrogenase	Q8Y6V2	2.2	39586	5.24	157	67	23
	Lmo1579	Similar to alanine dehydrogenase	Q8Y6V2	2.2	39586	5.24	153	64	23
103	Lmo1603	Similar to aminopeptidase	Q8Y6T0	2.2	41210	4.67	151	46	21
104	Lmo1611	Similar to aminopeptidase	Q8Y6S2	2.2	38795	5.57	128	73	21
	Lmo1611	Similar to aminopeptidase	Q8Y6S2	2.2	38795	5.57	190	63	15
	Lmo1611	Similar to aminopeptidase	Q8Y6S2	2.2	38795	5.57	163	74	22
105	DaaA	D-alanine aminotransferase	O85046	2.2	32554	5.12	111	61	14
106	Lmo1620	Similar to Xaa-His dipeptidase	Q8Y6R4	2.2	51874	4.96	139	56	23
	Lmo1620	Similar to Xaa-His dipeptidase	Q8Y6R4	2.2	51874	4.96	173	56	26

	Lmo1620	Similar to Xaa-His dipeptidase	Q8Y6R4	2.2	51874	4.96	146	57	25
	Lmo1620	Similar to Xaa-His dipeptidase	Q8Y6R4	2.2	51874	4.96	159	61	28
107	TrpF	Phosphoribosyl anthranilate isomerase	Q8Y6Q5	2.2	21976	5.14	112	59	11
108	Lmo1812	Similar to L-serine dehydratase	Q8Y685	2.2	31123	4.84	145	56	26
109	AspB	Similar to aspartate aminotransferases	Q8Y606	2.2	43388	5.39	119	61	23
110	DapB	Similar to dihydrodipicolinate reductase	Q8Y5Z6	2.2	28939	5.21	62	58	16
	DapB	Similar to dihydrodipicolinate reductase	Q8Y5Z6	2.2	28939	5.21	77	55	11
111	Lmo2075	Similar to glycoprotein endopeptidase	Q8Y5I7	2.2	36860	5.04	81	52	13
	Lmo2075	Similar to glycoprotein endopeptidase	Q8Y5I7	2.2	36860	5.04	95	58	21
112	Lmo2719	Conserved protein with unknown functions (cytidine/deoxycytidylate deaminase family protein)	Q8Y3W2	5.2	17606	5.42	89	71	12
113	Lmo2363	Similar to glutamate decarboxylase	Q9EYW9	2.2	53908	5.07	113	38	20
	Lmo2363	Similar to glutamate decarboxylase	Q9EYW9	2.2	53908	5.07	108	46	21
114	ThrC	Highly similar to threonine synthase	Q8Y4A5	2.2	37111	5.32	124	57	14
	ThrC	Highly similar to threonine synthase	Q8Y4A5	2.2	37111	5.32	108	57	15
115	GuaA	Highly similar to GMP synthetase	Q8Y822	2.3	58363	4.96	189	54	30
	GuaA	Highly similar to GMP synthetase	Q8Y822	2.3	58363	4.96	122	56	32
116	Lmo1463	Similar to cytidine deaminase	Q8Y749	2.3	14359	4.95	88	80	10
117	Lmo1494	Similar to 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase	Q8Y729	2.3	25422	4.5	121	69	17
118	Apt	Similar to adenine phosphoribosyltransferase	P0A2X5	2.3	19284	5.17	125	78	15
119	Lmo1691	Similar to deoxyuridine triphosphate nucleotidohydrolases	Q8Y6J3	2.3	16705	5.21	125	54	10
120	PurM	Phosphoribosylaminoimidazole synthetase	Q8Y6C3	2.3	37609	4.59	73	37	9
121	PurQ	Phosphoribosylformylglycinamidine synthetase I	Q8Y6C1	2.3	80689	4.75	99	43	28
122	PurE	Phosphoribosylaminoimidazole carboxylase I	Q8Y6B6	2.3	17034	5.36	89	61	16
123	Sod	Orotidine 5'-phosphate decarboxylase	P58641	2.3	25413	5.65	144	78	22
124	PyrD	Highly similar to dihydroorotase dehydrogenase	Q8Y667	2.3	32389	5.99	104	46	18
125	PyrDII	Highly similar to dihydroorotate dehydrogenase (electron transfer subunit)	Q8Y666	2.3	28115	4.86	74	51	14
126	PyrAB	Highly similar to carbamoyl-phosphate synthetase (catalytic subunit)	Q8Y665	2.3	118272	4.79	156	39	40
127	Ndk	Similar to nucleoside diphosphate kinase	Q8Y5X4	2.3	16411	5.21	64	60	12

128	Pnp	Similar to purine-nucleoside phosphorylase	Q8Y5V2	2.3	29514	4.86	82	42	12
	Pnp	Similar to purine-nucleoside phosphorylase	Q8Y5V2	2.3	29514	4.86	82	42	12
129	Drm	Similar to phosphopentomutase	Q8Y5V1	2.3	43746	4.84	198	55	24
	Drm	Similar to phosphopentomutase	Q8Y5V1	2.3	43746	4.84	101	64	19
130	Dra	Similar to deoxyribose-phosphate aldolase	Q8Y5R1	2.3	23750	5.29	100	50	10
131	Upp	Highly similar to uracil phosphoribosyltransferase	Q8Y4B3	2.3	23044	5.7	73	59	12
132	Adk	Highly similar to adenylate kinases	Q8Y449	2.3	24419	5.08	61	53	14
	Adk	Highly similar to adenylate kinases	Q8Y449	2.3	24419	5.08	61	53	14
133	Lmo2693	Similar to thymidylate kinase	Q8Y3Y6	2.3	23126	5.14	81	67	16
134	Lmo0970	Similar to enoyl- acyl-carrier protein reductase	Q8Y8D5	2.4	28391	5.46	90	63	16
135	Lmo1372	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha subunit)	Q8Y7B4	2.4	36327	4.81	81	43	19
136	Lmo1373	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase beta subunit)	Q8Y7B3	2.4	35828	4.81	177	66	28
	Lmo1373	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase beta subunit)	Q8Y7B3	2.4	35828	4.81	140	66	22
137	Lmo1414	Similar to Acetyl-CoA:acetyltransferase	Q8Y782	2.4	41165	5.09	160	59	22
	Lmo1414	Similar to Acetyl-CoA:acetyltransferase	Q8Y782	2.4	41165	5.09	71	45	12
138	FabG	Similar to 3-ketoacyl-acyl carrier protein reductase	Q8Y690	2.4	26289	5.38	106	56	16
139	FabD	Similar to malonyl CoA-acyl carrier protein transacylase	Q8Y689	2.4	32970	4.79	81	52	16
	FabD	Similar to malonyl CoA-acyl carrier protein transacylase	Q8Y689	2.4	32970	4.79	71	53	18
140	Lmo2201	Similar to 3-oxoacyl-acyl-carrier protein synthase	Q8Y574	2.4	44423	5.26	102	51	17
	Lmo2201	Similar to 3-oxoacyl-acyl-carrier protein synthase	Q8Y574	2.4	44423	5.26	136	60	22
	Lmo2201	Similar to 3-oxoacyl-acyl-carrier protein synthase	Q8Y574	2.4	44423	5.26	133	64	22
141	Lmo2202	Similar to 3-oxoacyl- acyl-carrier protein synthase	Q8Y573	2.4	34006	5.06	72	24	8
	Lmo2202	Similar to 3-oxoacyl- acyl-carrier protein synthase	Q8Y573	2.4	34006	5.06	70	29	7
142	Lmo2433	Similar to acetylesterase	Q8Y4K5	2.4	28943	5	100	53	17
143	Lmo2829	Similar to yeast protein Frm2p involved in fatty acid signaling	Q8Y3K6	2.4	22196	4.7	79	45	11
144	ThiD	Highly similar to phosphomethylpyrimidine kinase thiD	Q8Y971	2.5	28915	5.25	118	54	19
	ThiD	Highly similar to phosphomethylpyrimidine kinase thiD	Q8Y971	2.5	28915	5.25	130	50	15
145	Lmo0786	Similar to acyl-carrier protein phosphodiesterase and to NAD(P)H dehydrogenase	Q8Y8V6	2.5	23081	5.28	99	59	11

146	Lmo1093	Similar to NH(3)-dependent NAD(+) synthetases, nitrogen regulatory protein	Q8Y825	2.5	30626	5.07	73	53	15
147	FolD	Highly similar to methylenetetrahydrofolate dehydrogenase and methenyltetrahydrofolate cyclohydrolase	Q8Y7C5	2.5	31002	5.44	106	61	16
148	MenB	Similar to dihydroxynapthoic acid synthetase	Q8Y6L1	2.5	30176	5.15	100	56	17
	MenB	Similar to dihydroxynapthoic acid synthetase	Q8Y6L1	2.5	30176	5.15	112	53	14
149	Lmo1873	Similar to dihydrofolate reductases	Q8Y627	2.5	18406	5.15	88	55	12
150	Lmo1877	Similar to formyl-tetrahydrofolate synthetase N-terminal part	Q8Y624	2.5	60333	5.39	131	41	21
151	Lmo2101	Similar to a protein required for pyridoxine synthesis	Q8Y5G2	2.5	31720	5.12	83	35	10
	Lmo2101	Similar to a protein required for pyridoxine synthesis	Q8Y5G2	2.5	31720	5.12	78	57	19
152	DnaN	DNA polymerase III, beta chain	Q8YAW1	3.1	42403	4.7	73	39	12
153	Ssb	Highly similar to single-strand binding protein (SSB)	Q8YAR8	3.1	19538	4.98	65	47	7
154	Lmo1881	Similar to protein with unknown functions	Q8Y621	3.1	32514	4.84	65	50	16
155	Lmo0168	Similar to *B. subtilis* transcription regulatory protein AbrB	Q8YAF8	3.5.2	10584	6.29	87	58	7
156	Lmo0287	Similar to two-component response regulator	Q8YA72	3.5.2	27386	4.97	90	48	13
157	Lmo1022	Similar to two-component response regulator, in particular *B. subtilis* YvqC protein	Q8Y892	3.5.2	23657	4.56	79	59	13
158	CodY	HIghly similar to *B. subtilis* CodY protein	Q8Y7J7	3.5.2	28727	4.9	74	30	7
	CodY	HIghly similar to *B. subtilis* CodY protein	Q8Y7J7	3.5.2	28727	4.9	74	30	7
159	CcpA	Catabolite control protein A	Q8Y6T3	3.5.2	36960	5.31	133	65	20
160	PyrR	Highly similar to pyrimidine operon regulatory protein	Q8Y660	3.5.2	20491	6.23	140	79	16
161	Lmo1859	Similar to transcriptional regulator (PilB family)	Q8Y641	3.5.2	16597	4.91	73	57	6
162	ClpL	ATP-dependent Clp protease proteolytic subunit	Q71WV9	3.5.2	21619	4.71	75	50	17
163	Lmo1496	Similar to transcription elongation factor GreA	P64277	3.5.3	17474	4.61	85	43	10
	Lmo1496	Similar to transcription elongation factor GreA	P64277	3.5.3	17474	4.61	85	43	10
164	Tsf	Translation elongation factor	Q8Y6M7	3.5.3	32675	5.11	124	55	13
	Tsf	Translation elongation factor	Q8Y6M7	3.5.3	32675	5.11	157	61	21
	Tsf	Translation elongation factor	Q8Y6M7	3.5.3	32675	5.11	151	61	16
165	RpoA	Highly similar to RNA polymerase (alpha subunit)	P66699	3.5.3	34941	4.8	94	44	14
	RpoA	Highly similar to RNA polymerase (alpha subunit)	P66699	3.5.3	34941	4.8	68	50	16
166	NusA	Highly similar to N utilization substance protein A (NusA protein)	Q8Y7F9	3.5.4	47815	4.82	145	57	23

167	Lmo0935	Similar to *B. subtilis* CspR protein, rRNA methylase homolog	Q8Y8G9	3.6	19708	4.78	174	93	15
168	Fmt	Similar to methionyl-tRNA formyltransferase	Q8Y676	3.6	34122	5.44	142	68	24
169	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	74	82	11
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	84	84	12
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	84	87	10
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	121	79	13
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	81	75	11
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	69	79	8
	RpsF	Ribosomal protein S6	Q8YAR9	3.7.1	11500	5.08	97	84	12
170	RplJ	Ribosomal protein L10	P66042	3.7.1	17739	5.36	80	80	13
171	Lmo1938	Similar to similar to ribosomal protein S1 like protein	Q8Y5W7	3.7.1	41406	4.47	128	47	16
	Lmo1938	Similar to similar to ribosomal protein S1 like protein	Q8Y5W7	3.7.1	41406	4.47	139	42	13
	Lmo1938	Similar to similar to ribosomal protein S1 like protein	Q8Y5W7	3.7.1	41406	4.47	121	45	14
172	AspS	Aspartyl-tRNA synthetase	Q8Y709	3.7.2	66455	4.97	201	62	33
173	GatA	Glutamyl-tRNA(Gln) amidotransferase (subunit A)	Q8Y6D2	3.7.2	52434	4.92	80	50	24
	GatA	Glutamyl-tRNA(Gln) amidotransferase (subunit A)	Q8Y6D2	3.7.2	52434	4.92	127	49	20
174	TufA	Highly similar to translation elongation factor EF-Tu	Q8Y422	3.7.4	43429	4.81	205	70	32
	TufA	Highly similar to translation elongation factor EF-Tu	Q8Y422	3.7.4	43429	4.81	130	54	25
	TufA	Highly similar to translation elongation factor EF-Tu	Q8Y422	3.7.4	43429	4.81	123	54	24
	TufA	Highly similar to translation elongation factor EF-Tu	Q8Y422	3.7.4	43429	4.81	65	22	8
175	Fus	Highly similar to translation elongation factor G	Q8Y421	3.7.4	76973	4.85	238	55	37
	Fus	Highly similar to translation elongation factor G	Q8Y421	3.7.4	76973	4.85	214	55	38
	Fus	Highly similar to translation elongation factor G	Q8Y421	3.7.4	76973	4.85	141	55	34
176	Lmo0938	Similar to protein-tyrosine-phosphatase	Q8Y8G6	3.8	17002	5.2	63	59	13
177	Lmo1471	Similar to ribosomal protein L11 methyltransferase	Q9S5A2	3.8	34847	4.46	157	53	18
178	Lmo1709	Similar to methionine aminopeptidases	Q8Y6H5	3.8	27979	5.39	101	60	11
179	Lmo1860	Similar to peptidyl methionine sulfoxide reductases	Q8Y640	3.8	20092	5.41	167	88	13
180	Lmo1935	Similar to protein-tyrosine/serine phosphatase	Q8Y5X0	3.8	36726	4.94	106	58	19
181	Lmo2481	Similar to *B. subtilis* P-Ser-HPr phosphatase	Q8Y4G3	3.8	24696	4.55	93	55	16

182	Tig	Trigger factor (prolyl isomerase)	Q8Y7L0	3.9	47824	4.5	86	31	17
183	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	181	46	25
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	109	39	18
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	220	44	24
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	186	52	30
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	174	54	28
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	105	45	19
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	80	41	19
	DnaK	Class I heat-shock protein (molecular chaperone) DnaK	Q9S5A4	3.9	66104	4.57	68	43	21
184	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	169	61	32
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	124	47	24
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	133	53	24
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	181	53	25
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	177	61	29
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	154	51	24
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	80	22	10
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	128	56	26
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	64	44	16
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	102	51	26
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	145	57	28
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	80	44	16
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	80	40	18
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	130	47	22
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	127	48	23
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	103	40	21
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	76	40	16
	GroEL	Class I heat-shock protein (chaperonin) GroEL	Q9AGE6	3.9	57332	4.72	81	49	23
185	GroES	Class I heat-shock protein (chaperonin) GroES	Q9AGE7	3.9	10058	4.6	71	53	4
	GroES	Class I heat-shock protein (chaperonin) GroES	Q9AGE7	3.9	10058	4.6	79	74	7

186	Lmo2376	Similar to peptidyl-prolyl cis-trans isomerase	Q8Y4Q8	3.9	21407	4.59	71	43	9
	Lmo2376	Similar to peptidyl-prolyl cis-trans isomerase	Q8Y4Q8	3.9	21407	4.59	75	47	9
	Lmo2376	Similar to peptidyl-prolyl cis-trans isomerase	Q8Y4Q8	3.9	21407	4.59	73	42	8
187	Ctc	Similar to *B. subtilis* general stress protein	Q8YAD3	4.1	22641	4.44	96	65	13
	Ctc	Similar to *B. subtilis* general stress protein	Q8YAD3	4.1	22641	4.44	133	75	11
	Ctc	Similar to *B. subtilis* general stress protein	Q8YAD3	4.1	22641	4.44	96	46	7
188	RsbV	Anti-anti-sigma factor (antagonist of RsbW)	P0A4J8	4.1	12791	4.58	73	71	8
189	Lmo0983	Similar to glutathione peroxidase	Q8Y8C5	4.1	18167	5.18	141	66	14
190	Lmo1138	Similar to ATP-dependent Clp protease proteolytic component	Q8Y7Y1	4.1	21334	4.98	87	53	9
191	CspL	Similar to cold shock protein	P0A355	4.1	7261	4.45	78	70	4
192	Lmo2369	Similar to *B. subtilis* general stress protein 13 containing a ribosomal S1 protein domain	Q8Y4R5	4.1	13011	5.39	70	52	8
193	ClpP	ATP-dependent Clp protease proteolytic subunit	Q9RQI6	4.1	21605	4.94	68	37	10
194	Lmo1018	Similar to *E. coli* copper homeostasis protein CutC	Q8Y896	4.2	25624	5.34	114	56	12
195	Sod	Superoxide dismutase	P28764	4.2	22601	5.23	70	68	11
	Sod	Superoxide dismutase	P28764	4.2	22601	5.23	110	61	11
	Sod	Superoxide dismutase	P28764	4.2	22601	5.23	110	67	12
	Sod	Superoxide dismutase	P28764	4.2	22601	5.23	94	50	10
196	Lmo1583	Similar to thiol peroxidases	Q8Y6U8	4.2	18236	5.21	70	50	9
	Lmo1583	Similar to thiol peroxidases	Q8Y6U8	4.2	18236	5.21	84	52	9
197	Lmo1967	Similar to toxic ion resistance proteins	Q8Y5T8	4.2	45465	5.26	64	37	13
198	Lmo2067	Similar to conjugated bile acid hydrolase	Q8Y5J3	4.2	37167	5.02	111	59	20
	Lmo2067	Similar to conjugated bile acid hydrolase	Q8Y5J3	4.2	37167	5.02	101	60	20
199	Lmo2748	Similar to *B. subtilis* stress protein YdaG	Q8Y3T3	5.2	15725	4.57	85	70	11
200	Lmo0164	Similar to *B. subtilis* YabA protein	Q8YAG2	5.2	14946	4.75	117	96	18
201	Lmo0222	Conserved protein with unknown function	Q8YAC4	5.2	32123	4.59	111	60	21
202	Lmo0256	Conserved protein with unknown function	Q8YA98	5.2	22604	5.98	73	51	15
203	Lmo0260	Similar to unknown proteins	Q8YA95	5.2	20583	4.71	70	50	9
204	Lmo0273	Acetyltransferase, GNAT family	Q724D1	5.2	18815	5.48	69	50	6
205	Lmo0387	Similar to *B. subtilis* YhdG protein	Q8Y9Y0	5.2	14253	5.59	61	56	8

	Lmo0387	Similar to *B. subtilis* YhdG protein	Q8Y9Y0	5.2	14253	5.59	61	56	8
206	Lmo0406	Similar to *B. subtilis* YyaH protein	Q8Y9W3	5.2	14236	4.69	116	83	13
207	Lmo0515	Conserved protein with unknown function	Q8Y9L2	5.2	15515	5.23	74	65	8
208	Lmo0663	Conserved protein with unknown functions	Q8Y970	5.2	32406	4.85	73	66	20
209	Lmo0760	Protein with unknown function	Q8Y8Y2	5.2	22250	5.53	165	89	19
210	Lmo0796	Conserved protein with unknown function	Q8Y8U6	5.2	19326	4.69	84	84	13
	Lmo0796	Conserved protein with unknown function	Q8Y8U6	5.2	19326	4.69	142	85	13
	Lmo0796	Conserved protein with unknown function	Q8Y8U6	5.2	19326	4.69	127	91	14
	Lmo0796	Conserved protein with unknown function	Q8Y8U6	5.2	19326	4.69	129	76	12
211	Lmo0927	Hypothetical transmembrane protein	Q8Y8H6	5.2	74661	6.01	66	34	17
212	Lmo0930	Conserved protein with unknown function, similar to *B. subtilis* YhfI protein	Q8Y8H4	5.2	26931	5.44	64	33	10
213	Lmo0965	Similar to *B. subtilis* YjbK protein	Q8Y8D9	5.2	23138	4.97	96	64	16
214	Lmo0966	Protein with unknown function	Q8Y8D8	5.2	17495	4.68	96	72	12
215	Lmo1001	Similar to *B. subtilis* protein YkvS	Q8Y8A7	5.2	10722	4.47	85	69	8
216	Lmo1008	Similar to *B. subtilis* YkuJ protein	Q92D14	5.2	9007	4.29	68	83	7
217	Lmo1028	Similar to *B. subtilis* YkzG protein	Q92CZ4	5.2	8287	4.81	61	80	6
	Lmo1028	Similar to *B. subtilis* YkzG protein	Q92CZ4	5.2	8287	4.81	98	88	5
218	Lmo1029	Similar to conserved protein with unknown functions	Q8Y888	5.2	28960	5.23	122	68	19
219	Lmo1236	Similar to *B. subtilis* YslB protein	Q8Y7N8	5.2	19292	4.71	64	61	11
220	Lmo1401	Conserved protein with unknown function	Q8Y791	5.2	29937	5.47	120	75	19
221	Lmo1448	Conserved protein with unknown function	Q8Y757	5.2	33911	4.63	112	60	16
	Lmo1448	Conserved protein with unknown function	Q8Y757	5.2	33911	4.63	78	56	17
	Lmo1448	Conserved protein with unknown function	Q8Y757	5.2	33911	4.63	90	68	21
222	Lmo1468	Similar to unknown protein	Q92BP2	5.2	16511	5.96	82	59	10
223	Lmo1502	Similar to unknown proteins	Q8Y723	5.2	15396	5.31	71	60	7
224	Lmo1577	Similar to unknown proteins	Q8Y6V4	5.2	24804	4.81	119	58	14
225	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	97	68	9
	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	102	68	11

	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	79	59	8
	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	86	45	6
	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	67	70	10
	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	92	68	10
	Lmo1580	Similar to unknown protein	Q8Y6V1	5.2	16971	4.98	99	84	12
226	Lmo1608	Similar to unknown proteins	Q8Y6S5	5.2	30039	5.07	97	43	14
227	Lmo1750	Similar to unknown protein	Q8Y6D7	5.2	21160	4.82	102	73	13
228	Lmo1771	Similar to unknown protein	Q92AN7	5.2	9411	4.82	68	66	10
229	Lmo1814	Similar to unknown proteins	Q8Y683	5.2	59431	4.54	95	57	24
230	Lmo1830	Similar to conserved hypotheticl proteins	Q8Y669	5.2	20904	5.91	92	57	11
231	Lmo1888	Cell cycle protein gpsB	Q8Y614	5.2	12875	4.57	91	62	6
232	Lmo1965	Similar to unknown proteins	Q8Y5U0	5.2	21292	4.46	86	48	13
233	Lmo2048	Similar to unknown proteins	Q8Y5L1	5.2	20676	4.52	78	65	13
234	Lmo2072	Similar to a putative DNA binding proteins	P60384	5.2	24185	5.78	113	50	10
235	Lmo2078	Similar to unknown proteins	Q8Y5I4	5.2	17498	4.81	95	69	19
236	Lmo2256	Similar to unknown proteins	Q929B9	5.2	19118	5.22	138	70	11
	Lmo2256	Similar to unknown proteins	Q929B9	5.2	19118	5.22	105	61	9
237	Lmo2340	Similar to *Erwinia chrysanthemi* IndA protein	Q8Y4U2	5.2	32682	4.76	93	52	14
238	Lmo2391	Conserved protein with unknown function similar to *B. subtilis* YhfK protein	Q8Y4P4	5.2	22683	6	82	42	7
239	Lmo2401	Similar to conserved protein with unknown function and to *B. subtilis* YutF protein	Q8Y4N4	5.2	27877	4.74	94	35	11
240	Lmo2411	Similar to conserved protein with unknown functions	Q928M6	5.2	52711	4.87	202	62	38
241	Lmo2426	Conserved protein with unknown functions (ArsC family protein)	Q8Y4L1	5.2	13756	5.47	62	60	8
242	Lmo2487	Similar to *B. subtilis* YvlB protein	Q8Y4F7	5.2	47295	5.37	98	55	25
243	Lmo2511	Similar to conserved protein with unknown functions like to *B. subtilis* YvyD protein	Q927Y2	5.2	21620	5.25	68	53	10
244	Lmo2577	Conserved protein with unknown function (Cof-like hydrolase, HAD-superfamily, subfamily IIB)	Q8Y478	5.2	30337	4.71	67	54	15
245	Lmo2692	Protein with unknown function	Q8Y3Y7	5.2	11863	5	68	80	10

[1] See annotated spots on the 2-D proteome map shown in Figure 2.

[2] Protein name, description and functional categories from ListiList, a database dedicated to the analysis of the genomes of the food-borne pathogen, *Listeria monocytogenes* (available on http://genolist.pasteur.fr/ListiList/).

[3] Accession Number from the Universal Protein resource (Uniprot; http://www.uniprot.org/)

[4] Theoretical Mr and pI (calculated from the amino acid sequence of the translated gene), score, sequence coverage and number of peptides matches as per Mascot Search results

* similar refers to genomic similarity (based on ListiList) and does not imply similarity of phenotypes.