



HAL
open science

Two-dimensional electrophoresis database of *Listeria monocytogenes* EGDe proteome and proteomic analysis of mid-log and stationary growth phase cells

Patrice Folio, Patrick Chavant, Ingrid Chafsey, Abdel Belkorchia, Christophe C. Chambon, Michel Hébraud

► To cite this version:

Patrice Folio, Patrick Chavant, Ingrid Chafsey, Abdel Belkorchia, Christophe C. Chambon, et al.. Two-dimensional electrophoresis database of *Listeria monocytogenes* EGDe proteome and proteomic analysis of mid-log and stationary growth phase cells. *Proteomics*, 2004, 4 (10), pp.3187-3201. 10.1002/pmic.200300841 . hal-02681938

HAL Id: hal-02681938

<https://hal.inrae.fr/hal-02681938>

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Two-dimensional electrophoresis database of *Listeria monocytogenes* EGDe proteome and proteomic analysis of mid-log and stationary growth phase cells

Patrice Folio¹, Patrick Chavant¹, Ingrid Chafsey¹, Abdel Belkorchia¹,
Christophe Chambon² and Michel Hébraud^{1,2}

¹Station de Recherches sur la Viande-Microbiologie

²Plate Forme Protéomique,
Saint-Genès Champanelle, France

Listeria monocytogenes is the causative agent of listeriosis, one of the most significant food-borne diseases in industrialized countries. The complete genome of the *L. monocytogenes* EGDe strain, belonging to the serogroup 1/2a, has been sequenced and is comprised of 2853 open reading frames. The objective of the current study was to construct a two-dimensional (2-D) database of the proteome of this strain. The soluble protein fractions of the microorganism were recovered either in the mid-log or in the stationary phase of growth at 37°C. These fractions were analyzed by 2-D electrophoresis (2-DE), using immobilized pH gradient strips of various pH values (3–10, 3–6, and 5–8) for the first-dimensional separations and 12.5% acrylamide gels for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). 201 protein spots corresponding to 126 different proteins were identified by matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS). The 2-DE maps presented here provide a first basis for further investigations of protein expression in *L. monocytogenes*. In this way, the comparison of proteome between cells in the exponential or stationary phase of growth at 37°C allowed us to characterize 161 variations in protein spot intensity, of which 38 were identified. Among the differentially expressed proteins were ribosomal proteins (RpsF, RplJ, and RpmE), proteins involved in cellular metabolism (GlpD, PdhD, Pgm, Lmo1372, Lmo2696, and Lmo2743) or in stress adaptation (GroES and ferritin), a fructose-specific phosphotransferase enzyme IIB (Lmo0399) and different post-translational modified forms of listeriolysin (LLO).

Keywords: *Listeria monocytogenes* / Matrix assisted laser desorption/ionization-time of flight-mass spectrometry / Mid-log and stationary phase of growth / Proteome / Two-dimensional gel electrophoresis database

Received	1/12/03
Revised	5/3/04
Accepted	8/3/04

1 Introduction

Listeria monocytogenes is a Gram-positive facultative anaerobic rod that can be isolated from a large diversity of biotopes including soil, water and hydraulic networks, vegetables, human and animal faeces, foodstuffs and food industrial environments. It is one of the major food-borne pathogenic bacteria and the causative agent of listeriosis, a very serious localized or generalized infection

which can be lethal, particularly for high-risk groups such as elderly, pregnant women, neonates, and immunocompromised individuals [1]. A series of epidemic outbreaks in the last two decades has initiated research efforts to determine the role of foods and food processing in the epidemiology. The raw materials are extensively contaminated and, consequently, so are the food production plants and foodstuffs. Obviously, these bacteria are able to survive hostile environments and stress conditions such as those encountered in food-processing technologies [2–5]. They tolerate high salt concentrations (up to 15%), a wide range of pHs (from 4.5 to 9.6) and temperatures (from 0 to 45°C), and low water availability (A_w down to 0.91–0.93). Therefore, it is very important to under-

Correspondence: Dr. Michel Hébraud, Station de Recherches sur la Viande-Microbiologie/Plate Forme Protéomique, INRA site de Theix, F-63122 Saint-Genès Champanelle, France
E-mail: hebraud@clermont.inra.fr
Fax: +33(0)-473-624268

stand the mechanisms of survival and adaptation of *L. monocytogenes*, as well as the molecular mechanisms underlying its virulence.

For several years, we have been involved in research concerning the mechanisms of adaptation of foodborne bacteria to the environmental stresses encountered in the food industry [6–8] and the properties of bacteria growing in biofilms [9–11]. These studies were carried out by different approaches and particularly by the proteomic approach that consisted of the comparison of proteome variations and the identification of selected proteins by microsequencing or Western blots. However, the combination of recent advances in protein identification, by mass spectrometry (MS), and the knowledge of the whole *L. monocytogenes* genome sequence [12], has allowed identification on a large number of protein spots on 2-DE gels. To date, several 2-DE databases have been established on proteome of human or animal fluids and organs [13–15] and on proteomes of microorganisms, such as *Bacillus subtilis* [16], *Escherichia coli* [17], *Haemophilus influenzae* [18], *Streptococcus thermophilus* [19], *Mycobacterium tuberculosis* [20], *Corynebacterium glutamicum* [21], and *Saccharomyces cerevisiae* [22]. Most of these 2-DE maps are available on the Internet and give an important complement to the genetic information.

The aim of the present study was to establish 2-DE reference maps of *L. monocytogenes* soluble proteins, which could aid basic physiological investigations on mechanisms involved in environmental survival, adaptation, and in virulence. In order to obtain a larger recovery and a better resolution of the bacterial proteome, cells were recovered either in mid-log or stationary phases of growth and three different pH gradients were carried out for the first-dimensional separation. The identification of protein spots was by MALDI-TOF-MS. In this communication, we describe the present status of this proteome project and we point out some important variations in protein expression between mid-log and stationary growth phase.

2 Materials and methods

2.1 Materials

Immobilized pH gradient (IPG) strips, acrylamide solution and ampholytes were obtained from Bio-Rad (Hercules, CA, USA). Ammonium persulfate, TEMED, 3-(3-cholamido-propyl)dimethylammonio-1-propane sulfonate (CHAPS), EDTA, glycine, iodoacetamide, glycerol, sodium thiosulfate, Coomassie Brilliant Blue G, deoxyribonuclease I (Dnase I), ribonuclease A (Rnase A) and sodium carbonate were purchased from Sigma-Aldrich

(St. Louis, MO, USA). Tris, tributylphosphine (TBP), acetonitrile, and trifluoroacetic acid (TFA) were from Fluka (Buchs, Switzerland). Urea was purchased from Calbiochem (EMD Biosciences, San Diego, CA, USA) and thiourea and silver nitrate from Merck (NJ, USA). Sodium dodecyl sulfate (SDS) was purchased from USB (Cleveland, OH, USA).

2.2 Bacterial growth conditions

The *L. monocytogenes* EGDe strain, serogroup 1/2a, was kindly provided by P. Cossard (Institut Pasteur, Paris). The strain was precultured and cultured in an orbital shaking water bath (150 rpm) at 37°C with brain heart infusion (BHI) medium (Difco, Detroit, MI, USA). Growth was monitored by measuring the absorbance at 600 nm (OD_{600}). Precultured cells in stationary phase were used to inoculate cultures in order to obtain a final OD_{600} of 0.1 (approximately 10^7 CFU/mL). The strain was grown until the mid-log or the stationary phase, *i.e.*, 3 h ($OD_{600} \approx 0.7$) and 17 h ($OD_{600} \approx 1.3$), respectively.

2.3 Preparation of *L. monocytogenes* soluble protein samples

After sampling by centrifugation ($7500 \times g$, 15 min), cell pellets were washed twice with TE buffer (20 mM Tris-HCl, pH 7.5; 5 mM EDTA; 5 mM $MgCl_2$), then resuspended in TE buffer, pH 9.0, and stored at $-20^\circ C$ until their treatment. To optimize the yield of protein extraction, different protocols were evaluated, in particular enzymatic lysis using different concentrations of lysosyme, combined or not with sonication or a mechanical treatment using glass beads. The extraction buffer was also tested by using TBP instead of DTT as reducing agent. The best results, in terms of quantity of extracted proteins and number of spots resolved by 2-DE, were obtained by the following method. The bacteria were sonicated with a Vibracell sonifier (Bioblock Scientific, Illkirch, France) three times for 2 min at power level 5 and 50% of the active cycle. The samples were then treated with Dnase I and Rnase A for 30 min at room temperature. A solution was added at a final concentration of 4 M urea, 2 M thiourea, 2% w/v CHAPS, and 2 mM TBP, and the incubation was allowed to continue for 30 min on ice with intermittent agitation. The soluble protein fractions were separated from cell debris by centrifugation at $13\,000 \times g$ for 20 min. The protein concentrations were determined for each fraction by the method of Bradford [23] using the Bio-Rad protein assay kit. Bovine serum albumin was used as the standard. The protein samples were precipitated with three volumes of cold acetone at $-20^\circ C$ during at least 2 h and then pelleted by centrifugation ($13\,000 \times g$, 40 min).

Pellets were resuspended in isoelectric focusing (IEF) buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, and a trace of bromophenol blue) at a concentration of 5 mg/mL proteins and stored at -20°C .

2.4 Two-dimensional electrophoresis

2-DE was performed essentially according to the principles of O'Farrell [24] with few adaptations. For IEF, pre-cast IPG strips with linear gradients of pH 3–10, pH 3–6 or pH 5–8 were passively rehydrated overnight in a reswelling tray with 400 μL IEF buffer containing 1.5% v/v ampholytes adapted to the pH gradient, 2 mM TBP, and 60, 50 or 50 μg of proteins, respectively. Proteins were separated using a Multiphor II electrophoresis unit (Amersham Biosciences, Uppsala, Sweden). To improve the protein separation, low voltages (50 V, then 200 or 500 V according to the IPG strips) were applied at the beginning. IEF was then continued with maximum setting up to 3500 V at a focusing temperature of 19°C . The time necessary to reach a steady state was dependent on the pH gradient: 36 500 Vh for pH 3–10 IPG gels, 59 000 Vh for pH 3–6 IPG gels, and 79 000 Vh for pH 5–8 IPG gels. Focused IPG strips were stored at -20°C in plastic bags. Prior to running the second dimension, strips were equilibrated twice for 15 min in an equilibration solution (50 mM Tris-HCl, pH 8.8, 6 M urea, 2% w/v SDS, 30% v/v glycerol) containing 5 mM TBP for the first step and 2.5% w/v iodoacetamide and a trace of bromophenol blue for the second step. The second dimension (SDS-PAGE) was carried out with 12.5% T, 3.3% C polyacrylamide gels in a Multicell Protean II XL system (Bio-Rad). Six gels (190 \times 185 mm) of 1 mm width, without stacking gel, were run simultaneously until the marker dye had reached the bottom of the gel.

2.5 Staining of 2-DE gels and image analysis

Routinely, proteins in gels used to establish reference maps were silver stained according to a modified procedure [25] of Blum *et al.* [26]. For the identification of protein spots, semipreparative conditions were used. The 2-DE gels were carried out with 800 μg of proteins and spots were detected using colloidal Coomassie blue stain according to the protocol of Neuhoff *et al.* [27]. Stained 2-DE gels were scanned by a GS-700 imaging densitometer (Bio-Rad) and image analysis was performed using Melanie III (release 3.03) 2-DE analysis software (Genebio, Geneva, Switzerland). The reference 2-DE maps were established with at least four gels per growth condition and pH gradient from two independent bacterial cultures and protein extractions. For the image analysis and comparison, only spots present in at least $n-1$ gels,

n being the number of gels run for each condition, were taken into account. The molecular masses were determined by running standard protein markers (Bio-Rad), covering the range of 6.5–200 kDa, during the second dimension. The pI values were determined according to information provided by the supplier of IPG strips.

2.6 Peptide mass fingerprinting

2.6.1 In-gel digestion

The protein spots on semipreparative 2-DE gels were removed using pipette cones, cut to the diameter of each spot. The gel pieces were put into a 96-well plate then destained and digested using the Montage In-Gel Digest₉₆ Kit (Millipore, Bedford, MA, USA) according to the supplier protocol except for the trypsin which was purchased from Promega (Madison, WI, USA). Briefly, removed spots were washed several times with the destaining solution, then dehydrated with 100% acetonitrile. The dried gels were reswollen in 50 μL of trypsin solution containing 300 ng trypsin and incubated overnight at 37°C . Resulting peptides were extracted with 50 μL of extraction solution, then the mixtures were concentrated in a Speedvac evaporator to a maximum volume of 10 μL . The samples were desalted using $\mu\text{C}18$ -ZipTip[™] (Millipore, Bedford, MA, USA) as recommended by the manufacturer. Finally, the peptide mixture was eluted from the ZipTip with 60% acetonitrile, 0.1% TFA.

2.6.2 MALDI-TOF-MS

For MALDI-TOF-MS, 1 μL of each sample and 1 μL saturated α -cyano-4-hydroxycinnamic acid matrix (10 mg/mL) prepared in 50% acetonitrile, 0.1% TFA were mixed onto the target and the mixture was allowed to dry. Positive ion MALDI mass spectra were recorded in the reflectron mode of a MALDI-TOF-MS (Voyager DE-Pro; PerSeptive BioSystems, Framingham, MA, USA) using Voyager software for data collection and analysis. The MS was calibrated with a standard peptide solution (Proteomix; LaserBio Labs, Sophia-Antipolis, France). Internal calibration of samples was achieved by using trypsin autolysis peptides. Monoisotopic peptide masses were assigned and used for NCBI database searches with the "Mascot" software (<http://www.matrixscience.com>). The following parameters were considered for the searches: a maximum fragment ion mass tolerance of ± 25 ppm, a maximum of one missed enzymatic cleavage, modification of cysteines by carbamidomethylation and possible oxidation of methionine.

3 Results and discussion

3.1 Hypothetical 2-DE map of *L. monocytogenes* EGDe proteins

A hypothetical 2-DE map was prepared using theoretical masses and *pI* of amino acid sequences of the bacteria provided from the database of Pasteur Institut (<http://genolist.pasteur.fr/ListiList/>). This virtual 2-DE map is shown in Fig. 1. As in a similar display for *B. subtilis* [16] and *E. coli* [28], this virtual map showed two clusters, with a clear border around pH 8. The majority of *L. monocytogenes* proteins were localized in a region between pH 4 and 10 and between M_r 7.5 and 100 kDa.

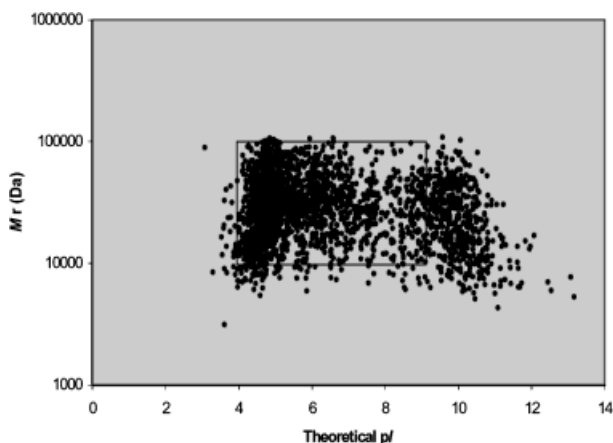


Figure 1. Hypothetical 2-DE map of *L. monocytogenes* EGDe proteins. Theoretical mass and *pI* of the 2853 ORF of the strain were plotted. The boxed area indicates the separation range under the 2-DE conditions used in this study.

3.2 Analysis of the soluble protein fraction of *L. monocytogenes* EGDe

The soluble protein fraction of *L. monocytogenes* EGDe grown until the stationary phase in BHI broth at 37°C was used to establish the reference 2-DE maps. A large view of the *L. monocytogenes* protein content was obtained by using pH 3–10 IPG strips for IEF separation. A total of about 900 spots were detected in the silver-stained gels, the majority of them were localized in the pH range 4–8 (Fig. 2A). Considering this result and the theoretical distribution of all the predicted proteins of *L. monocytogenes* EGDe, we used two other overlapping and narrowest pH gradients, pH 3–6 IPG strips (Fig. 2B) and pH 5–8 IPG strips (Fig. 2C), to gain protein resolution. The three combined pH gradients theoretically allowed the separation and resolution of about 2220 protein precursors (over 2853 annotated

ORF) covering 78% of the strain proteome (Fig. 1). Experimentally, the combination of the three pH gradients resolved about 1300 different silver-stained spots for the cytoplasmic fraction.

The most prominent and well-separated spots were analyzed by MALDI-TOF-MS for identification on the basis of peptide mass fingerprint matching. The 126 identified proteins, representing 4.4% of the *L. monocytogenes* potential gene products, were localized on the 2-DE reference maps (Fig. 2), and referenced with an identification number. The names and specific information on each of the proteins are listed in Table 1 (see Addendum). An alphanumeric code was assigned to each protein according to the *pI* (a to f from acidic to basic range) and M_r (1 to 5 from high to low M_r) which allowed to easily localize them on the 2-DE maps. The flagellin (spot 7), the constitutive protein of *L. monocytogenes* flagella, was added to the reference maps although it was only resolved as a strong spot on 2-DE gels with protein samples from bacterial cultures at 20°C (data not shown). This observation was consistent with the morphological particularity of the bacterium, which is mobile between 20° and 28°C using flagella.

Numerous proteins were resolved as two or more spots on the 2-D gels (e.g., spots 8, 9, 10, 31, 73, 76 in Fig. 2B). In fact, the 126 identified proteins corresponded to 201 spots on the 2-D gels due to post-translational modifications. This suggests that, in our growth conditions, a theoretical average value of 1.59 separate spots could be resolved on 2-DE for each open reading frame of *L. monocytogenes* EGDe strain, which is a similar value to that obtained for *E. coli* [17]. By extrapolating, we can estimate that our 2-DE reference maps (about 1300 spots) covered 28.8% of the *L. monocytogenes* EGDe potential gene products.

3.3 Proteome comparison between cells in exponential and stationary phase of growth

The proteomic approach was used to assess the differences in *L. monocytogenes* EGDe protein expression between two phases of growth in BHI at 37°C. Protein extracts were prepared from cells in mid-log phase and in late-stationary phase, which corresponded to 3 h and 17 h (i.e., 8 h after entry in stationary state) of growth in BHI medium. A statistical analysis was performed using Student's *t*-test (95% confidence interval) to select only significant changes in spot intensity between the two phases of growth. Moreover, only spots in this confidence interval which exhibited an over- or underexpression ratio of at least twofold were taken into consideration. Spots

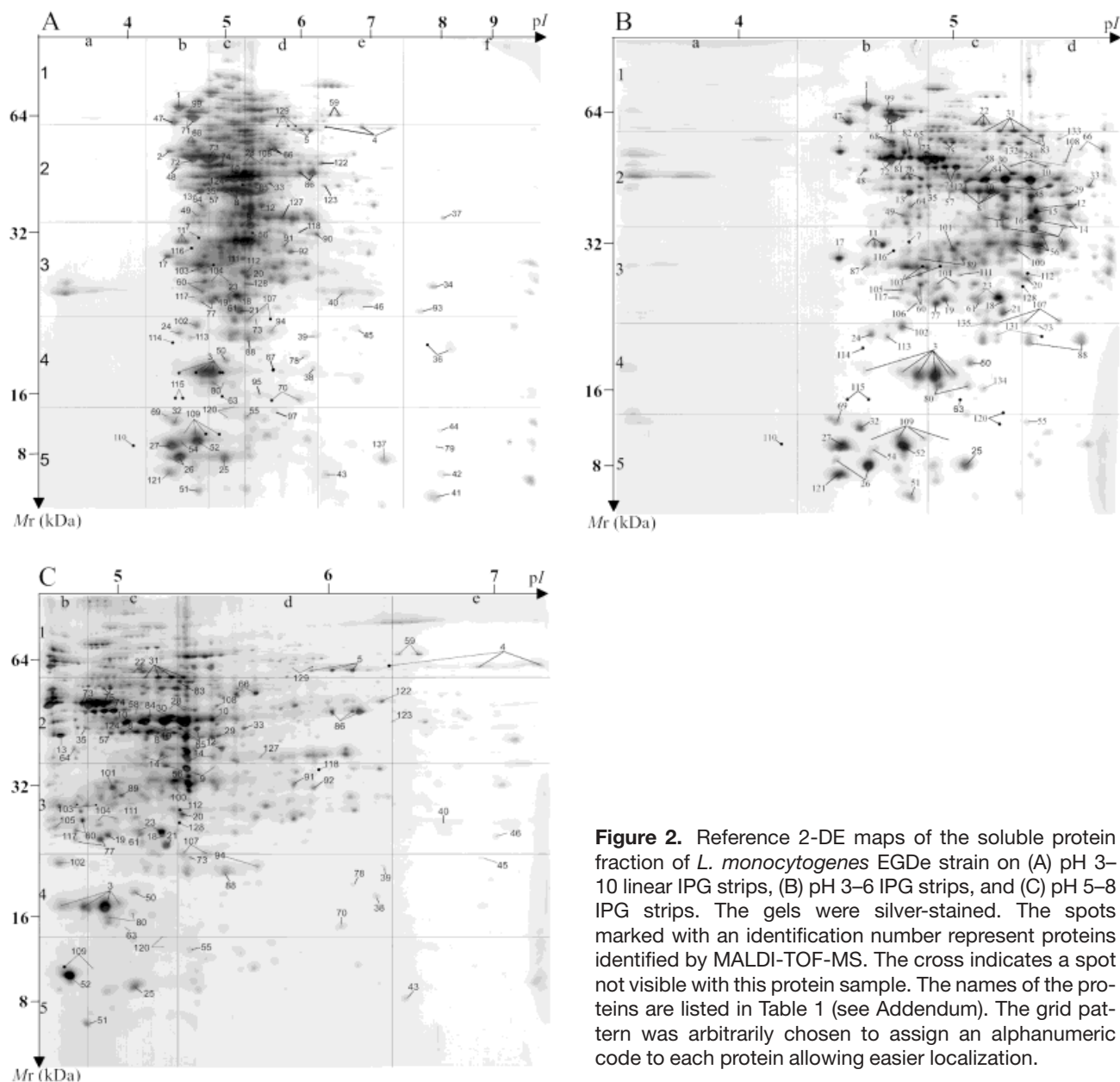


Figure 2. Reference 2-DE maps of the soluble protein fraction of *L. monocytogenes* EGDe strain on (A) pH 3–10 linear IPG strips, (B) pH 3–6 IPG strips, and (C) pH 5–8 IPG strips. The gels were silver-stained. The spots marked with an identification number represent proteins identified by MALDI-TOF-MS. The cross indicates a spot not visible with this protein sample. The names of the proteins are listed in Table 1 (see Addendum). The grid pattern was arbitrarily chosen to assign an alphanumeric code to each protein allowing easier localization.

from 2-DE gels obtained with pH 3–6 IPG strips and resolved in the pH range 6–9.5 of pH 3–10 IPG strips, representing a total of 1149 detected spots, were selected for the comparative analysis. About 14% (161 spots) of the proteome was affected by the growth-phase. 9.2% (106 spots) of the protein spots corresponded to overexpressed or neosynthesized proteins while 4.8% (55 spots) were underexpressed in the stationary phase by comparison to the mid-log phase. In fact, 39.6% (42 over 106 spots) of the overexpressed spots in stationary phase corresponded to neosynthe-

sized proteins. Among the observed 161 variations in spot intensities (Fig. 3), 38 protein spots were identified by MALDI-TOF-MS and corresponded to 23 different proteins (Table 2, see Addendum).

When cells are exposed to some environmental variations or aggressions such as starvation or sudden thermal shock, they sometimes undergo growth arrest and enter in stationary phase. Due to the quorum sensing, the same event takes place when the bacterial density reaches a certain level. Stationary phase may therefore mimic a

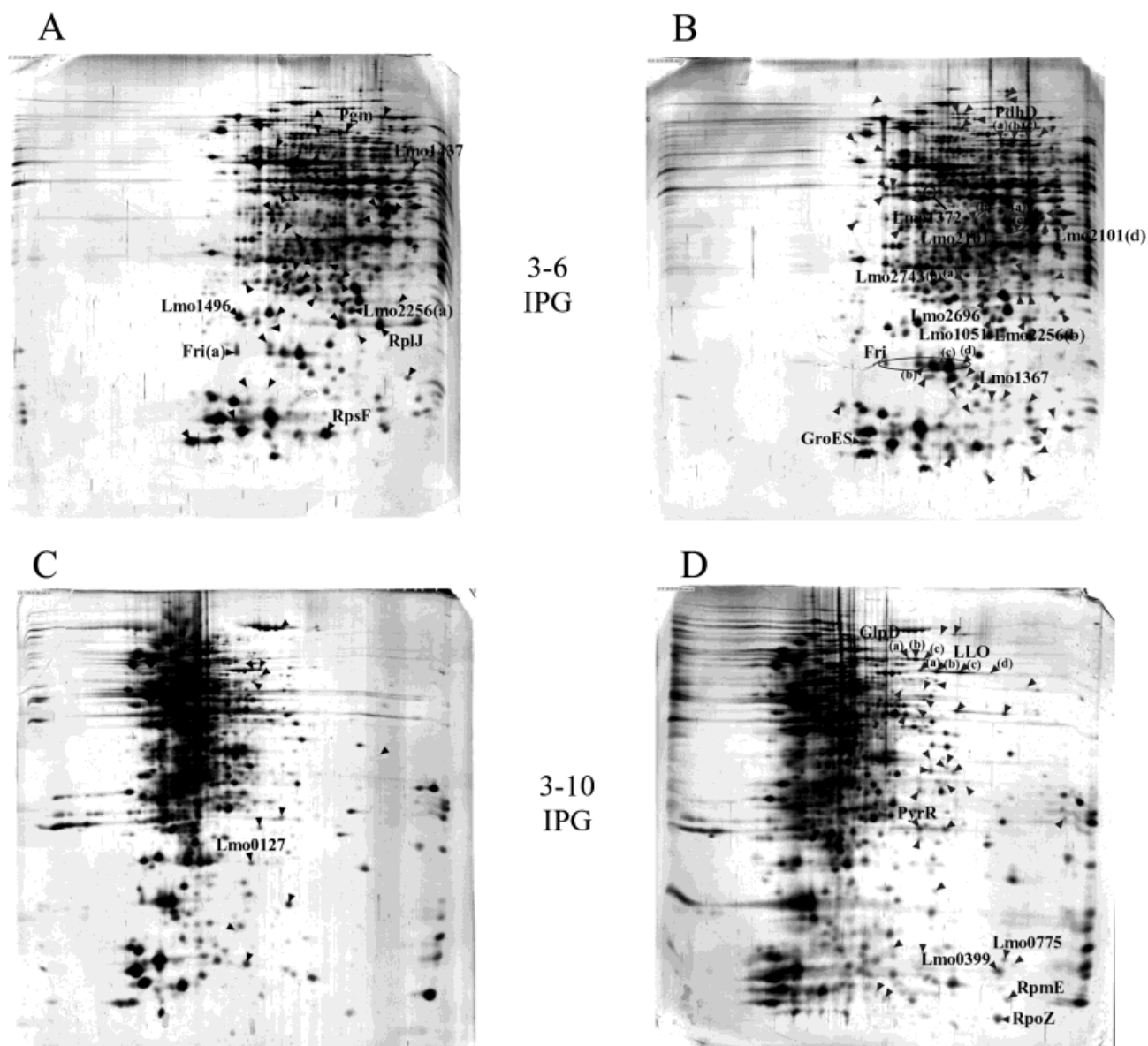


Figure 3. 2-DE maps of the soluble protein fractions of *L. monocytogenes* EGDe obtained in (A), (C) exponential or in (B), (D) stationary phase of growth. The protein extracts were separated in (A), (B) pH 3–6 IPG strips and (B), (D) pH 3–10 IPG strips. Arrows indicate the overexpressed proteins in each protein sample and numbers point out the identified spots listed in Table 2 (see Addendum). The two circled areas indicate multiple spots corresponding to two putative post-translational modified proteins.

starvation state (starvation for nutrient, oxygen, amino acid, *etc.*). The transition to stationary phase is accompanied by many physiological changes [31, 32] due in part to modifications that occur in medium composition during the bacterial growth. Two major bacterial cell responses generally take place during this transition. The first is the stringent response, which is a pleiotropic bacterial global regulatory event that is usually triggered by various nutritional and metabolic stresses in order to conserve energy

[33, 34]. It is mediated by [(p)ppGpp] and affects the expression of many genes with the general effect of an indirect reduction of protein synthesis. The second is a general stress response mediated by the alternative sigma factors RpoS and sigmaB (σ^B) in Gram-negative and Gram-positive bacteria, respectively. It is well-known that these factors regulate the expression of numerous genes under various environmental stress conditions and upon entry into the stationary phase [35–38]. In *L. mono-*

cytogenes, Becker *et al.* [38] demonstrated that there is increased *L. monocytogenes sigB* transcription, which implies that there is increased σ^B activity, following exposure of exponentially-growing cells to different stresses as well as upon entry into the stationary phase. In *B. subtilis*, it has been shown that the σ^B general stress sigma factor regulates its own expression [39] but no evidence exists for such an auto-regulation of *L. monocytogenes* σ^B .

Our analyses of 2-DE gels did not reveal any variation in protein spot intensity in the area corresponding to the putative localization of σ^B . Based on previous reports [40, 41], we search for putative σ^B -dependent promoter regions among the genes encoding the differentially expressed proteins upon entry into the stationary phase. Interestingly, we found σ^B consensus patterns for several genes including *rpmE* (GTTTTA-N₁₆-GGG at -205 from the start codon), *rplJ* (GCTTAA-N₁₃-GGGATTTC at -157), *lmo2696* (GTTTTG-N₁₃-GGGAAA at -66), *lmo1372* (GTTTTA-N₁₃-GGGAAG at -138), and *fri* (GAATAA-N₁₃-GGGATT at -157). This suggests a possible involvement of σ^B in the regulation of the expression of some *L. monocytogenes* gene during transition to the stationary phase.

Among the differentially-expressed protein spots, we found proteins involved in cellular metabolisms, RNA and protein biosynthesis, and stress adaptation. It is widely known that transition to stationary phase induces a modification in the transcriptional and translational machineries due to the stringent response. RpsF and RplJ, the 30S ribosomal protein S6 and the 50S ribosomal protein L10 respectively, were underexpressed in the stationary phase. It has been shown that the *L. monocytogenes* ribosome number rapidly decreased during the stationary phase [42]. Similar downregulation during the stationary phase has been reported for the *E. coli rpsF* [43] and the *Streptomyces coelicolor rplJ* gene [44]. In contrast, the 50S ribosomal protein L31, named RpmE, was overexpressed. An adaptative reprogramming of the ribosomes is necessary under certain stressing growth conditions [45] and it can be assumed that the entry into a stationary phase also necessitates similar changes. Ribosomes may also represent a valuable source of metabolites to starved cells, but maintenance of an active ribosomal pool would appear to be essential for cell survival and recovery [46]. In addition, two transcriptional repressors, Lmo1367 and PyrR, were also overexpressed in the *L. monocytogenes* stationary phase. These proteins were involved in bacterial response to starvation, one in *B. subtilis* where PyrR represses the expression of genes involved in the pyrimidine nucleotide biosynthesis [47], and the other in *E. coli*, where the arginine repressor, corresponding to the *lmo1367* gene product, reduces the synthesis of the arginine-biosynthesis enzymes [48]. A third protein,

Lmo1496, similar to the transcription elongation factor GreA, was found to be underexpressed. These results suggest that transition to stationary phase leads to a global reduction of RNA and protein synthesis.

Several proteins involved in cellular metabolism were overexpressed in the stationary phase. This is the case for Lmo0399, a fructose-specific phosphotransferase enzyme IIB required for the uptake of fructose and for five proteins (GlpD, PdhD, Lmo1372, Lmo2696, and Lmo2743) implicated in different metabolic pathways. In contrast, the phosphoglycerate mutase Pgm, involved in the main glycolytic pathway, was downregulated in the stationary phase. These physiological events are certainly triggered by the modifications which occur in the medium during the bacterial growth (glucose and nutrient consumption, O₂ limitation, etc.).

Some proteins generally involved in stress adaptation also appeared to be affected during the stationary phase of growth. It was especially the case for ferritin which is a homohexamer in *L. monocytogenes* [8] able to oxidize and sequester about 500 iron atoms. The induction of ferritin in stationary phase has been previously reported [49] but here we show that at least two of the five spots of ferritin increased whereas one decreased in the stationary phase. This protein has also been characterized as a stress protein induced by cold-shock [8], heat-shock, SDS, deoxycholate, or ethanol [50]. The respective involvement of the different molecules of ferritin appears quite difficult to explain and needs further investigation. Another stress protein, GroES, was found to be upregulated in stationary phase. This class I heat-shock protein is an ubiquitous bacterial chaperonin induced in numerous bacteria in response to various stresses such as heat-shock, acid-shock, salt, ethanol, or hydrogen peroxide. In this study, the induction of GroES could be an unrelated consequence of the transition to stationary phase. Indeed, the pH of BHI broth decreased from 7.5 to 5.5 during growth under our culture conditions. Therefore, as the induction of GroES following an acidic treatment has been already reported in *L. monocytogenes* [51], the higher level of expression observed in stationary phase could only be due to the medium acidification.

In some species, such as *Clostridium difficile* [52] or *Streptococcus pyogenes* [53], the virulence factors are differentially regulated according to the growth phase. For *L. monocytogenes*, it is known that *inlA* and *inlB* genes, encoding internalin proteins involved in the cellular internalization step of the virulence process, are maximally expressed during the exponential phase of growth [54]. However, the expression of the transcriptional activator PrfA, which positively regulates most of the virulence genes, increases during the stationary phase [55] and starvation [56]. Due to their

extracellular or membraneous localization and/or to their basic *pI*, these proteins could not have been found in our study. However, we observed that the level of expression of listeriolysin O precursor (LLO), which is under the control of PrfA, was increased in the stationary phase. The four spots surrounded in Fig. 3D were identified as LLO and all appeared overexpressed in the stationary phase. These spots most likely correspond to different levels of protein phosphorylation of LLO.

It is surprising to note that the level of expression of RpoZ, a probable DNA-RNA polymerase omega subunit [57, 58], increased in stationary phase. Little is known of *rpoZ* regulation and its possible involvement in bacterial physiology except that a *w*-null mutant of *E. coli* exhibits a slow-growth phenotype [59] and that the *YloH* gene encoding *w* subunit in *Bacillus cereus* is induced during heat stress response [60]. Therefore, the reason for the increase in *L. monocytogenes* RpoZ in the stationary phase of growth remains unclear and requires further investigation.

4 Concluding remarks

In summary, we have established a 2-DE database of the intracellular soluble proteins of *L. monocytogenes* EGDe by using three overlapping pH gradients. The annotated 2-DE reference maps with detailed information on the 201 identified spots can be found on our website at <http://www.clermont.inra.fr/teome>. This first 2-DE database is an important tool for all further proteomic studies developed on biological events in *L. monocytogenes* strains. In this way, the comparative proteomic analysis between *L. monocytogenes* EGDe cells in mid-log and stationary growth phases revealed some interesting results. A deepened characterization of the reprogramming of gene expression pattern in the stationary phase will be undertaken through a kinetic study with protein radiolabelling.

We are greatly indebted to R. Taylor for critical reading of the manuscript. This work was supported in part by a Fundamental Research Program in Microbiology, Infectious and Parasitology of the French Minister of National Education Research and Technology.

5 References

- [1] Vazquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T. *et al.*, *Clin. Microbiol. Rev.* 2001, 14, 584–640.
- [2] Cheroutre-Vialette, M., Lebert, I., Hébraud, M., Labadie, J., Lebert, A., *Int. J. Food Microbiol.* 1998, 42, 71–77.
- [3] Vasseur, C., Baverel, L., Hébraud, M., Labadie, J., *J. Appl. Microbiol.* 1999, 8, 469–476.
- [4] Vasseur, C., Rigaud, N., Hébraud, M., Labadie, J., *J. Food Protect.* 2001, 64, 1442–1445.
- [5] Michel, V., Lehoux, I., Depret, G., Anglade, P. *et al.*, *J. Bacteriol.* 1997, 179, 7331–7342.
- [6] Vasseur, C., Labadie, J., Hébraud, M., *Electrophoresis* 1999, 20, 2204–2213.
- [7] Perrot, F., Hébraud, M., Junter, G.-A., Jouenne, T., *Electrophoresis* 2000, 21, 1625–1629.
- [8] Hébraud, M., Guzzo, J., *FEMS Microbiol. Lett.* 2000, 190, 19–34.
- [9] Perrot, F., Hébraud, M., Charlonet, R., Junter, G.-A. *et al.*, *Electrophoresis* 2000, 21, 645–653.
- [10] Perrot, F., Hébraud, M., Junter, G.-A., Jouenne, T., *Electrophoresis* 2001, 22, 2110–2119.
- [11] Chavant, P., Martinie, B., Meylheuc, T., Bellon-Fontaine, M. N. *et al.*, *Appl. Environ. Microbiol.* 2002, 68, 728–737.
- [12] Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C. *et al.*, *Science* 2001, 294, 849–852.
- [13] Golaz, O., Hughes, G. J., Frutiger, S., Paquet, N. *et al.*, *Electrophoresis* 1993, 14, 1223–1231.
- [14] Karlsson, K., Cairns, N., Lubec, G., Fountoulakis, M., *Electrophoresis* 1999, 20, 2970–2976.
- [15] Corbett, J. M., Why, H. J., Wheeler, C. H., Richardson, P. J. *et al.*, *Electrophoresis* 1998, 19, 2031–2042.
- [16] Büttner, K., Bernhardt, J., Scharf, C., Schmid, R. *et al.*, *Electrophoresis* 2001, 22, 2908–2935.
- [17] Tonella, L., Walsh, B. J., Sanchez, J. C., Ou, K. *et al.*, *Electrophoresis* 1998, 19, 1960–1971.
- [18] Langen, H., Takacs, B., Evers, S., Berndt, P. *et al.*, *Electrophoresis* 2000, 21, 411–429.
- [19] Perrin, C., Gonzalez-Marquez, H., Gaillard, J. L., Bracquart, P. *et al.*, *Electrophoresis* 2000, 21, 949–955.
- [20] Rosenkrands, I., *Electrophoresis* 2000, 21, 935–948.
- [21] Hermann, T., *Electrophoresis* 2001, 22, 1712–1723.
- [22] Wildgruber, R., Harder, A., Obermaier, C., Boguth, G. *et al.*, *Electrophoresis* 2000, 21, 2610–2616.
- [23] Bradford, M. M., *Anal. Biochem.* 1976, 72, 248–254.
- [24] O'Farrell, P. H., *J. Biol. Chem.* 1975, 250, 4007–4021.
- [25] Rabilloud, T., *Electrophoresis* 1992, 13, 429–439.
- [26] Blum, H., Beier, H., Gross, H. J., *Electrophoresis* 1987, 8, 93–99.
- [27] Neuhoff, V., Arold, N., Taube, D., Ehrhardt, W., *Electrophoresis* 1988, 9, 255–262.
- [28] VanBogelen, R. A., Abshire, K. Z., Moldover, B., Olson, E. R. *et al.*, *Electrophoresis* 1997, 18, 1243–1251.
- [29] Rabilloud, T., Adessi, C., Giraudel, A., Lunardi, J., *Electrophoresis* 1997, 18, 307–316.
- [30] Herbert, B. R., Molloy, M. P., Gooley, A. A., Walsh, B. J. *et al.*, *Electrophoresis* 1998, 19, 845–851.
- [31] Siegele, D. A., Kolter, R., *J. Bacteriol.* 1992, 174, 345–348.
- [32] Ishihama, A., *Curr. Opin. Genet. Dev.* 1997, 7, 582–588.
- [33] Chatterji, D., Ojha, A. K., *Curr. Opin. Microbiol.* 2001, 4, 160–165.
- [34] Godfrey, H. P., Bugrysheva, J. V., Cabello, F. C., *Trends Microbiol.* 2002, 10, 349–351.
- [35] Lange, R., Hengge-Aronis, R., *Mol. Microbiol.* 1991, 5, 49–59.
- [36] Hecker, M., Völker, U., *Mol. Microbiol.* 1998, 29, 1129–1136.
- [37] Ferreira, A., O'Byrne, C. P., Boor, K. J., *Appl. Environ. Microbiol.* 2001, 67, 4454–4457.
- [38] Becker, L. A., Cetin, M. S., Hutkins, R. W., Benson, A. K., *J. Bacteriol.* 1998, 180, 4547–4554.
- [39] Petersohn, A., Brigulla, M., Haas, S., Hoheisel, J. D. *et al.*, *J. Bacteriol.* 2001, 183, 5617–5631.

- [40] Kazmierczak, M. J., Mithoe, S. C., Boor, K. J., Wiedmann, M., *J. Bacteriol.* 2003, **185**, 5722–5734.
- [41] Milohanic, E., Glaser, P., Coppée, J.-Y., Frangeul, L. *et al.*, *Mol. Microbiol.* 2003, **47**, 1613–1625.
- [42] Milner, M. G., Saunders, J. R., McCarthy, A. J., *Microbiology* 2001, **147**, 2689–2696.
- [43] Chang, D. E., Smalley, D. J., Conway, T., *Mol. Microbiol.* 2002, **45**, 289–306.
- [44] Blanco, G., Rodicio, M. R., Puglia, A. M., Mendez, C. *et al.*, *Mol. Microbiol.* 1994, **12**, 375–385.
- [45] Kaan, T., Homuth, G., Mader, U., Bandow, J. *et al.*, *Microbiology* 2002, **148**, 3441–3455.
- [46] Davis, B. D., Luger, S. M., Tai, P. C., *J. Bacteriol.* 1986, **166**, 439–445.
- [47] Switzer, R. L., Turner, R. J., Lu, Y., *Prog. Nucleic Acid Res. Mol. Biol.* 1999, **62**, 329–367.
- [48] Maas, W. K., *Microbiol. Rev.* 1994, **58**, 631–640.
- [49] Polidoro, M., De Biase, D., Montagnini, B., Guarrera, L. *et al.*, *Gene* 2002, **296**, 121.
- [50] Phan-Thanh, L., Gormon, T., *Electrophoresis* 1997, **18**, 1464–1471.
- [51] Gahan, C. G., O'Mahony, J., Hill, C., *Infect. Immun.* 2001, **69**, 3924–3932.
- [52] Dupuy, B., Sonenshein, A. L., *Mol. Microbiol.* 1998, **27**, 107–120.
- [53] Unnikrishnan, M., Cohen, J., Sriskandan, S., *Infect. Immun.* 1999, **67**, 5495–5499.
- [54] Dramsi, S., Kocks, C., Forestier, C., Cossart, P., *Mol. Microbiol.* 1993, **9**, 931–941.
- [55] Mengaud, J., Dramsi, S., Gouin, E., Vazquez-Boland, J. A. *et al.*, *Mol. Microbiol.* 1991, **5**, 2273–2283.
- [56] Sokolovic, Z., Schuller, S., Bohne, J., Baur, A. *et al.*, *Infect. Immun.* 1996, **64**, 4008–4019.
- [57] Kojima, I., Kasuga, K., Kobayashi, M., Fukasawa, A. *et al.*, *J. Bacteriol.* 2002, **184**, 6417–6423.
- [58] Gentry, D. R., Burgess, R. R., *J. Bacteriol.* 1989, **171**, 1271–1277.
- [59] Mukherjee, K., Nagai, H., Shimamoto, N., Chatterji, D., *Eur. J. Biochem.* 1999, **266**, 228–235.
- [60] Periago, P. M., Van Schaik, W., Abee, T., Wouters, J. A., *Appl. Envir. Microbiol.* 2002, **68**, 3486–3495.

6 Addendum

Table 1. List of *L. monocytogenes* EGDe soluble proteins localized on the 2-DE maps

(Map localization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M _r (kDa) (obs) (calc)	pI (obs) (calc)	Peptides matched	Coverage (%)
(b2)48	<i>ddlA</i>	D-Alanine-D-alanine ligase	1.1	gi/16802896	LM00855	45.3 40.7	4.7 4.20	3	7
(d2)15	<i>Imo0096</i>	Similar to PTS system mannose-specific, factor IIAB	1.2	gi/16802144	LM00096	38.9 34.9	5.3 5.15	9	25
(d4)38	<i>Imo0783</i>	Similar to mannose-specific phosphotransferase system (PTS) component IIB	1.2	gi/16802825	LM00783	19.9 17.9	6.2 6.55	10	45
(b5)52	<i>ptsH</i>	PTS phosphocarrier protein Hpr (histidine containing protein)	1.2	gi/16803042	LM01002	10.9 9.4	4.7 4.46	5	43
(c4)50	<i>Imo1017</i>	Similar to phosphotransferase system glucose-specific enzyme IIA	1.2	gi/16803057	LM01017	20.9 17.6	5.1 5.02	4	37
(b3)11	<i>Imo2415</i>	Similar to ABC transporter, ATP-binding protein	1.2	gi/16804453	LM02415	33.1 29.1	4.6 4.27	5	22
(f5)79	<i>Imo0399</i>	Similar to fructose-specific phosphotransferase enzyme IIB	1.2	gi/16802444	LM00399	13.3 11.3	7.8 8.23	3	21
(d3)90	<i>Imo1636</i>	Similar to similar to ABC transporter (ATP-binding protein)	1.2	gi/16803676	LM01636	33.3 33.8	6.3 6.52	4	12
(b1)99	<i>Imo1003</i>	Phosphotransferase system enzyme I	1.2	gi/16803043	LM01003	67.9 63.2	4.6 4.44	11	12
(e2)122	<i>gbuA</i>	Highly similar to glycine betaine ABC transporter (ATP-binding protein)	1.2	gi/16803054	LM01014	46.7 43.6	6.3 6.40	9	28

Table 1. Continued

(Map local- ization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M_r (kDa)		pI		Peptides matched	Cover- age (%)
						(obs)	(calc)	(obs)	(calc)		
(b2)68	<i>atpD</i>	Highly similar to H ⁺ -transporting ATP synthase chain beta	1.4	gi/16804567	LM02529	53.3	51.6	4.7	4.47	18	32
(d2)83	<i>atpA</i>	Highly similar to H ⁺ -transporting ATP synthase chain alpha	1.4	gi/16804569	LM02531	56.3	55.1	5.4	5.17	11	24
(f3)93	<i>atpH</i>	Highly similar to H ⁺ -transporting ATP synthase chain delta	1.4	gi/16804570	LM02532	25.9	20.3	7.8	8.75	8	47
(b2)49	<i>trxB</i>	Thioredoxin reductase	1.4	gi/16804516	LM02478	37.3	34.2	4.7	4.52	10	32
(e2)123	<i>Imo2471</i>	Similar to NADH oxidase	1.4	gi/16804509	LM02471	42.4	37.0	6.4	6.87	6	22
(b3)7	<i>flaA</i>	Flagellin protein	1.5	gi/16802732	LM00690	33.6	30.4	4.7	4.67	13	48
(b5)32	<i>Imo0197</i>	Similar to <i>B. subtilis</i> SpoVG protein	1.7	gi/16802243	LM00197	12.5	11.4	4.6	4.24	4	35
(b5)69	<i>Imo0196</i>	Similar to <i>B. subtilis</i> SpoVG protein	1.7	gi/16802242	LM00196	13.2	11.2	4.5	4.20	7	47
(b2)82	<i>ftsZ</i>	Highly similar to cell-division initiation protein FtsZ	1.7	gi/16804071	LM02032	47.7	41.3	4.8	4.48	10	30
(b4)102	<i>div IVA</i>	Similar to cell division initiation protein (septum placement)	1.7	gi/16804059	LM02020	23.6	20.3	4.7	4.48	8	45
(d3)92	<i>Imo1086</i>	Similar to CDP-ribitol pyrophosphorylase	2.1	gi/16803126	LM01086	31.0	26.7	6.0	5.96	13	50
(d2)12	<i>pfk</i>	Highly similar to 6-phosphofructokinase	2.1.1	gi/16803611	LM01571	39.4	34.4	5.6	5.44	7	24
(e1)59	<i>glpD</i>	Similar to glycerol 3 phosphate dehydrogenase	2.1.1	gi/16803333	LM01293	66.3	63.2	6.4	6.77	14	29
(d2)28	<i>ackA</i>	Highly similar to acetate kinase	2.1.1	gi/16803621	LM01581	46.5	43.8	5.4	5.19	5	14
(c3)61	<i>Imo2696</i>	Similar to hypothetical dihydroxyacetone kinase	2.1.1	gi/16804733	LM02696	25.9	21.5	5.1	4.96	7	27
(d2)16	<i>ldh</i>	Similar to L-lactate dehydrogenase	2.1.1	gi/16802256	LM00210	38.0	34.2	5.3	5.05	6	16
(d3)56	<i>fbaA</i>	Similar to fructose-1,6-bis-phosphate aldolase	2.1.1	gi/16804594	LM02556	33.1	30.1	5.2	5.05	16	59
(c3)100	<i>Imo2592</i>	Similar to oxydoreductase, aldo-keto reductase family	2.1.1	gi/16804630	LM02592	32.5	31.5	5.2	4.95	12	34
(c3)104	<i>Imo2743</i>	Similar to transaldolase	2.1.1	gi/16804780	LM02743	28.0	23.1	4.9	4.73	11	49
(d3)118	<i>Imo2700</i>	Similar to aldo/keto reductase	2.1.1	gi/16804737	LM02700	34.1	32.6	6.1	6.15	10	44
(d1/e1)129	<i>Imo0913</i>	Similar to succinate semialdehyde dehydrogenase	2.1.1	gi/16802954	LM00913	60.3	53.2	5.9	5.87	13	27
(b2)13	<i>pdhB</i>	Highly similar to pyruvate dehydrogenase (E1 beta subunit)	2.1.2	gi/16803093	LM01053	41.5	35.3	4.8	4.51	9	27
(c1)22	<i>pgm</i>	Highly similar to phosphoglycerate mutase	2.1.2	gi/16804494	LM02456	62.0	56.1	5.1	4.89	9	27
(c2)10	<i>gap</i>	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	2.1.2	gi/16804497	LM02459	43.8	36.3	5.3	5.00	6	11
(c2)31	<i>pdhD</i>	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	2.1.2	gi/16803095	LM01055	58.4	49.5	5.2	5.01	6	24

Table 1. Continued

(Map local- ization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M_r (kDa)		pI		Peptides matched	Cover- age (%)
						(obs)	(calc)	(obs)	(calc)		
(d2)86	<i>pdhA</i>	highly similar to pyruvate dehydrogenase (E1 alpha subunit)	2.1.2	gi/16803092	LMO1052	45.0	41.3	6.1	6.42	17	48
(b2)72	<i>eno</i>	Highly similar to enolase	2.1.2	gi/16804493	LMO2455	47.3	46.5	4.7	4.42	22	66
(c2)74	<i>pgk</i>	Highly similar to phosphoglycerate kinase	2.1.2	gi/16804496	LMO2458	46.0	42.1	4.9	4.72	9	30
(b3)103	<i>tpi</i>	Highly similar to triose phosphate isomerase	2.1.2	gi/16804495	LMO2457	29.1	26.9	4.7	4.46	7	25
(c2)132	<i>pgi</i>	Glucose-6-phosphate isomerase	2.1.2	gi/16804405	LMO2367	52.6	49.9	5.2	5.07	20	47
(d2)66	<i>Imo2414</i>	Similar to aminotransferase	2.2	gi/16804452	LMO2414	49.9	48.1	5.9	5.79	15	36
(d3)9	<i>cysK</i>	Highly similar to cysteine synthase	2.2	gi/16802269	LMO0223	36.0	32.2	5.3	5.08	7	21
(c2)58	<i>Imo0265</i>	Similar to succinyldiaminopimelate desuccinylase	2.2	gi/16802311	LMO0265	45.3	41.9	5.1	4.71	6	24
(d2)33	<i>Imo1611</i>	Similar to aminopeptidase	2.2	gi/16803651	LMO1611	42.7	38.8	5.8	5.66	8	29
(c2)30	<i>Imo1579</i>	Similar to alanine dehydrogenase	2.2	gi/16803619	LMO1579	44.7	39.6	5.2	5.08	13	43
(c2)75	<i>Imo1620</i>	Similar to Xaa-His dipeptidase	2.2	gi/16803660	LMO1620	53.2	51.8	4.9	4.71	15	45
(c3)89	<i>Imo1011</i>	Similar to tetrahydrodipicolinate succinylase	2.2	gi/16803051	LMO1011	30.6	24.8	5.5	4.70	9	33
(d2)108	<i>Imo1437</i>	Similar to aspartate-semialdehyde dehydrogenase	2.2	gi/16803477	LMO1437	46.7	37.6	5.5	5.29	8	15
(d2)5	<i>guaB</i>	Similar to inosine-monophosphate dehydrogenase	2.3	gi/16804795	LMO2758	58.4	52.5	6.1	6.45	13	30
(b3)60	<i>deoD</i>	Purine nucleoside phosphorylase	2.3	gi/16803896	LMO1856	27.0	29.5	4.8	4.86	8	43
(b2)65	<i>drm</i>	Similar to phosphopentomutase	2.3	gi/16803993	LMO1954	50.4	43.7	4.8	4.84	12	43
(d4)67	<i>purE</i>	Phosphoribosylaminoimidazole carboxylase I	2.3	gi/16803815	LMO1775	20.4	16.9	5.5	5.21	6	49
(b2)81	<i>pdp</i>	Similar to pyrimidine-nucleoside phosphorylase	2.3	gi/16804032	LMO1993	49.4	46.1	4.8	4.50	13	32
(d3)128	<i>dra</i>	Similar to deoxyribose-phosphate aldolase	2.3	gi/16804034	LMO1995	28.2	23.5	5.5	5.02	6	31
(c4)134	<i>ndk</i>	Similar to nucleoside diphosphate kinase	2.3	gi/16803968	LMO1929	16.9	16.5	5.1	5.02	8	63
(c2)35	<i>Imo1372</i>	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase alpha subunit)	2.4	gi/16803412	LMO1372	42.7	36.3	4.8	4.54	9	25
(b2)64	<i>Imo1373</i>	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit (2-oxoisovalerate dehydrogenase beta subunit)	2.4	gi/16803413	LMO1373	38.9	35.7	4.8	4.55	7	35
(b3)117	<i>Imo2829</i>	Similar to yeast protein Frm2p involved in fatty acid signaling	2.4	gi/16804866	LMO2829	26.6	22.2	4.7	4.44	11	49

Table 1. Continued

(Map local- ization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M_r (kDa)		pI		Peptides matched	Cover- age (%)
						(obs)	(calc)	(obs)	(calc)		
(c2)124	<i>lmo0931</i>	Similar to lipoate protein ligase A	2.4	gi/16802971	LM00931	43.3	37.9	5.0	4.74	11	21
(d3)20	<i>lmo0786</i>	Similar to acyl-carrier protein phosphodiesterase and to NAD(P)H dehydrogenase	2.5	gi/16802828	LM00786	28.6	23.1	5.3	5.08	7	20
(e3)46	<i>lmo1336</i>	Similar to 5-formyltetrahydro- folate cyclo-ligase	2.5	gi/16803376	LM01336	26.5	20.7	7.1	7.18	4	20
(c2/d2)14	<i>lmo2101</i>	Similar to a protein required for pyridoxine synthesis	2.5	gi/16804140	LM02101	37.8	31.7	5.1	5.12	11	18
(c2)84	<i>recA</i>	Recombination protein recA	3.3	gi/16803438	LM01398	44.5	37.9	5.1	4.81	10	23
(f2)37	<i>parB</i>	Partition protein ParB homolog	3.4	gi/16804827	LM02790	37.4	32.2	7.8	8.74	8	21
(c4)63	<i>lmo1367</i>	Similar to arginine repressor	3.5.2	gi/16803407	LM01367	15.6	16.8	5.0	5.24	2	26
(e3)40	<i>pyrR</i>	Highly similar to pyrimidine operon regulatory protein	3.5.2	gi/16803880	LM01840	27.2	20.5	6.5	6.69	4	15
(d4)78	<i>fur</i>	Similar to transcriptional regulator (Fur family)	3.5.2	gi/16803995	LM01956	21.5	17.3	6.2	6.40	5	45
(d2)85	<i>ccpA</i>	Catabolite control protein A	3.5.2	gi/16803639	LM01599	42.6	36.9	5.45	5.05	11	32
(c3)101	<i>codY</i>	Highly similar to <i>B. subtilis</i> Cod Y protein	3.5.2	gi/16803320	LM01280	31.9	28.7	4.9	4.60	11	35
(b4)24	<i>lmo1496</i>	Similar to transcription elongation factor GreA	3.5.3	gi/16803536	LM01496	22.8	17.5	4.6	4.31	5	41
(c2)8	<i>tsf</i>	Translation elongation factor	3.5.3	gi/16803697	LM01657	41.6	32.6	5.1	4.85	14	36
(b2)76	<i>rpoA</i>	Highly similar to RNA polymerase (alpha subunit)	3.5.3	gi/16804644	LM02606	44.0	34.9	4.8	4.53	8	21
(d2)29	<i>fmt</i>	Similar to methionyl-tRNA formyltransferase	3.6	gi/16803863	LM01823	41.4	34.1	5.6	5.26	5	22
(b5)27	<i>rplL</i>	Ribosomal protein L12	3.7.1	gi/16802297	LM00251	10.5	12.5	4.5	4.23	7	49
(c5)25	<i>rpsF</i>	Ribosomal protein S6	3.7.1	gi/16802092	LM00044	8.8	11.5	5.0	4.83	4	34
(b2)2	<i>lmo1938</i>	Similar to similar to ribosomal protein S1 like protein	3.7.1	gi/16803977	LM01938	49.4	41.4	4.5	4.21	7	20
(f5)42	<i>rpmE</i>	Ribosomal protein L31	3.7.1	gi/16804586	LM02548	9.7	9.2	7.8	9.45	4	33
(d4)88	<i>rplJ</i>	Ribosomal protein L10	3.7.1	gi/16802296	LM00250	22.2	17.7	5.3	5.09	8	37
(d2)127	<i>rpsB</i>	30S ribosomal protein S2	3.7.1	gi/16803698	LM01658	36.4	28.4	6.0	6.08	14	36
(e5)137	<i>infA</i>	Highly similar to initiation factor IF-I	3.7.3	gi/16804648	LM02610	8.9	8.2	7.5	7.69	3	31
(c2)73	<i>tufA</i>	Highly similar to translation elongation factor EF-Tu	3.7.4	gi/16804690	LM02653	47.7	43.3	4.9	4.55	27	73
(c3)18	<i>frr</i>	Highly similar to ribosome recycling factors	3.7.5	gi/16803354	LM01314	25.9	20.7	5.2	4.95	13	62
(c4)135	<i>lmo1051</i>	Similar to formylmethionine deformylase and to <i>B. subtilis</i> YkrB protein	3.8	gi/16803091	LM01051	24.0	20.6	5.1	4.94	7	40
(b1)47	<i>tig</i>	Trigger factor (prolyl isomerase)	3.9	gi/16803307	LM01267	60.3	47.8	4.6	4.20	12	22
(b1)1	<i>dnaK</i>	Class I heat-shock protein (molecular chaperone) DnaK	3.9	gi/16803513	LM01473	69.9	66.1	4.6	4.30	24	28
(b5)26	<i>groES</i>	Class I heat-shock protein (chaperonin) GroES	3.9	gi/16804108	LM02069	8.9	10.1	4.6	4.31	5	61

Table 1. Continued

(Map local- ization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M_r (kDa)		pI		Peptides matched	Cover- age (%)
						(obs)	(calc)	(obs)	(calc)		
(b1)71	<i>groEL</i>	Class I heat-shock protein (chaperonin) GroEL	3.9	gi/16804107	LM02068	65.1	57.4	4.7	4.43	14	37
(b3)17	<i>ctc</i>	Similar to <i>B. subtilis</i> general stress protein	4.1	gi/16802257	LM00211	30.6	22.6	4.5	4.14	7	46
(b4/c4)3	<i>fri</i>	Non-heme iron-binding ferritin	4.1	gi/16802983	LM00943	19.6	18.0	4.9	4.57	7	58
(c3)19	<i>lmo1138</i>	Similar to ATP-dependent Clp protease proteolytic component	4.1	gi/16803178	LM01138	26.4	21.3	4.9	4.71	7	36
(f4)36	<i>lmo1601</i>	Similar to general stress protein	4.1	gi/16803641	LM01601	23.2	18.4	7.8	8.80	4	25
(c3)77	<i>clpP</i>	ATP-dependent Clp protease proteolytic subunit	4.1	gi/16804506	LM02468	25.9	21.6	4.9	4.71	13	33
(b3)87	<i>grpE</i>	Heat shock protein GrpE	4.1	gi/16803514	LM01474	29.4	21.9	4.7	4.25	6	33
(b4)114	<i>RsbW</i>	Sigma B activity negative regulator RsbW	4.1	gi/16802935	LM00894	21.7	17.5	4.6	4.22	12	70
(b5)121	<i>cspB</i>	Similar to major cold-shock protein	4.1	gi/16804055	LM02016	8.2	7.3	4.5	4.15	3	28
(c3)23	<i>sod</i>	Superoxide dismutase	4.2	gi/16803479	LM01439	26.5	22.6	5.2	5.10	6	33
(c4/d4)131	<i>lmo1583</i>	Similar to thiol peroxidases	4.2	gi/16803623	LM01583	22.8	18.1	5.2	5.02	9	56
(d4)39	<i>lmo0127</i>	Weakly similar to protein gp20 from bacteriophage A118	4.3	gi/16802175	LM00127	23.5	19.7	6.3	6.52	4	28
(e2)4	<i>hly</i>	Listeriolysin O precursor	4.5	gi/16802248	LM00202	60.3	58.7	6.9	8.23	12	25
(d5)55	<i>lmo0903</i>	Conserved hypothetical protein	5.2	gi/16802944	LM00903	12.8	13.9	5.3	5.17	3	41
(c3)21	<i>lmo2511</i>	Similar to conserved hypothetical proteins like to <i>B. subtilis</i> YvyD protein	5.2	gi/16804549	LM02511	25.0	21.6	5.2	5.03	4	19
(b5)51	<i>lmo1028</i>	Similar to <i>B. subtilis</i> YkzG protein	5.2	gi/16803068	LM01028	7.2	8.3	4.8	4.52	6	88
(e5)43	<i>lmo1058</i>	Similar to <i>B. subtilis</i> YktA protein	5.2	gi/16803098	LM01058	9.7	10.4	6.3	6.86	4	31
(f5)41	<i>rpoZ</i>	Probable DNA-directed RNA polymerase omega chain	5.2	gi/16803866	LM01826	7.2	7.6	7.8	8.90	4	40
(b5)54	<i>lmo1888</i>	Similar to hypothetical proteins	5.2	gi/16803927	LM01888	10.5	12.9	4.6	4.25	6	73
(d4)95	<i>lmo1515</i>	Similar to unknown protein	5.2	gi/16803555	LM01515	17.0	15.6	5.7	5.26	8	61
d(5)97	<i>lmo2426</i>	Conserved hypothetical proteins	5.2	gi/16804464	LM02426	15.7	13.6	5.9	5.29	6	69
(e4)45	<i>lmo2021</i>	Similar to unknown protein	5.2	gi/16804060	LM02021	24.2	19.1	6.9	7.18	8	34
(f3)34	<i>lmo2853</i>	Highly similar to <i>B. subtilis</i> Jag protein	5.2	gi/16804890	LM02853	28.0	23.1	7.8	8.75	6	22
(d4)70	<i>lmo1468</i>	Similar to unknown proteins	5.2	gi/16803508	LM01468	16.6	16.5	5.8	6.04	7	49
(c4)80	<i>lmo1580</i>	Similar to unknown protein	5.2	gi/16803620	LM01580	16.6	16.9	4.9	4.72	5	25
(d3)91	<i>lmo2072</i>	Similar to a putative DNA binding proteins	5.2	gi/16804111	LM02072	31.7	24.2	6.0	6.03	11	50
(c2)57	<i>lmo1726</i>	Similar to hypothetical proteins	5.2	gi/16803766	LM01726	41.7	36.8	4.9	4.71	9	34
(d2)133	<i>lmo2487</i>	Similar to <i>B. subtilis</i> YvIB protein	5.2	gi/16804525	LM02487	54.1	47.3	5.5	5.13	21	54
(d3)107	<i>lmo2256</i>	Similar to unknown protein	5.2	gi/16804295	LM02256	24.2	19.1	5.3	5.04	12	61
(b5)109	<i>lmo2199</i>	Unknown protein	5.2	gi/16804238	LM02199	11.4	14.5	4.6	4.52	8	47

Table 1. Continued

(Map local-ization) Spot No.	Gene name	Protein description	Functional category	NCBI protein accession	Swiss-Prot protein accession	M_r (kDa)		pI		Peptides matched	Coverage (%)
						(obs)	(calc)	(obs)	(calc)		
(a5)110	<i>Imo2223</i>	Unknown protein	5.2	gi/16804262	LMO2223	11.3	13.5	4.3	3.99	5	47
(c3)111	<i>Imo2113</i>	Unknown protein	5.2	gi/16804152	LMO2113	28.9	28.8	5.0	4.80	8	23
(d3)112	<i>Imo0794</i>	Similar to <i>B. subtilis</i> YwnB protein	5.2	gi/16802836	LMO0794	29.0	23.3	5.3	5.11	6	28
(b4)113	<i>Imo0796</i>	Conserved hypothetical protein	5.2	gi/16802838	LMO0796	22.3	19.3	4.7	4.45	11	72
(b4)115	<i>Imo2748</i>	Similar to <i>B. subtilis</i> stress protein YdaG	5.2	gi/16804785	LMO2748	15.7	15.7	4.5	4.31	8	30
(b3)116	<i>Imo0437</i>	Conserved hypothetical protein	5.2	gi/16802481	LMO0437	31.6	30.4	4.7	4.85	11	37
(c5)120	<i>Imo0515</i>	Conserved hypothetical protein	5.2	gi/16802558	LMO0515	14.3	15.4	5.2	4.99	4	30
(d4)94	<i>Imo0592</i>		6	gi/16802635	LMO0592	23.8	19.9	5.8	5.71	7	31
(f5)44	<i>Imo0775</i>		6	gi/16802817	LMO0775	14.2	12.5	7.8	8.86	3	20
(b3)105	<i>Imo1059</i>		6	gi/16803099	LMO1059	27.2	19.9	4.7	4.54	8	34

Table 2. Identified proteins whose level of expression is modified during the stationary phase of growth in rich medium (BHI) at 37°C

Protein spot name	Functional classes	Gene name	Description	% Vol.		Ratio (SP/EP)
				Stationary phase (SP)	Exponential phase (EP)	
Overexpressed protein spots in stationary phase						
Lmo0399	Transport/binding proteins and lipoproteins	<i>Imo0399</i>	Similar to fructose-specific phosphotransferase enzyme IIB	1.248	0.210	+5.95
Lmo26966	Metabolism of carbohydrates and related molecules (specific pathways)	<i>Imo2696</i>	Similar to hypothetical dihydroxyacetone kinase	0.293	0.145	+2.03
GlpD(a)	Metabolism of carbohydrates and related molecules (specific pathways)	<i>glpD</i>	Similar to glycerol-3-phosphate dehydrogenase	1.163	0.574	+2.03
GlpD(b)				2.411	0.564	+4.27
GlpD(c)				0.453	0.000	N
Lmo2743(a)	Metabolism of carbohydrates and related molecules (specific pathways)	<i>Imo2743</i>	Similar to transaldolase	0.113	0.052	+2.18
Lmo2743(b)				0.069	0.040	+1.70
PdhD(a)	Metabolism of carbohydrates and related molecules (main glycolytic pathways)	<i>pdhD</i>	Highly similar to dihydrolipoamide dehydrogenase, E3 subunit of pyruvate dehydrogenase complex	0.276	0.135	+2.04
PdhD(b)				0.413	0.202	+2.04
PdhD(c)				0.203	0.137	+1.48
Lmo1372	Metabolism of lipids	<i>Imo1372</i>	Similar to branched-chain alpha-keto acid dehydrogenase E1 subunit	0.266	0.102	+2.62

Table 2. Continued

Protein spot name	Functional classes	Gene name	Description	% Vol.		Ratio (SP/EP)
				Stationary phase (SP)	Exponential phase (EP)	
Lmo2101(a)	Metabolism of coenzymes and prosthetic groups	<i>lmo2101</i>	Similar to a protein required for pyridoxine synthesis	0.242	0.121	+2.00
Lmo2101(b)				0.171	0.049	+3.47
Lmo2101(c)				0.226	0.104	+2.17
Lmo2101(d)				0.125	0.043	+2.87
Lmo1367	RNA synthesis (regulation)	<i>lmo1367</i>	Similar to arginine repressor	0.014	0.000	N
PyrR	RNA synthesis (regulation)	<i>pyrR</i>	Highly similar to pyrimidine operon regulatory protein	1.919	0.425	+4.51
RpmE	Protein synthesis (ribosomal proteins)	<i>rpmE</i>	50S ribosomal protein L31 type B	0.574	0.140	+4.11
GroES	Protein folding	<i>groES</i>	Class I heat-shock protein (chaperonin) GroES	0.206	0.081	+2.55
Lmo1051	Protein modification	<i>lmo1051</i>	Similar to formylmethionine deformylase and to <i>B. subtilis</i> YkrB protein	0.240	0.120	+2.00
LLO(a)	Miscellaneous	<i>hly</i>	Listeriolysin O precursor	1.096	0.000	N
LLO(b)				2.666	0.815	+3.27
LLO(c)				2.145	0.479	+4.48
LLO(d)				0.161	0.000	N
Fri(d)	Adaptation to atypical conditions	<i>fri</i>	Non-heme iron-binding ferritin	0.169	0.041	+4.15
Fri(b)				0.058	0.000	N
Fri(c)				0.196	0.058	+3.37
Lmo2256(b)	Similar to unknown proteins (from other organisms)	<i>lmo2256</i>	Similar to unknown protein	0.206	0.092	+2.25
RpoZ	Similar to unknown proteins (from other organisms)	<i>rpoZ</i>	Probable DNA-directed RNA polymerase omega chain	2.387	0.590	+4.05
Lmo0775	No similarity	<i>lmo0775</i>		0.586	0.243	+2.41
Underexpressed protein spots in stationary phase						
Pgm	Metabolism of carbohydrates and related molecules (main glycolytic pathways)	<i>pgm</i>	Highly similar to phosphoglycerate mutase	0.077	0.163	−2.11
Lmo1437	Metabolism of amino acids and related molecules	<i>lmo1437</i>	Similar to aspartate-semialdehyde dehydrogenase	0.035	0.102	−2.90
Lmo1496	RNA synthesis (elongation)	<i>lmo1496</i>	Similar to transcription elongation factor GreA	0.292	0.595	−2.04
RpsF	Protein synthesis (ribosomal proteins)	<i>rpsF</i>	30S ribosomal protein S6	0.635	1.266	−2.00
RplJ	Protein synthesis (ribosomal proteins)	<i>rplJ</i>	50S ribosomal protein L10	0.398	0.812	−2.04
Fri(a)	Adaptation to atypical conditions	<i>fri</i>	Non-heme iron-binding ferritin	0.056	0.149	−2.68
Lmo0127	Phage-related functions	<i>lmo0127</i>	Weakly similar to protein gp20 from bacteriophage A118	0.303	1.107	−3.66
Lmo2256(a)	Similar to unknown proteins (from other organisms)	<i>lmo2256</i>	Similar to unknown proteins	0.128	0.264	−2.10

% Vol., volume of the spot divided by the total volume of all the spots in the 2-DE gels (Melanie III software)

N: neosynthesized proteins in stationary phase of growth