Microbial Pathogenesis 102 (2017) 148–159

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Acyl homoserine lactone changes the abundance of proteins and the levels of organic acids associated with stationary phase in *Salmonella* Enteritidis

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ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form
22 November 2016
Accepted 29 November 2016
Available online 1 December 2016

Keywords:
Autoinducer
Differentially abundant proteins
Formic acid
Starvation
Stationary phase

ABSTRACT

Quorum sensing (QS) is cell-cell communication mechanism mediated by signaling molecules known as autoinducers (AIs) that lead to differential gene expression. *Salmonella* is unable to synthesize the AI-1 acyl homoserine lactone (AHL), but is able to recognize AHLs produced by other microorganisms through SdiA protein. Our study aimed to evaluate the influence of AI-1 on the abundance of proteins and the levels of organic acids of *Salmonella* Enteritidis. The presence of *N*-dodecyl-homoserine lactone (C12-HSL) did not interfere on the growth or the total amount of extracted proteins of *Salmonella*. However, the abundance of the proteins PheT, HtpG, PtsI, Adi, TalB, PmgI (or GpmI), Eno, and PykF enhanced while the abundance of the proteins RplB, RplE, RpsB, Tsf, OmpA, OmpC, OmpD, and GapA decreased when *Salmonella* Enteritidis was anaerobically cultivated in the presence of C12-HSL. Additionally, the bacterium produced less succinic, lactic, and acetic acids in the presence of C12-HSL. However, the concentration of extracellular formic acid reached 20.46 mM after 24 h and was not detected when the growth was in the absence of AI-1. Considering the cultivation period for protein extraction, their abundance, process and function, as well as the levels of organic acids, we observed in cells cultivated in presence of C12-HSL a correlation with what is described in the literature as entry into the stationary phase of growth, mainly related to nitrogen and amino acid starvation and acid stress. Further studies are needed in order to determine the specific role of the differentially abundant proteins and extracellular organic acids secreted by *Salmonella* in the presence of quorum sensing signaling molecules.

1. Introduction

Salmonella enterica serovar Enteritidis is the most common serotype responsible for salmonellosis in many countries and, this pathogen is primarily transmitted by food [1–4]. The high virulence of this pathogen is associated with the presence of many pathogenicity islands encoding virulence factors [5–7].

Quorum sensing (QS) is a mechanism of cell-cell communication mediated by signaling molecules known as autoinducers (AIs) that leads to differential gene expression in response to changes in the population density among microbial cells or microbial and host cells [8–11]. In *Salmonella*, this mechanism can be achieved through three types of autoinducers (AIs) called AI-1, AI-2, and AI-3. Complete sets of QS systems composed of signal synthase and signal receptors are present in many bacteria [12].

The QS system mediated by AI-1 is present in Gram-negative

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http://dx.doi.org/10.1016/j.micpath.2016.11.027
0882-4010/

bacteria, but in *Salmonella* it is incomplete. This pathogen is unable of synthesizing the AI-1 called acyl homoserine lactone (AHL) since neither *luxI* gene nor other homologues that codify for the AI-1 synthase are present in the bacterial genome. However, *Salmonella* is able to recognize AHLs synthesized by other microorganisms through SdiA protein, a transcriptional regulator homologous to LuxR which is the signal receptor [13–15]. The AHLs are internalized and bind to the ligand-binding domain (LBD) of SdiA which dimerizes and binds to DNA by using its DNA-binding domain (DBD) regulating expression of target genes [16]. The AI-2 is found in *Salmonella* where it is synthesized by LuxS and internalized by using products of the *lsr* operon [17–19]. In the QS mediated by AI-3 in *Salmonella*, the signal molecules are the hormones epinephrine and norepinephrine, synthesized by animal cells, which are sensed through proteins coded by the *qseBC* operon and *qseE* gene [12,20].

Autoinducers influence gene expression and protein abundance in *Salmonella,* consequently generating different phenotypes, including motility, biofilm formation as well as adhesion, invasion and survival in eukaryotic cells [12,15,21–27]. Campos-Galvão et al. [26] showed enhanced biofilm formation by *Salmonella* Enteritidis PT4 578 growing in the presence of 50 nM of AHLs with six, eight, ten and twelve carbons. However, the effect of *N*-dodecanoyl-DL-homoserine lactone (C12-HSL) on this phenotype was statistically higher than the other AHLs evaluated. These data were reinforced by the recently published study on molecular docking in which AHLs with twelve carbons presented greater affinity to SdiA of *Salmonella* Enteritidis PT4 578 than AHLs with ten, eight, six and four carbons side chains [28]. Liu et al. [25] reported that the presence of AI-1 increased the adhesion of *S. enterica* serovar Typhi to HeLa cells and biofilm formation in polystyrene. Similar results were observed for *Salmonella* Enteritidis in presence of AI-1, where biofilm formation in polystyrene was increased when cells were cultivated in anaerobic conditions [26,27]. Moreover, the *rck* operon of *Salmonella*, which is related to virulence, was more expressed in presence of the AI-1 as previously observed by other groups [14,29]. So far, only two studies evaluated the influence of QS in the abundance of proteins in *Salmonella*. In both cases, the effect of AI-2 on two strains of *S. enterica* serovar Typhimurium was tested, but no studies evaluated the influence of AI-1 on this bacterium [30,31].

Proteomics allows the determination of the global picture of proteins expressed by the genome and gives new insights into the behavior of bacteria during the QS phenomena [32]. Conventional two-dimensional gel electrophoresis (2-DE) in combination with advanced mass spectrometric techniques has facilitated the characterization of thousands of proteins using a single polyacrylamide gel. The 2-DE procedure allows easy visualization of protein isoforms and posttranslational modifications (PTMs) based on protein separation using two physical parameters such as isoelectric point and molecular weight, rendering this technology extremely informative [33].

Thus, considering the scarcity of information about the influence of AI-1 in *Salmonella*, our study aimed to evaluate the effect of this signaling molecule in the abundance of proteins and the levels of organic acids of *Salmonella* Enteritidis. The comparative analysis helps to understand the QS mechanism dependent upon AHL on the physiology of this pathogen.

2. Materials and methods

2.1. Bacterial strain

Salmonella enterica sorovar Enteritidis PT4 578, isolated from chicken meat, was provided by Fundação Oswaldo Cruz (FIOCRUZ, Rio de Janeiro, Brazil) and has been previously described [26,27,34]. Cultures were stored at −20 °C in Luria-Bertani (LB) broth [35] supplemented with 20% (v/v) of sterile glycerol.

2.2. Preparation of inoculum

Tryptone soy broth (TSB; Merck, Germany) was prepared under O_2-free conditions with a CO_2 filling and was dispensed into anaerobic bottles that were sealed with butyl rubber stoppers and then, autoclaved (anaerobic TSB) [36]. Before each experiment, cells were cultivated in anaerobic bottles containing 20 mL of anaerobic TSB for 24 h at 37 °C in a static-model anaerobic chamber (Coy Laboratory, USA) containing a mixture of H_2 (3–5%) and CO_2 (95–97%). Then, 1 mL of culture was transferred into 10 mL of anaerobic TSB and incubated at 37 °C in anaerobic chamber. After incubation for 4 h, exponentially growing cells were harvested by centrifugation at 5000*g* at 4 °C for 10 min (Sorvall, USA), washed with 0.85% saline, and the pellet resuspended in 0.85% saline. The inoculum was standardized to 0.1 of optical density at 600 nm (OD_{600nm}), approximately 10^7 colonies forming units per milliliter (CFU/mL), using a spectrophotometer (Thermo Fisher Scientific, Finland).

2.3. Preparation of HSL solution

N-dodecanoyl-DL-homoserine lactone (C12-HSL; PubChem CID: 11565426; Fluka, Switzerland) was suspended in acetonitrile (PubChem CID: 6342; Merck, Germany) at a concentration of 10 mM and further diluted to a working solution of 10 μM in acetonitrile. Control experiment was performed using acetonitrile. The final concentration of acetonitrile in the media was always less than 1% (v/v) to avoid interference in the growth and response of *Salmonella* to C12-HSL [14].

2.4. Effect of HSL on the growth of Salmonella

To evaluate the effect of C12-HSL on the growth of *Salmonella*, bottles containing 20 mL of anaerobic TSB supplemented with 50 nM of C12-HSL were inoculated with 2 mL of the standardized inoculum. Bottles were incubated at 37 °C for up to 12 h in anaerobic chamber. In established time points, the OD_{600nm} was determined using a spectrophotometer (Thermo Fisher Scientific, Finland).

2.5. Extraction and quantification of proteins of Salmonella

A standardized inoculum was added into anaerobic bottles containing 30 mL of anaerobic TSB supplemented with 50 nM of C12-HSL or the equivalent volume of acetonitrile as control and then, incubated at 37 °C in anaerobic chamber. After 7 h of incubation, the OD_{600nm} and CFU/mL were determined. Concomitantly, an aliquot of the media was centrifuged at 5000*g* at 4 °C for 15 min (Sorvall, USA). The cells in the pellet were resuspended in 1 mL of sterilized distilled water, transferred to 1.5 mL microtubes and once again centrifuged at 9500*g* at 4 °C for 30 min (Brikmann Instruments, Germany). The pellet was resuspended in 50 mM ammonium bicarbonate, 1 mM phenylmethylsulfonyl fluoride (PMSF) added of 1 mL of 2:1 trifluoroethanol:chloroform (TFE:$CHCl_3$), followed by vigorous agitation. Next, the mixture was kept at 0 °C for 1 h in ultrasound bath (100 W MSE 20 KHz), with mixing every 10 min. The material was centrifuged at 6500*g* at 4 °C for 4 min (Brikmann Instruments, Germany) to obtain three phases. The upper phase (composed by proteins soluble in TFE) and the central phase (composed by proteins insoluble in TFE) were collected. The mixture of both phases were dried in SpeedVac (Genevac, England) and resolubilized in 500 μL of a mixture of 5 M urea, 2 M tiourea, 4% (w/v) 3-[(3-cholamidopropyl)

dimethylammonio]-1-propanesulfonate (CHAPS), 40% (v/v) TFE, and 10 mM Tris-HCl (pH 8.8–9.0). Disulfide bonds of the proteins were reduced by 50 mM dithiothreitol (DTT) for 1 h at room temperature, and subsequently, alkylation was carried out by adding 100 mM acrylamide [37–39]. The protein extract was precipitated with 10% (w/v) trichloroacetic acid (TCA) and kept in ice for 30 min and then the material was centrifuged at 9500*g* for 10 min. The supernatant was discarded and the precipitate washed three times with cold acetone. After evaporation of the residual acetone at room temperature, the precipitate was resuspended in 700 μL of a mixture containing 7 M urea, 2 M thiourea, and 2% (w/v) CHAPS. Proteins were quantified using Coomassie blue dye [40] and then, the protein extracts were stored at −20 °C.

2.6. Separation of proteins by two-dimensional gel electrophoresis (2-DE)

2.6.1. Rehydration and sample loading

The protein extracts with 150 μg of proteins were loaded during the re-swelling process in a rehydration apparatus IPG BOX (GE Healthcare, Sweden) at 20 °C for 12 h. For the first dimension, 7 cm gel strips were used with a linear pH gradient ranging from 3 to 10 (Immobiline™ DryStrip; GE Healthcare, Sweden). To rehydrate each strip, 125 μL of a mixture containing the solubilized proteins in 40 mM DTT and 2% (v/v) IPG buffer plus DeStreak solution (GE Healthcare, Sweden) were used.

2.6.2. Isoelectric focusing (IEF)

The IEF was conducted in the equipment IPGphor III (GE Healthcare, Sweden). Electrophoresis conditions were: (i) 300 V for 12 h; (ii) 300 Vh in gradient until 1000 V; (iii) 2000 V in gradient for 1 h; (iv) 2000 Vh in one step of 2000 V; (v) 3000 V in gradient for 1.5 h; (vi) 3000 Vh in one step of 3000 V; (vii) 5000 V in gradient for 1.5 h; (viii) 3000 Vh in one step of 5000 V.

2.6.3. Equilibration of the gel strips

After the IEF, the strips were equilibrated in 10 mL equilibrating buffer 75 mM Tris-HCl pH 8.8, 6 M urea, 29.3% (v/v) glycerol, 2% (w/v) SDS and 0.002% (w/v) bromophenol blue in two 30 min stages in order to reduce and alkylate the proteins. In the first stage, 1% (w/v) DTT was added to the equilibrating buffer. In the second stage, 2.5% (w/v) iodoacetamide was added to the equilibrating buffer. The strips were then briefly incubated in running buffer and submitted to the second dimension of the 2-DE (SDS-PAGE).

2.6.4. Electrophoresis in polyacrylamide gel (SDS-PAGE)

The SDS-PAGE was based on Laemmli [41] in a 12% polyacrylamide gel with a Mini-Protean II Electrophoresis System (Bio-Rad Laboratories, EUA), using the broad range weight marker (6.5–200.0 kDa, Bio-Rad, USA). Separation was performed at 80 V for 30 min and then, at 60 V until the bromophenol blue reached the gel lower limit.

2.6.5. Fixation and staining of the gel

The 2D gels were fixed in solution containing 10% (v/v) acetic acid and 50% (v/v) methanol for 30 min and were stained in a solution containing 8% (w/v) ammonium sulfate, 0.8% (v/v) phosphoric acid, 0.08% (w/v) Coomassie blue G-250, and 30% (v/v) methanol for 72 h. After this step, the gels were washed and maintained in 5% (v/v) acetic acid.

2.6.6. Analysis of the protein spots

The 2D stained gels were then photo-digitalized in an Image Scanner III (GE Healthcare, Sweden) in transparent mode, resolution of 300 dpi, green color filter and with updated calibration. For the comparative analysis of the images, the software ImageMaster 2D Platinum 7.5 (GE Healthcare, Sweden) was used. Image analysis included spot detection, spot measurement, background subtraction and spot matching of three biological replicate gels. Prior to performing spot matching between gel images, one gel image was selected as reference and the used parameters were: contrast equal to - 1, smooth equal to 2, minimum area equal to 20 and saliency equal to 30. The amount of protein of each spot was expressed as the volume of that spot which was defined as the sum of the intensities of all the pixels that make up that spot. To correct the variability and to reflect the quantitative variations of protein spots, the spot volumes were normalized as the percentage (%) of the total volume considering all the spots in the gel. The analysis of variance (ANOVA) for protein spots were followed by the software ImageMaster 2D Platinum 7.5 (GE Healthcare, USA) and the fold change was calculated as the ratio of the % volume of the treatment with C12-HSL by the control. The spots with *p*-value less than 0.05 ($p < 0.05$) and intensities less than 0.8-fold or more than 1.2-fold (fold changed <0.8 or >1.2), as well as when the protein spot was not detected in one of the treatments, which we defined as less than 1.0-fold or more than 1.0-fold (fold changed <1.0 or >1.0), were considered differentially abundant proteins [42].

2.7. Identification of differentially abundant proteins by mass spectrometry

2.7.1. In-gel protein digestion

The protein spots with significant expression changes were excised from stained 2D gels and were submitted to trypsinolysis according to Shevchenko et al. [43] with modifications suggested by Pereira et al. [44]. For the tryptic digestion Sequencing Grade Modified Trypsin, Porcine (Promega, USA) was used. The samples, containing tryptic peptides, were concentrated until about 5 μL in a SpeedVac Concentrator (Thermo Fisher Scientific, Finland) and were added of 5 μL of 0.1% (v/v) trifluoroacetic acid (TFA). Subsequently, samples were desalted in ZipTip® C18 columns (Millipore, USA), concentrated in a SpeedVac Concentrator and resuspended in 5 μL of 0.1% (v/v) TFA.

2.7.2. Mass spectrometry

Mass spectra of the tryptic peptides were performed using a matrix assisted laser desorption/ionization – time of flight/time of flight (MALDI-TOF/TOF) mass spectrometer, model Ultraflex III (Bruker Daltonics, Germany). The samples of tryptic peptides were mixed with α-cyano-4-hydroxycinnamic acid (Bruker Daltonics, Germany) in a proportion of 1:1.

2.7.3. Identification of proteins

The mass spectra obtained were processed using Flex Analysis software (version 3.3; Bruker Daltonics, Germany) and the peaks lists in *.xml* and *.mgf* format were generated by BioTools software (version 3.2; Bruker Daltonics, Germany). These were used for identification of the proteins by the peptide mass fingerprinting (PMF) method and by the peptide fragment fingerprinting (PFF) method using the Mascot software (version 2.4.0; Matrix Science, United Kingdom) against the knowledgebase UniProtKB (http://www.uniprot.org/). For the search, the following parameters were considered: taxonomy *Salmonella* and all entries (separately), monoisotopic mass, trypsin, allow up to one missed cleavage site, peptide tolerance equal to 0.5 Da, MS/MS tolerance equal to 0.5 Da, peptide charge equal to +1, fixed modification for carbamidomethylation of cysteine residues and variable modification for oxidation of methionine residues. The identifications of the proteins from the PFF by Mascot software were validated by Scaffold software (version 3.6.4; Proteome Software, USA). Peptide

identifications were accepted if they could be established at greater than 90% probability as specified by the Peptide Prophet algorithm [45]. Protein identifications were accepted if they could be established at greater than 90% probability and contained at least one identified peptide by the Protein Prophet algorithm [46].

2.8. Gene ontology (GO) analysis and protein-protein interactions (PPI) network

The GO annotations for differentially abundant proteins were acquired by European Bioinformatics Institute (http://www.ebi.ac.uk/QuickGO/). Then, the PPI network was generated for proteins of the *Salmonella* Typhimurium LT2 with the confidence interactions greater than 0.4 using the STRING database version 10.0 (http://string-db.org/ [47]).

2.9. Quantification of extracellular organic acids and ethanol

A standardized inoculum of *Salmonella* was added into anaerobic bottles containing 20 mL of anaerobic TSB supplemented with 50 nM C12-HSL or acetonitrile as control and incubated at 37 °C for up to 36 h in anaerobic chamber. After incubation for 2, 7, 24, and 36 h, 2 mL samples were centrifuged at 12,000*g* for 10 min and the cell-free culture supernatants were treated as described by Siegfried et al. [48]. The extracellular organic acids and ethanol were determined as described by Bento et al. [49]. The high-performance liquid chromatography (HPLC) apparatus coupled to a refractive index (Dionex Corporation, USA) was calibrated with the standard curve of the following organic acids: succinic, lactic, acetic, formic, propionic, valeric, isovaleric, butyric, and isobutyric acids as well as ethanol. All acids were prepared to a final concentration of 10 mM, except isovaleric acid (5 mM) and acetic acid (20 mM). Ethanol was prepared to a final concentration of 150 mM.

2.10. Statistics

Experiments were carried out in three biological replicates. All data were subjected to analysis of variance (ANOVA) followed by Tukey's test using the Statistical Analysis System and Genetics Software® [50] and the software ImageMaster 2D Platinum 7.5 (GE Healthcare, USA) for proteins analysis. A $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Growth of Salmonella in the presence of HSL

The presence of 50 nM of C12-HSL in the medium did not interfere on the *Salmonella* Enteritidis PT4 578 growth during 12 h of incubation (Fig. 1). The concentration of C12-HSL used in this study is much higher than the necessary concentration to induce *sdiA* gene expression in *Salmonella* Typhimurium which, according to Michael et al. [14], can be as low as 1.0 nM. These results corroborate with those found by Campos-Galvão et al. [34] for the same pathogen growing in the presence of different AHLs. Others studies have shown that the growth rate of different serovars of *S. enterica* during the exponential phase decreased in the presence of the cell free supernatants (CFSs) of *Yersinia enterocolitica* and *Serratia proteamaculans* [51] or *Pseudomonas aeruginosa* [52] containing AHLs. However, it is necessary to consider that these CFSs had other unknown metabolites and the inhibitory effect cannot be exclusively attributed to the AHLs.

3.2. HSL changes the abundance of proteins in Salmonella

Changes in the abundance of proteins in *Salmonella* Enteritidis PT4 578 were evaluated in anaerobic TSB supplemented with C12-HSL at 37 °C for 7 h. At this time, *Salmonella* Enteritidis was in early stationary phase of growth and the OD_{600nm} and log CFU/mL in the presence of C12-HSL (0.167 ± 0.008 and 9.1 ± 0.1, respectively) did not differ ($p > 0.05$) to the control treatment (0.165 ± 0.001 and 9.1 ± 0.1, respectively) (Fig. 1). The total proteins extracted from the cells supplemented with C12-HSL (3.98 ± 0.58 μg/μL) did not differ ($p > 0.05$) to the control treatment (4.07 ± 0.21 μg/μL), without this AI-1.

Analysis of the 2-DE gel prepared with protein extracted from early stationary phase growing cells in the presence or absence of C12-HSL showed a total of 184 protein spots, with 39 spots differentially abundant between treatments (Fig. 2). Of these, the abundance of 10 spots was decreased in the presence of C12-HSL ($p < 0.05$ and fold changed < 0.8 as well as < 1.0) whereas, the abundance rate of 29 spots was increased ($p < 0.05$ and fold changed > 1.2 as well as > 1.0) when compared with the control treatment (Table 1). From the 39 differentially abundant protein spots removed from the gels, 21 were identified (53.8%) and validated by Scaffold software (Table 2).

Of the 21 identified proteins, 90.5% (19 proteins) had theoretical and experimental isoeletric points (pI) ranging between 4.0 and 7.0. However, three proteins were identified in more than one spot such as OmpA (spots 41 and 42), Adi (spots 100, 101 and 102), and PykF (spots 171, 173 and 174) (Table 2). Kint et al. [31] also reported the identification of the proteins LuxS and FljB, differentially abundant in wild-type and *luxS* mutant *Salmonella* Typhimurium, in two different spots. These authors observed that the molecular mass (MM) of LuxS was similar in both spots where the protein was identified, but the pI values were different and *a posteriori* analysis revealed that this protein showed posttranslational modifications (PTMs) [31]. PTMs of proteins can change their pI and/or their MM resulting in isoforms that separate in different spots in 2D gels [31,53].

The abundance of the proteins PheT, HtpG, PtsI, Adi, TalB, PmgI (or GpmI), Eno, and PykF enhanced while the abundance of the proteins RplB, RplE, RpsB, Tsf, OmpA, OmpC, OmpD, and GapA decreased when *Salmonella* Enteritidis was cultivated in the

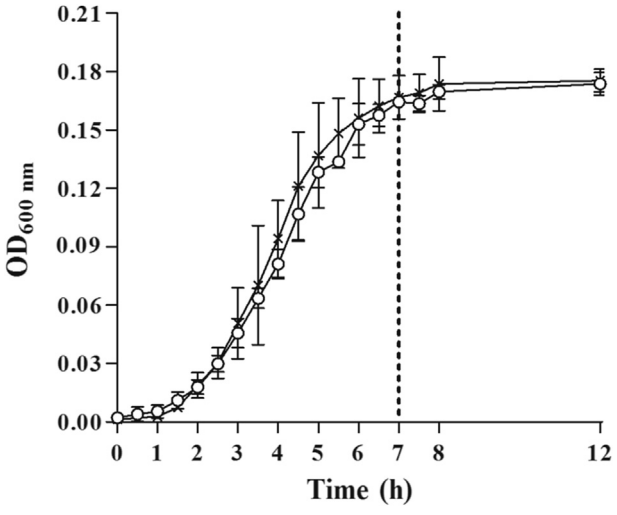

Fig. 1. Growth of *Salmonella* Enteritidis PT4 578 in the presence of C12-HSL. *Salmonella* was anaerobically cultivated in TSB at 37 °C for 12 h in the presence of acetonitrile (open cycle) or 50 nM of C12-HSL (crossed line). The dashed line indicates the time of collection of cells for protein extraction. Error bars indicate standard error.

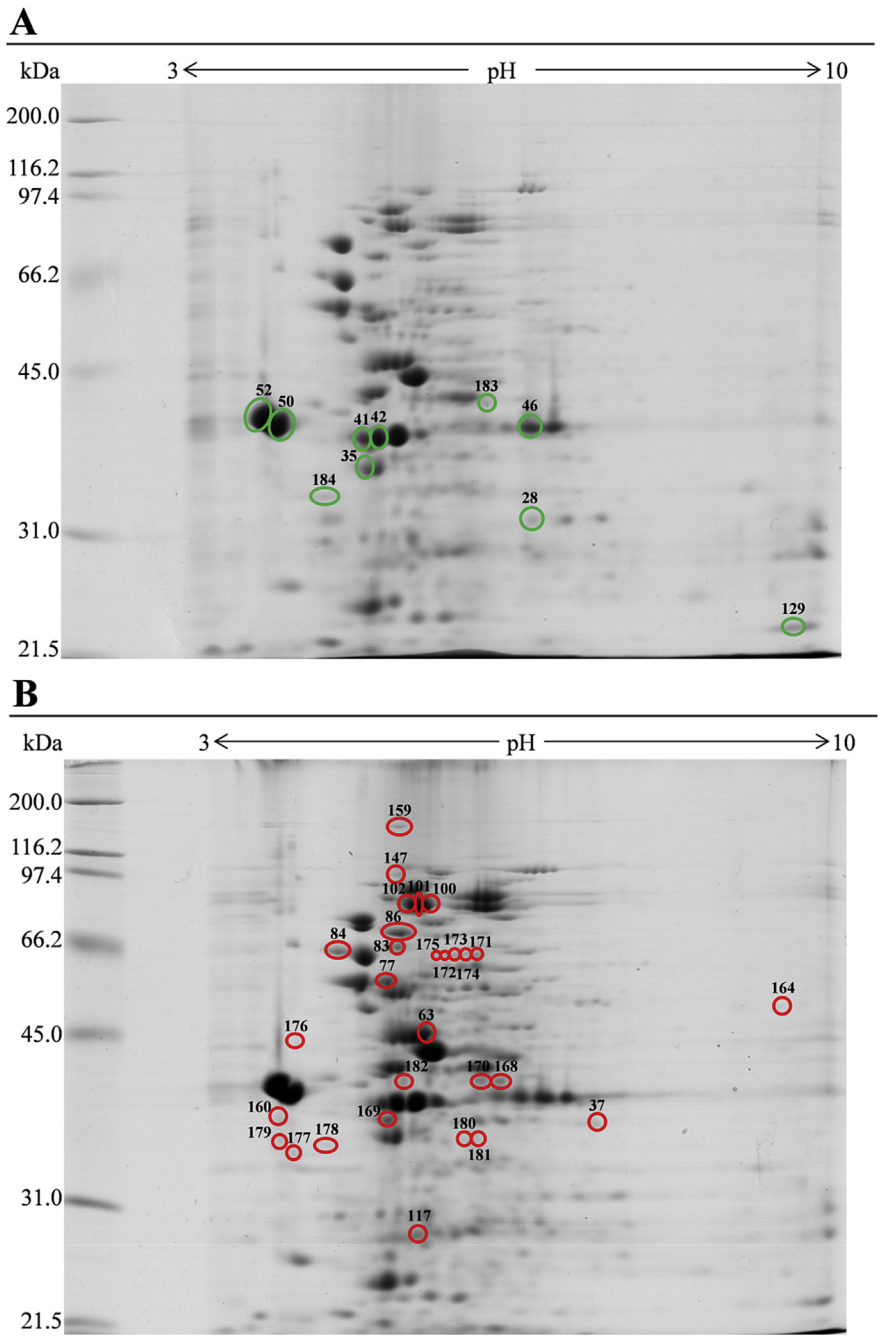

Fig. 2. Representative 2D gels of protein extracts of *Salmonella* Enteritidis PT4 578 anaerobically cultivated in TSB at 37 °C for 7 h in the absence (A) or presence (B) of 50 nM of C12-HSL. Proteins with significantly ($p < 0.05$ and fold changed > 1.2 as well as > 1.0) increased abundance rates following C12-HSL treatment are surrounded by red circle. The green-encircled spots represent proteins whose abundance was significantly ($p < 0.05$ and fold changed < 0.8 as well as < 1.0) decreased. The numbers on the circles are the references of each protein spot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presence of C12-HSL (Fig. 3). We have only found proteomic studies related to the role of AI-2 mediated QS system in *Salmonella*. Soni et al. [30] showed that the LuxS/AI-2-mediated system in *Salmonella* Typhimurium controls the increased abundance of proteins such as Tig, YaeT, PhoP, GpmI, TrxB, and an unidentified protein, while PfkA decreased in wild-type *Salmonella* Typhimurium when compared to *luxS* mutant. On the other hand, when mutant cells were cultivated in the presence of AI-2, the abundance of PfkA, Tig, YaeT, PhoP, TalB, GpmI, TrxB, Pta, YiiM, and an unidentified protein enhanced while the abundance of GroL, OmpF and RpoA descreased [30]. Kint et al. [31] observed a decrease in the abundance of FljB in *luxS* mutant of *Salmonella* Typhimurium compared to wild-type cells.

The proteins identified in the present study were classified into the following four categories, based on their process according to the GO annotations: (i) translation: PheT, RplB, RplE, RpsB, and Tsf, (ii) transport: OmpA, OmpC, OmpD, and PtsI (iii) metabolic process: TalB, GapA, PmgI, Eno, and PykF, and (iv) stress response: HtpG and Adi. We then grouped the proteins based on their functions (Fig. 3).

Thus, considering the cultivation period for extracting the proteins as well as their abundance and function, we observed a correlation with what is described in the literature for cells entering into

Table 1
Differentially abundant protein spots of *Salmonella* Enteritidis PT4 growing in TSB for 7 h in the presence of C12-HSL.

Spot	Experimental	Mean % spot volume ± SE		Fold changed (% spot's volume with C12-HSL/Control)	Total
	pI/MM (kDa)	Control	C12-HSL		
184	4.47/33.882	0.409 ± 0.073[a]	ND[b]	<1.00	10
183	6.27/41.739	0.097 ± 0.016[a]	ND[b]	<1.00	
129	9.52/23.452	0.330 ± 0.079[a]	0.106 ± 0.027[b]	0.32	
28	6.77/32.188	0.312 ± 0.015[a]	0.160 ± 0.037[b]	0.51	
41	4.91/38.583	1.805 ± 0.099[a]	1.061 ± 0.112[b]	0.59	
46	6.73/39.653	2.914 ± 0.118[a]	1.931 ± 0.147[b]	0.66	
35	4.96/35.910	0.965 ± 0.048[a]	0.678 ± 0.097[b]	0.70	
42	5.10/38.583	3.992 ± 0.134[a]	2.909 ± 0.198[b]	0.73	
50	4.03/39.653	7.355 ± 0.407[a]	5.509 ± 0.538[b]	0.75	
52	3.86/40.475	10.110 ± 0.322[a]	7.964 ± 0.138[b]	0.79	
37	7.33/36.905	0.059 ± 0.003[b]	0.079 ± 0.004[a]	1.34	29
63	5.42/46.910	1.378 ± 0.125[b]	1.875 ± 0.112[a]	1.36	
77	4.97/58.092	0.575 ± 0.087[b]	0.843 ± 0.019[a]	1.47	
102	5.21/82.975	0.768 ± 0.069[b]	1.127 ± 0.126[a]	1.47	
101	5.31/82.975	0.881 ± 0.075[b]	1.334 ± 0.113[a]	1.51	
84	4.47/65.808	0.353 ± 0.022[b]	0.555 ± 0.024[a]	1.57	
86	5.09/72.248	0.630 ± 0.061[b]	1.050 ± 0.097[a]	1.67	
100	5.41/82.975	0.910 ± 0.183[b]	1.680 ± 0.246[a]	1.85	
164	9.30/51.893	0.028 ± 0.009[b]	0.055 ± 0.006[a]	1.99	
160	3.84/37.542	0.076 ± 0.003[b]	0.153 ± 0.024[a]	2.01	
147	5.05/95.991	0.047 ± 0.007[b]	0.104 ± 0.018[a]	2.21	
117	5.34/28.944	0.096 ± 0.029[b]	0.216 ± 0.023[a]	2.25	
83	5.07/65.808	0.134 ± 0.013[b]	0.399 ± 0.035[a]	2.98	
159	5.09/150.199	0.045 ± 0.013[b]	0.162 ± 0.016[a]	3.61	
180	5.83/35.437	ND[b]	0.040 ± 0.008[a]	>1.00	
175	5.49/62.593	ND[b]	0.047 ± 0.016[a]	>1.00	
174	5.86/62.204	ND[b]	0.052 ± 0.004[a]	>1.00	
178	4.31/35.100	ND[b]	0.053 ± 0.016[a]	>1.00	
172	5.59/62.204	ND[b]	0.066 ± 0.020[a]	>1.00	
171	5.97/62.593	ND[b]	0.072 ± 0.015[a]	>1.00	
181	5.97/35.550	ND[b]	0.073 ± 0.014[a]	>1.00	
176	3.99/43.728	ND[b]	0.078 ± 0.028[a]	>1.00	
173	5.72/62.204	ND[b]	0.080 ± 0.014[a]	>1.00	
168	6.23/40.381	ND[b]	0.092 ± 0.037[a]	>1.00	
179	3.79/35.437	ND[b]	0.095 ± 0.010[a]	>1.00	
170	6.02/40.381	ND[b]	0.099 ± 0.031[a]	>1.00	
182	5.15/40.253	ND[b]	0.129 ± 0.047[a]	>1.00	
177	3.97/34.326	ND[b]	0.164 ± 0.037[a]	>1.00	
169	4.99/37.053	ND[b]	0.242 ± 0.005[a]	>1.00	
Total differentially abundant proteins					**39**

MM = Molecular Mass; pI = Isoeletric point; Mean = mean of the three biological replicates; SE = Standard Error; ND = not detected.
Average followed by different letters in the same for each spot differ at 5% probability ($p < 0.05$) by Tukey's test.
< 1.00 or >1.00 indicate that the protein was not detected in one of the conditions.

stationary phase, mainly related to nitrogen and amino acid starvation and, acid stress. These data corroborate with those reported for *E. coli* in response to nutrient limitation in the presence of AHL, where the $\sigma^{s/38}$ (RpoS) of RNA polymerase, which is specific in stationary phase, was more abundant [54,55]. In *Burkholderia pseudomallei*, 60 genes have been shown to be controlled by AHL and most of these genes are also co-regulated by RpoS and associated with stationary phase [56]. In addition, in three species of the genus *Burkholderia*, AHLs have been shown to anticipate the responses to the stresses of stationary phase leading to increased cellular survival [57]. Van Delden et al. [58] demonstrated that the expression of both *lasR* and *rhlR* genes and AHL synthesis are prematurely activated during the stringent response induced by overexpression of *relA* in *P. aeruginosa* PAO1, independently of cell density.

Initially, the abundance of PheT (Phenylalanine-tRNA ligase beta subunit or Phenylalanine-tRNA synthetase beta subunit) was enhanced by 2.21 fold in *Salmonella* Enteritidis PT4 growing in the presence of the autoinducer (Fig. 3). This protein is the β subunit of the enzyme $\alpha_2\beta_2$ heterotetrameric and PheS is the α subunit of that enzyme, responsible for charging the $tRNA^{Phe}$ with the L-phenylalanine [59]. PheS is located in the same operon of PheT. However, this last protein was not identified in our gels. In *E. coli*, the synthesis of phenylalanyl-soluble RNA synthetase enhanced twice when cells were cultivated under phenylalanine limitation [60]. Putzer and Laalami [61] reported that *pheST* genes were repressed 2.5 fold under conditions in which the cellular concentration of charged $tRNA^{Phe}$ was decreased. Thus, the arrival of uncharged $tRNA^{Phe}$ in the ribosomes enhances the synthesis of nucleotides guanosine tetraphosphate (ppGpp) and pentaphosphate (pppGpp) by RelA resulting in stringent response [62–66].

The incapacity of charging tRNA caused by nitrogen and amino acid starvation leads to a reduction in ribosomal protein synthesis and rRNA, as well as degradation of rRNA, dimerization, inactivation and ultimately degradation of the ribosomes [67–70]. Thus, four proteins related to translation processes were less abundant in the presence of C12-HSL, namely RplB (50S ribosomal protein L2), RplE (50S ribosomal protein L5), and RpsB (30S ribosomal protein S2) with the function of structural constituent of ribosome, and the Tsf or EF-Ts (Elongation factor Ts) with the function of translational elongation (Fig. 3). Aseev et al. [71] showed that the promoter of the operon *rpsB-tsf* in *E. coli* was negatively regulated by (p)ppGpp during amino acid starvation. Moreover, during the stringent response, the cell blockage of DNA synthesis inhibits stable RNAs and membrane components as well as leads to a rapid production

Table 2
Identification of differentially abundant proteins by *Salmonella* Enteritidis PT4 growing for 7 h in the presence of C12-HSL.

No. Spot	Access number[a]	Identified protein[a]	Protein name[a]	Gene name[a]	Gene locus[a]	Theoretical[b]	Experimental	Peptide mass fingerprinting (PMF)			Peptide fragment fingerprinting (PFF)			
						pI/MM (kDa)		Score	Match	Coverage (%)	Score	Match	Exclusive peptide	Coverage (%)
28	P66541	30S ribosomal protein S2	RpsB	*rpsB*	STM0216	6.61/26.741	6.77/32.188	33	12	42	135	3	2	13
35	P64052	Elongation factor Ts	Tsf	*tsf*	STM0217	5.13/30.338	4.96/35.910	37	9	27	229	4	1	15
41	P02936	Outer membrane protein A	OmpA	*ompA*	STM1070	5.60/37.491	4.91/38.583	47	18	52	205	3	2	9
42	P02936	Outer membrane protein A	OmpA	*ompA*	STM1070	5.60/37.491	5.10/38.583	84	15	47	255	4	2	9
46	P0A1P0	Glyceraldehyde-3-phosphate dehydrogenase	GapA	*gapA*	STM1290	6.33/35.564	6.73/39.653	41	11	38	290	5	2	16
50	P37592	Outer membrane porin protein OmpD	OmpD	*nmpC*	STM1572	4.66/39.671	4.03/39.653	56	10	32	211	5	2	13
52	P0A263	Outer membrane protein C	OmpC	*ompC*	STM2267	4.61/41.311	3.86/40.475	35	8	25	440	4	4	14
63	P64076	Enolase	Eno	*eno*	STM2952	5.25/45.570	5.42/46.910	46	9	26	91	3	1	6
77	Q8ZL56	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	PmgI or GpmI	*pmgI*	STM3704	5.05/56.219	4.97/58.092	71	21	50	171	3	2	5
84	P0A249	Phosphoenolpyruvate-protein phosphotransferase	PtsI or EI	*ptsI*	STM2432	4.75/63.344	4.47/65.808	115	24	40	278	8	2	9
86	P58480	Chaperone protein HtpG	HtpG	*htpG*	STM0487	5.11/71.457	5.09/72.248	65	22	39	450	6	5	13
100	Q8ZKE3	Arginine decarboxylase	Adi	*adi*	STM4296	5.24/84.250	5.41/82.975	85	25	38	348	7	4	10
101	Q8ZKE3	Arginine decarboxylase	Adi	*adi*	STM4296	5.24/84.250	5.31/82.975	90	25	39	262	5	2	7
102	Q8ZKE3	Arginine decarboxylase	Adi	*adi*	STM4296	5.24/84.250	5.21/82.975	80	18	30	334	8	4	9
129	P62405	50S ribosomal protein L5	RplE	*rplE*	STM3428	9.40/20.304	9.52/23.452	102	18	71	392	9	3	38
147	P15434	Phenylalanine-tRNA ligase beta subunit	PheT	*pheT*	STM1338	5.12/87.226	5.05/95.991	28	16	21	92	2	1	3
169	P66955	Transaldolase	TalB	*talB*	STM0007	5.10/35.149	4.99/37.053	62	13	47	151	3	1	7
171	P77983	Pyruvate kinase	PykF	*pykF*	STM1378	5.52/48.622	5.97/62.593	38	11	18	104	3	1	2
173	P77983	Pyruvate kinase	PykF	*pykF*	STM1378	5.52/48.622	5.72/62.204	36	6	11	117	1	1	2
174	P77983	Pyruvate kinase	PykF	*pykF*	STM1378	5.52/48.622	5.86/62.204	41	8	19	100	1	1	2
184	P60428	50S ribosomal protein L2	RplB	*rplB*	STM3437	10.93/29.801	4.47/33.882	56	17	50	212	6	2	21

PI/MM = Isoeletric point/Molecular Mass.
[a] Corresponds to knowledgebase UniProtKB (http://www.uniprot.org/) reference of *Salmonella enterica* serovar Typhimurium LT2 [96].
[b] Theoretical pI/MM values for monoisotopic obtained from ExPASy bioinformatics tool "Compute pI/Mw" (http://web.expasy.org/compute_pi/).

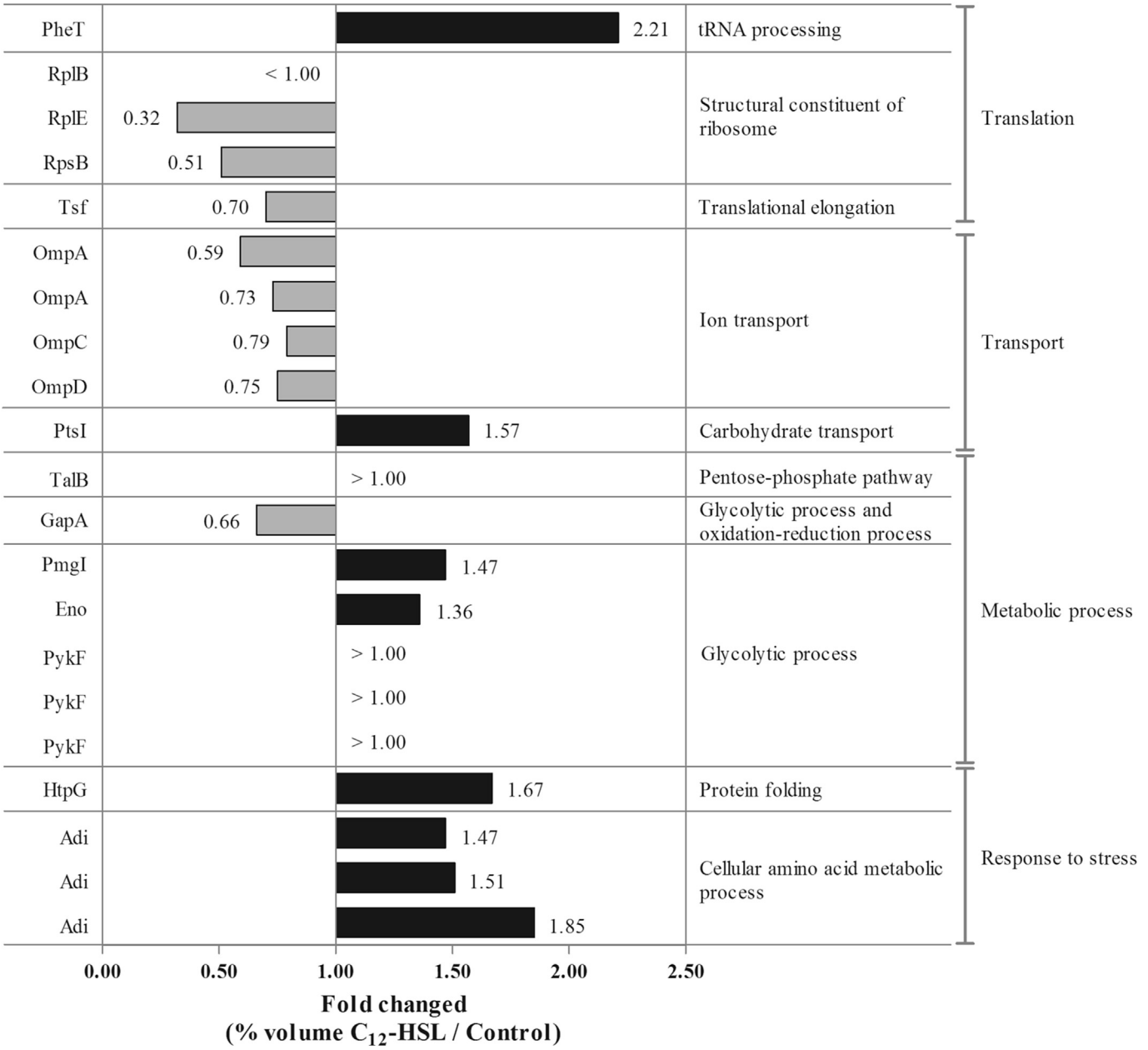

Fig. 3. Differentially abundant proteins identified and grouped as to the process and function according to Gene Ontology (GO) annotations (European Bioinformatics Institut). Proteins with enhanced abundance following C12-HSL treatments ($p < 0.05$ and fold changed > 1.2 as well as > 1.0) are shown in black bars and the proteins which abundance decreased following C12-HSL treatments ($p < 0.05$ and fold changed < 0.8 as well as < 1.0) are shown in grey bars.

of factors that are crucial for stress resistance, amino acid biosynthesis and directs the cellular energy resources [62,63,65,66].

One way for the cell to obtain energy is by increasing glucose uptake by the phosphotransferase system (PTS), where the enzyme I (EI) receives phosphate from phosphoenolpyruvate (PEP) and then, phosphorylates the histidine protein, HPr. The phosphorylation cascade continues with the phosphorylation of different domains of the enzyme II (EII) and then, glucose into glucose-6-phosphate (G6P) [72]. In *Salmonella* Enteritidis PT4, the presence of C12-HSL enhanced in 1.57 fold the abundance of PtsI protein (Phosphoenolpyruvate-protein phosphotransferase) or EI (Phosphotransferase system, enzyme I) (Fig. 3), suggesting an increase in the glucose uptake and therefore directing G6P to many metabolic pathways such as the pentose-phosphate pathway and the glycolytic pathway for amino acid biosynthesis. Nishio et al. [73] showed that the increased expression of *ptsI* gene enhanced the uptake rate and the specific usage of glucose by *E. coli*.

Interestingly, two proteins that have glyceraldehyde-3-phosphate (GAP) as a substrate in common in different metabolic pathways were differentially abundant. TalB (Transaldolase) from the pentose-phosphate pathway was more abundant in *Salmonella* Enteritidis growing in the presence of C12-HSL, while GapA (Glyceraldehyde-3-phosphate dehydrogenase) from the glycolytic pathway and related to the oxidation-reduction process was less abundant (Fig. 3). However, the abundance of the proteins PmgI or GpmI (2,3-bisphosphoglycerate-independent phosphoglycerate mutase), Eno (Enolase), and PykF (Pyruvate kinase) all belonging to the glycolytic pathway, but downstream of GAP, increased in the presence of C12-HSL (Fig. 3).

In the glycolytic pathway, GAP is transformed by GapA into 1,3-

bisphospho-D-glycerate (1,3PG), which in turn is transformed into PEP through the participation of several enzymes including PmgI (or GpmI) and Eno. Then, two pyruvate kinases, PykA and PykF, catalyze the conversion of PEP into pyruvate (PYR) coupled to the synthesis of ATP which is the point of flux control in the glycolytic pathway. Soni et al. [30] showed that GpmI and TalB were more abundant in wild-type *Salmonella* Typhimurium as well as in *luxS* mutant cultivated in the presence of AI-2 when compared with the *luxS* mutant in the absence of AI-2.

The abundance of outer membrane proteins (OMPs), OmpA (Outer membrane protein A), OmpC (Outer membrane protein C), and OmpD (Outer membrane porin protein OmpD) reduced when *Salmonella* Enteritidis was cultivated in the presence of C12-HSL (Fig. 3). The OMPs are involved in the ion transport, adhesion and invasion of macrophages, and are recognized by bacteriophages and by the immune system. Because of that, this group of proteins is widely used in the studies for vaccine elaboration [74–79]. Studies have reported that the level of expression of OMPs is controlled by membrane and environmental stresses such as nutrient limitation and concentration of some antibiotics [77,80–82].

The chaperone HtpG (Chaperone protein HtpG or Heat shock protein HtpG) was 1.67 fold more abundant in the presence of C12-HSL (Fig. 3). This protein is an *hsp*90 homologue more abundant in *Salmonella* Typhimurium in response to the bactericidal/permeability increasing protein (BPI) from human neutrophils [83], and the *htpG* gene was more abundant in *Salmonella* Typhimurium following exposure to antimicrobial peptides present in the tonsils, ileum and/or ileocaecal lymph nodes of pigs [84].

The Adi protein (Arginine decarboxylase) involved in the cellular amino acid metabolic process was 1.85 fold more abundant in *Salmonella* Enteritidis PT4 in medium supplemented with HSL (Fig. 3). This protein is part of the system of resistance to arginine-dependent acid stress (AR3) of *Salmonella* that is activated anaerobically in order to increase intracellular pH, since AdiA or SpeA (Arginine decarboxylase) decarboxylate L-arginine consuming an intracellular proton H^+ forming carbon dioxide (CO_2) and agmatine [85,86]. Van Houdt et al. [87] showed that in the presence of 0.5 mM C6-HSL, the *gadA* promoter (Glutamate decarboxylase A) in *E. coli* was strongly upregulated after 8 and 60 h of growth in LB broth acidified to pH 4.0 (with HCl) at 30 °C. This response is associated with increases in the acid tolerance dependent on *sdiA* [87]. Kieboom and Abee [86] showed that this system is important for the survival of *Salmonella* Typhimurium in mineral medium with pH 2.5 by adding arginine in anaerobiosis, inducing an enhancement in the expression of the gene *adiA*. Furthermore, this gene was positively controlled by $\sigma^{S/38}$ in *Salmonella* Typhimurium leading to synthesis of polyamines [88]. In addition, the polyamines increased the biofilm formation, stress resistance and virulence in *Salmonella* [89–92].

Moreover, the PPI network of these proteins showed a *p*-value of 1e-09 for enrichment (Fig. 4). This small value indicates that the

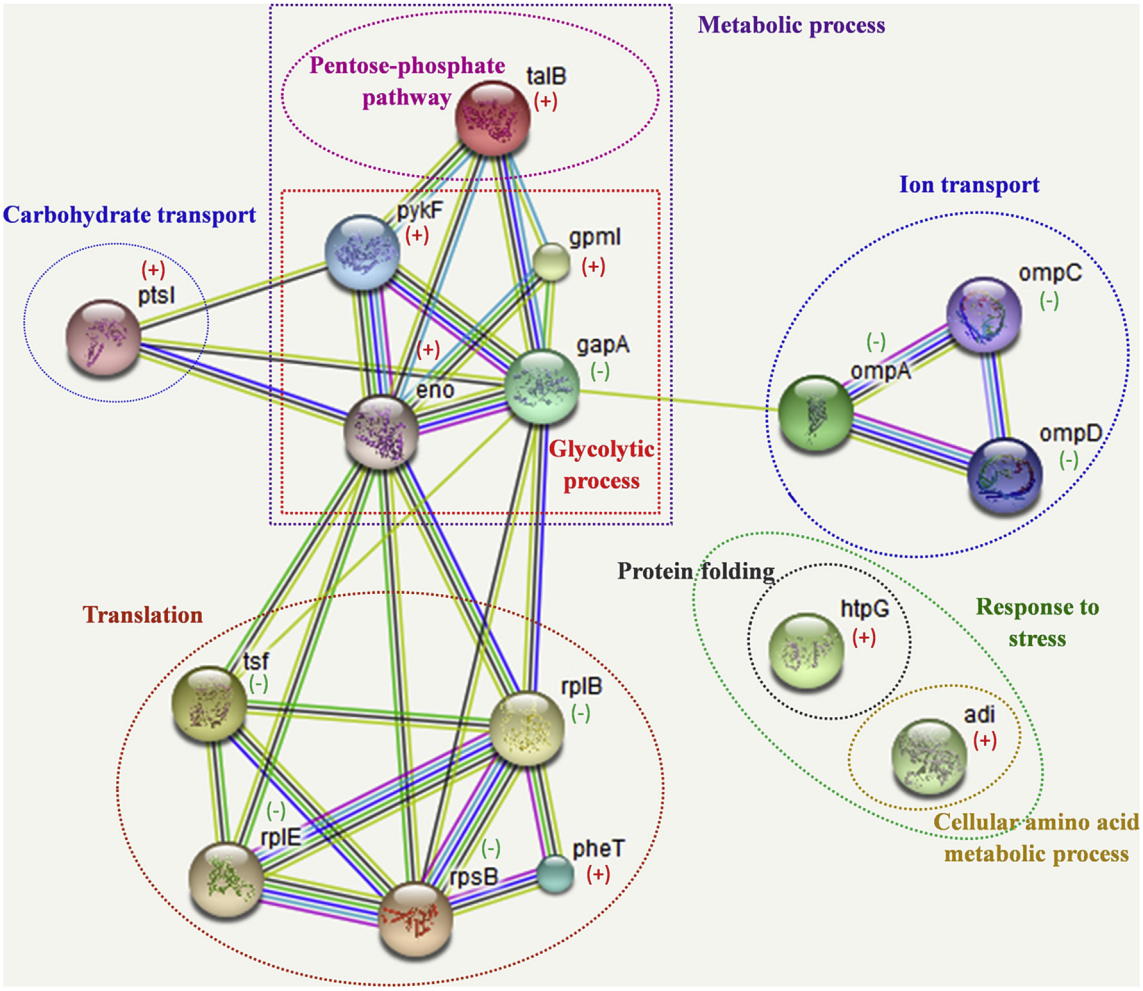

Fig. 4. The PPI network in relation to the differentially abundant proteins of *Salmonella* Enteritidis in the presence of C12-HSL. The boxes with dashed line delimite the proteins of the same process or with the same functions (written with the same colors of the boxes). (+), proteins with significantly ($p < 0.05$ and fold changed > 1.2 as well as > 1.0) increased abundance rates following C12-HSL treatment; (−), proteins whose abundance was significantly ($p < 0.05$ and fold changed < 0.8 as well as < 1.0) decreased.

Table 3
Concentration of extracellular organic acids and ethanol.

Time (h)	Succinic acid (mM)		Lactic acid (mM)		Acetic acid (mM)		Formic acid (mM)		Ethanol (mM)	
	Control	C12-HSL	Control	C12-HSL	Control	C12-HSL	Control	C12-HSL	Control	C12-HSL
2	0.87^{D}	0.93^{C}	0.11^{D}	0.74^{C}	5.03^{D}	4.97^{C}	0.00^{B}	0.00^{C}	15.46^{B}	15.18^{B}
7	8.33^{C}	8.65^{B}	9.57^{C}	10.16^{B}	30.89^{C}	30.97^{B}	60.13^{A}	57.59^{A}	17.02^{A}	16.52^{A}
24	10.61^{B}	10.73^{A}	27.02^{B}	27.74^{A}	34.29^{B}	34.91^{A}	0.00^{bB}	20.46^{aB}	17.26^{A}	16.84^{A}
36	12.22^{aA}	11.05^{bA}	31.84^{aA}	28.44^{bA}	37.13^{aA}	35.24^{bA}	0.00^{B}	0.00^{C}	12.91^{C}	13.09^{C}

The comparisons can be drawn between treatments or throughout time. Average followed by different lower case letters in the same line (between treatments) and followed by different capital letters in the columns (throughout time) differs at 5% probability ($p < 0.05$) by Tukey's test. Where a lower case letter is not shown, no statistical difference between control and C12-HSL was observed.

proteins have more interactions among themselves than what would be expected for a random set of proteins of similar size as well as the proteins are at least partially biologically connected as a group [47].

3.3. HSL changes the levels of organic acids and ethanol of *Salmonella*

Although C12-HSL alters the pattern of proteins expression, especially proteins related to metabolic processes in *Salmonella* Enteritidis PT4 growing at 37 °C for 7 h in anaerobic TSB, the levels of extracellular organic acids and ethanol were not altered in the same conditions and time cultivation (Table 3). Moreover, considering that succinic, lactic, acetic, and formic acids were identified in both conditions, *Salmonella* Enteritidis PT4 may activate the mixed-acid pathway fermentation. The organic acids: propionic, valeric, isovaleric, butyric, and isobutyric acids were not identified in the samples evaluated.

There was a gradual increase in the concentrations of succinic, lactic, and acetic acids along the growth of *Salmonella* (Table 3). However, the concentration of these acids reached a plateau after 24 h incubation in the presence of C12-HSL. In addition, formic acid and ethanol concentrations did not follow a regular pattern throughout time. On the other hand, comparing between the treatments, only at 36 h of incubation a significantly lower concentration of succinic, lactic, and acetic acids were produced in treatment containing AI-1 in relation to the control without this signaling molecule. Different to what was observed for the other extracellular organic acids, the concentration of formic acid had a pick at 7 h incubation and decreased in later time points. However, in the control treatment extracellular formic acid was not detected at 24 h of incubation while for cells treated with C12-HSL, extracellular formic acid reached 20.46 mM ($p < 0.05$).

Thus, the results show that C12-HSL interferes with energy metabolism of *Salmonella* Enteritidis PT4 during its growth and, consequently, in the extracellular levels of organic acids. These acids can also be present in the environment as in the mammalian gastrointestinal tract where they regulate invasion genes [93,94]. In *Salmonella* Typhimurium, formic acid enhances the expression of the regulators *hilA* and *hilD* of the *Salmonella* pathogenicity island 1 (SPI1) that contains genes associated with invasion thereby enhancing invasion of epithelial cells [94]. Furthermore, Barker et al. [95] showed that the organic acids, mainly formic acid, protect *E. coli* and *Salmonella* cells in stationary phase from a potent antimicrobial peptide. It would be interesting to test the infectivity of *Salmonella in vivo* in the presence and absence of C12-HSL.

4. Conclusion

The results show that AI-1 changes the abundance of proteins and the levels of organic acids of *Salmonella* Enteritidis PT4 in anaerobic condition. Thus, considering the cultivation period for extracting the proteins as well as their abundance and function, we observed a correlation with what is described in the literature for cells entering into stationary phase, mainly related to nitrogen and amino acid starvation and, acid stress. In addition, formic acid remains longer in the supernatant of cells growing in the presence of AHL. However, more studies are needed to determine the specific role of the differentially abundant proteins and the extracellular organic acids secreted by *Salmonella* growing in the presence of AHL. It is still not clear what is the advantage for *Salmonella* to control its proteins and organic acids synthesis through quorum sensing by exogenous AI-1. However, it is important to know and understand the effects of AHLs on the physiology of this pathogen in order to find ways to eliminate it and hence reduce the numbers of associated foodborne outbreaks.

Acknowledgements

Felipe Alves de Almeida was supported by a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico and this research was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Financiadora de Estudos e Projetos, Sistema Nacional de Laboratórios em Nanotecnologias (SisNANO) and Ministério da Ciência, Tecnologia e Informação (MCTI). The authors thank the Núcleo de Análise de Biomoléculas (Nubiomol) of Universidade Federal de Viçosa for the equipments and softwares for protein analysis.

References

[1] S.E. Majowicz, J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien, et al., The global burden of nontyphoidal *Salmonella* gastroenteritis, Clin. Infect. Dis. 50 (2010) 882–889, http://dx.doi.org/10.1086/650733.
[2] M.M. Nunes, A.L.A.A. Mota, E.D. Caldas, Investigation of food and water microbiological conditions and foodborne disease outbreaks in the Federal District, Brazil, Food Control. 34 (2013) 235–240, http://dx.doi.org/10.1016/j.foodcont.2013.04.034.
[3] M. Chironna, S. Tafuri, M.S. Gallone, A. Sallustio, D. Martinelli, R. Prato, et al., Outbreak of *Salmonella infantis* gastroenteritis among people who had eaten at a hash house in southern Italy, Public Health 128 (2014) 438–443, http://dx.doi.org/10.1016/j.puhe.2014.02.002.
[4] R. Freeman, G. Dabrera, C. Lane, N. Adams, L. Browning, T. Fowler, et al., Association between use of proton pump inhibitors and non-typhoidal salmonellosis identified following investigation into an outbreak of *Salmonella* Mikawasima in the UK, 2013, Epidemiol. Infect. 144 (2015) 968–975, http://dx.doi.org/10.1017/S0950268815002332.
[5] M. Hensel, Evolution of pathogenicity islands of *Salmonella enterica*, Int. J. Med. Microbiol. 294 (2004) 95–102, http://dx.doi.org/10.1016/j.ijmm.2004.06.025.
[6] S.Y. Ong, F.L. Ng, S.S. Badai, A. Yuryev, M. Alam, Analysis and construction of pathogenicity island regulatory pathways in *Salmonella enterica* serovar Typhi, J. Integr. Bioinform 7 (2010), http://dx.doi.org/10.2390/biecoll-jib-2010-145.
[7] M.R. Hayward, V.A.A. Jansen, M.J. Woodward, Comparative genomics of *Salmonella enterica* serovars Derby and Mbandaka, two prevalent serovars associated with different livestock species in the UK, BMC Genomics 14 (2013) 365, http://dx.doi.org/10.1186/1471-2164-14-365.
[8] W.C. Fuqua, S.C. Winans, E.P. Greenberg, Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators, J. Bacteriol. 176 (1994) 269–275.
[9] C. Fuqua, M.R. Parsek, E.P. Greenberg, Regulation of gene expression by cell-

to-cell communication: acyl-homoserine lactone quorum sensing, Annu. Rev. Genet. 35 (2001) 439–468, http://dx.doi.org/10.1146/annurev.genet.35.102401.090913.
[10] C. Fuqua, S.C. Winans, E.P. Greenberg, Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators, Annu. Rev. Microbiol. 50 (1996) 727–751, http://dx.doi.org/10.1146/annurev.micro.50.1.727.
[11] L. Keller, M.G. Surette, Communication in bacteria: an ecological and evolutionary perspective, Nat. Rev. Microbiol. 4 (2006) 249–258, http://dx.doi.org/10.1038/nrmicro1383.
[12] M. Walters, V. Sperandio, Quorum sensing in *Escherichia coli* and *Salmonella*, Int. J. Med. Microbiol. 296 (2006) 125–131, http://dx.doi.org/10.1016/j.ijmm.2006.01.041.
[13] B.M. Ahmer, J. Van Reeuwijk, C.D. Timmers, P.J. Valentine, F. Heffron, *Salmonella* Typhimurium encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid, J. Bacteriol. 180 (1998) 1185–1193.
[14] B. Michael, J.N. Smith, S. Swift, F. Heffron, B.M.M. Ahmer, SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities, J. Bacteriol. 183 (2001) 5733–5742, http://dx.doi.org/10.1128/JB.183.19.5733-5742.2001.
[15] J.N. Smith, B.M.M. Ahmer, Detection of other microbial species by *Salmonella*: expression of the SdiA regulon, J. Bacteriol. 185 (2003) 1357–1366, http://dx.doi.org/10.1128/JB.185.4.1357-1366.2003.
[16] Y. Nguyen, N.X. Nguyen, J.L. Rogers, J. Liao, J.B. Macmillan, Y. Jiang, et al., Structural and mechanistic roles of novel chemical ligands on the SdiA quorum-sensing transcription regulator, MBio 6 (2015) e02429, http://dx.doi.org/10.1128/mBio.02429-14.
[17] M.E. Taga, J.L. Semmelhack, B.L. Bassler, The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella* Typhimurium, Mol. Microbiol. 42 (2001) 777–793.
[18] M.E. Taga, B.L. Bassler, Chemical communication among bacteria, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 14549–14554, http://dx.doi.org/10.1073/pnas.1934514100.
[19] K.B. Xavier, B.L. Bassler, Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*, J. Bacteriol. 187 (2005) 238–248, http://dx.doi.org/10.1128/JB.187.1.238-248.2005.
[20] D.T. Hughes, V. Sperandio, Inter-kingdom signalling: communication between bacteria and their hosts, Nat. Rev. Microbiol. 6 (2008) 111–120, http://dx.doi.org/10.1038/nrmicro1836.
[21] M.B. Miller, B.L. Bassler, Quorum sensing in bacteria, Annu. Rev. Microbiol. 55 (2001) 165–199, http://dx.doi.org/10.1146/annurev.micro.55.1.165.
[22] A.M. Prouty, W.H. Schwesinger, J.S. Gunn, Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp, Infect. Immun. 70 (2002) 2640–2649, http://dx.doi.org/10.1128/IAI.70.5.2640-2649.2002.
[23] J.N. Smith, J.L. Dyszel, J.A. Soares, C.D. Ellermeier, C. Altier, S.D. Lawhon, et al., SdiA, an *N*-acylhomoserine lactone receptor, becomes active during the transit of *Salmonella enterica* through the gastrointestinal tract of turtles, PLoS One 3 (2008) e2826, http://dx.doi.org/10.1371/journal.pone.0002826.
[24] P.R. Jesudhasan, M.L. Cepeda, K. Widmer, S.E. Dowd, K.A. Soni, M.E. Hume, et al., Transcriptome analysis of genes controlled by *luxS*/autoinducer-2 in *Salmonella enterica* serovar Typhimurium, Foodborne Pathog. Dis. 7 (2010) 399–410, http://dx.doi.org/10.1089/fpd.2009.0372.
[25] Z. Liu, F. Que, L. Liao, M. Zhou, L. You, Q. Zhao, et al., Study on the promotion of bacterial biofilm formation by a *Salmonella* conjugative plasmid and the underlying mechanism, PLoS One 9 (2014) e109808, http://dx.doi.org/10.1371/journal.pone.0109808.
[26] M.E.M. Campos-Galvão, A.O.B. Ribon, E.F. Araújo, M.C.D. Vanetti, Changes in the *Salmonella enterica* Enteritidis phenotypes in presence of acyl homoserine lactone quorum sensing signals, J. Basic Microbiol. 56 (2015) 493–501, http://dx.doi.org/10.1002/jobm.201500471.
[27] F.A. Almeida, N.J. Pimentel-Filho, U.M. Pinto, H.C. Mantovani, L.L. Oliveira, M.C.D. Vanetti, Acyl homoserine lactone-based quorum sensing stimulates biofilm formation by *Salmonella* Enteritidis in anaerobic conditions (in press), Arch. Microbiol. (2016), http://dx.doi.org/10.1007/s00203-016-1313-6.
[28] F.A. Almeida, U.M. Pinto, M.C.D. Vanetti, Novel insights from molecular docking of SdiA from *Salmonella* Enteritidis and *Escherichia coli* with quorum sensing and quorum quenching molecules, Microb. Pathog. 99 (2016) 178–190, http://dx.doi.org/10.1016/j.micpath.2016.08.024.
[29] N. Abed, O. Grépinet, S. Canepa, G. a Hurtado-Escobar, N. Guichard, A. Wiedemann, et al., Direct regulation of the *pefI-srgC* operon encoding the Rck invasin by the quorum-sensing regulator SdiA in *Salmonella* Typhimurium, Mol. Microbiol. 94 (2014) 254–271, http://dx.doi.org/10.1111/mmi.12738.
[30] K.A. Soni, P.R. Jesudhasan, M. Cepeda, B. Williams, M. Hume, W.K. Russell, et al., Autoinducer AI-2 is involved in regulating a variety of cellular processes in *Salmonella* Typhimurium, Foodborne Pathog. Dis. 5 (2008) 147–153, http://dx.doi.org/10.1089/fpd.2007.0050.
[31] G. Kint, K.A. Sonck, G. Schoofs, D. De Coster, J. Vanderleyden, S.C. De Keersmaecker, 2D proteome analysis initiates new insights on the *Salmonella* Typhimurium LuxS protein, BMC Microbiol. 9 (2009) 198, http://dx.doi.org/10.1186/1471-2180-9-198.
[32] R. Di Cagno, M. De Angelis, M. Calasso, M. Gobbetti, Proteomics of the bacterial cross-talk by quorum sensing, J. Proteomics 74 (2011) 19–34, http://dx.doi.org/10.1016/j.jprot.2010.09.003.
[33] F. Chevalier, Highlights on the capacities of "Gel-based" proteomics, Proteome Sci. 8 (2010) 23, http://dx.doi.org/10.1186/1477-5956-8-23.
[34] M.E.M. Campos-Galvão, T.D.S. Leite, A.O.B. Ribon, E.F. Araújo, M.C.D. Vanetti, A new repertoire of informations about the quorum sensing system in *Salmonella enterica* serovar Enteritidis PT4, Genet. Mol. Res. 14 (2015) 4068–4084, http://dx.doi.org/10.4238/2015.April.27.22.
[35] J.H. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1972.
[36] J.R. Lima, A.O.B. Ribon, J.B. Russell, H.C. Mantovani, Bovicin HC5 inhibits wasteful amino acid degradation by mixed ruminal bacteria *in vitro*, FEMS Microbiol. Lett. 292 (2009) 78–84, http://dx.doi.org/10.1111/j.1574-6968.2008.01474.x.
[37] J.M.P. Deshusses, J.A. Burgess, A. Scherl, Y. Wenger, N. Walter, V. Converset, et al., Exploitation of specific properties of trifluoroethanol for extraction and separation of membrane proteins, Proteomics 3 (2003) 1418–1424, http://dx.doi.org/10.1002/pmic.200300492.
[38] R.O. Ebanks, A. Dacanay, M. Goguen, D.M. Pinto, N.W. Ross, Differential proteomic analysis of *Aeromonas salmonicida* outer membrane proteins in response to low iron and *in vivo* growth conditions, Proteomics 4 (2004) 1074–1085, http://dx.doi.org/10.1002/pmic.200300664.
[39] B.L. Nunn, S.A. Shaffer, A. Scherl, B. Gallis, M. Wu, S.I. Miller, et al., Comparison of a *Salmonella* Typhimurium proteome defined by shotgun proteomics directly on an LTQ-FT and by proteome pre-fractionation on an LCQ-DUO, Genomics Proteom. 5 (2006) 154–168, http://dx.doi.org/10.1093/bfgp/ell024.
[40] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254, http://dx.doi.org/10.1016/0003-2697(76)90527-3.
[41] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685, http://dx.doi.org/10.1038/227680a0.
[42] Y. Ye, H. Li, N. Ling, Y. Han, Q. Wu, X. Xu, et al., Identification of potential virulence factors of *Cronobacter sakazakii* isolates by comparative proteomic analysis, Int. J. Food Microbiol. 217 (2016) 182–188, http://dx.doi.org/10.1016/j.ijfoodmicro.2015.08.025.
[43] A. Shevchenko, H. Tomas, J. Havlis, J.V. Olsen, M. Mann, In-gel digestion for mass spectrometric characterization of proteins and proteomes, Nat. Protoc. 1 (2006) 2856–2860, http://dx.doi.org/10.1038/nprot.2006.468.
[44] M.P. Pereira, B.C. Gouvêa, F.C. Marcelino-Guimarães, H.J.O. Ramos, M.A. Moreira, E.G. Barros, Proteomic analysis of soybean leaves in a compatible and an incompatible interaction with *Phakopsora pachyrhizi*, Organelles Proteom. 1 (2013) 16–27, http://dx.doi.org/10.2478/orpr-2013-0004.
[45] A. Keller, A.I. Nesvizhskii, E. Kolker, R. Aebersold, Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search, Anal. Chem. 74 (2002) 5383–5392, http://dx.doi.org/10.1021/ac025747h.
[46] A.I. Nesvizhskii, A. Keller, E. Kolker, R. Aebersold, A statistical model for identifying proteins by tandem mass spectrometry, Anal. Chem. 75 (2003) 4646–4658, http://dx.doi.org/10.1021/ac0341261.
[47] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, et al., STRING v10: protein-protein interaction networks, integrated over the tree of life, Nucleic Acids Res. 43 (2015) D447–D452, http://dx.doi.org/10.1093/nar/gku1003.
[48] R. Siegfried, H. Ruckemann, G. Stumpf, Method for the determination of organic acids in silage by high performance liquid chromatography, Landwirt Forsch 37 (1984) 298–304.
[49] C.B.P. Bento, A.C. Azevedo, E. Detmann, H.C. Mantovani, Biochemical and genetic diversity of carbohydrate-fermenting and obligate amino acid-fermenting hyper-ammonia-producing bacteria from Nellore steers fed tropical forages and supplemented with casein, BMC Microbiol. 15 (2015) 28, http://dx.doi.org/10.1186/s12866-015-0369-9.
[50] D.F. Ferreira, SISVAR: a computer statistical analysis system, Cienc. e Agrotecnologia 35 (2011) 1039–1042.
[51] D. Dourou, M.S. Ammor, P.N. Skandamis, G.J.E. Nychas, Growth of *Salmonella* Enteritidis and *Salmonella* Typhimurium in the presence of quorum sensing signalling compounds produced by spoilage and pathogenic bacteria, Food Microbiol. 28 (2011) 1011–1018, http://dx.doi.org/10.1016/j.fm.2011.02.004.
[52] H.-H. Wang, K.-P. Ye, Q.-Q. Zhang, Y. Dong, X.-L. Xu, G.-H. Zhou, Biofilm formation of meat-borne *Salmonella enterica* and inhibition by the cell-free supernatant from *Pseudomonas aeruginosa*, Food Control. 32 (2013) 650–658, http://dx.doi.org/10.1016/j.foodcont.2013.01.047.
[53] B.D. Halligan, V. Ruotti, W. Jin, S. Laffoon, S.N. Twigger, E.A. Dratz, ProMoST (Protein Modification Screening Tool): a web-based tool for mapping protein modifications on two-dimensional gels, Nucleic Acids Res. 32 (2004) 638–644, http://dx.doi.org/10.1093/nar/gkh356.
[54] G.W. Huisman, R. Kolter, Sensing starvation: a homoserine lactone-dependent signaling pathway in *Escherichia coli*, Science 265 (1994) 537–539, http://dx.doi.org/10.1126/science.7545940.
[55] R. Hengge-Aronis, Signal transduction and regulatory mechanisms involved in control of the σ^S (RpoS) subunit of RNA polymerase, Microbiol. Mol. Biol. Rev. 66 (2002) 373–395, http://dx.doi.org/10.1128/MMBR.66.3.373-395.2002.
[56] P. Wongtrakoongate, S. Tumapa, S. Tungpradabkul, Regulation of a quorum sensing system by stationary phase sigma factor RpoS and their co-regulation of target genes in *Burkholderia pseudomallei*, Microbiol. Immunol. 56 (2012) 281–294, http://dx.doi.org/10.1111/j.1348-0421.2012.00447.x.
[57] E. Goo, C.D. Majerczyk, J.H. An, J.R. Chandler, Y.-S. Seo, H. Ham, et al., Bacterial

quorum sensing, cooperativity, and anticipation of stationary-phase stress, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 19775–19780, http://dx.doi.org/10.1073/pnas.1218092109.
[58] C. Van Delden, R. Comte, A.M. Bally, Stringent response activates quorum sensing and modulates cell density-dependent gene expression in *Pseudomonas aeruginosa*, J. Bacteriol. 183 (2001) 5376–5384, http://dx.doi.org/10.1128/JB.183.18.5376-5384.2001.
[59] M.M. Champion, C.S. Campbell, D.A. Siegele, D.H. Russell, J.C. Hu, Proteome analysis of *Escherichia coli* K-12 by two-dimensional native-state chromatography and MALDI-MS, Mol. Microbiol. 47 (2003) 383–396, http://dx.doi.org/10.1046/j.1365-2958.2003.03294.x.
[60] G. Nass, F.C. Neidhardt, Regulation of formation of aminoacyl-ribonucelic acid synthetases in *Escherichia coli*, Biochim. Biophys. Acta 134 (1967) 347–359.
[61] H. Putzer, S. Laalami, Regulation of the expression of aminoacyl-tRNA synthetases and translation factors, Transl. Mech. 107 (2003) 388–415.
[62] K. Potrykus, M. Cashel, ppGpp: still magical? Annu. Rev. Microbiol. 62 (2008) 35–51, http://dx.doi.org/10.1146/annurev.micro.62.081307.162903.
[63] Z.D. Dalebroux, M.S. Swanson, ppGpp: magic beyond RNA polymerase, Nat. Rev. Microbiol. 10 (2012) 203–212, http://dx.doi.org/10.1038/nrmicro2720.
[64] V. Shyp, S. Tankov, A. Ermakov, P. Kudrin, B.P. English, M. Ehrenberg, et al., Positive allosteric feedback regulation of the stringent response enzyme RelA by its product, EMBO Rep. 13 (2012) 835–839, http://dx.doi.org/10.1038/embor.2012.106.
[65] D.R. Brown, G. Barton, Z. Pan, M. Buck, S. Wigneshweraraj, Nitrogen stress response and stringent response are coupled in *Escherichia coli*, Nat. Commun. 5 (2014) 4115, http://dx.doi.org/10.1038/ncomms5115.
[66] D.R. Brown, G. Barton, Z. Pan, M. Buck, S. Wigneshweraraj, Combinatorial stress responses: direct coupling of two major stress responses in *Escherichia coli*, Microb. Cell. 1 (2014) 315–317, http://dx.doi.org/10.15698/mic2014.09.168.
[67] A. Wada, Growth phase coupled modulation of *Escherichia coli* ribosomes, Genes Cells 3 (1998) 203–208, http://dx.doi.org/10.1046/j.1365-2443.1998.00187.x.
[68] M.P. Deutscher, Degradation of stable RNA in bacteria, J. Biol. Chem. 278 (2003) 45041–45044, http://dx.doi.org/10.1074/jbc.R300031200.
[69] B.J. Paul, W. Ross, T. Gaal, R.L. Gourse, rRNA transcription in *Escherichia coli*, Annu. Rev. Genet. 38 (2004) 749–770, http://dx.doi.org/10.1146/annurev.genet.38.072902.091347.
[70] M.P. Deutscher, Degradation of RNA in bacteria: comparison of mRNA and stable RNA, Nucleic Acids Res. 34 (2006) 659–666, http://dx.doi.org/10.1093/nar/gkj472.
[71] L.V. Aseev, L.S. Koledinskaya, I.V. Boni, Dissecting the extended "-10" *Escherichia coli rpsB* promoter activity and regulation *in vivo*, Biochem. Biokhim. 79 (2014) 776–784, http://dx.doi.org/10.1134/S0006297914080057.
[72] P.W. Postma, J.W. Lengeler, G.R. Jacobson, Phosphoenolpyruvate: carbohydrate phosphotransferase systems of bacteria, Microbiol. Rev. 57 (1993) 543–594.
[73] Y. Nishio, Y. Usuda, K. Matsui, H. Kurata, Computer-aided rational design of the phosphotransferase system for enhanced glucose uptake in *Escherichia coli*, Mol. Syst. Biol. 4 (2008) 160, http://dx.doi.org/10.1038/msb4100201.
[74] R.S. Negm, T.G. Pistole, Macrophages recognize and adhere to an OmpD-like protein of *Salmonella* Typhimurium, FEMS Immunol, Med. Microbiol. 20 (1998) 191–199.
[75] H. Nikaido, Molecular basis of bacterial outer membrane permeability revisited, Microbiol. Mol. Biol. Rev. 67 (2003) 593–656.
[76] B. Hara-Kaonga, T.G. Pistole, OmpD but not OmpC is involved in adherence of *Salmonella enterica* serovar Typhimurium to human cells, Can. J. Microbiol. 50 (2004) 719–727, http://dx.doi.org/10.1139/w04-056.
[77] S.G.J. Smith, V. Mahon, M.A. Lambert, R.P. Fagan, A molecular swiss army knife: OmpA structure, function and expression, FEMS Microbiol. Lett. 272 (2007) 1–11, http://dx.doi.org/10.1111/j.1574-6968.2007.00778.x.
[78] S.B. Berger, X. Romero, C. Ma, G. Wang, W.A. Faubion, G. Liao, et al., SLAM is a microbial sensor that regulates bacterial phagosome functions in macrophages, Nat. Immunol. 11 (2010) 920–927, http://dx.doi.org/10.1038/ni.1931.
[79] J.S. Lee, I.D. Jung, C.-M. Lee, J.W. Park, S.H. Chun, S.K. Jeong, et al., Outer membrane protein a of *Salmonella enterica* serovar Typhimurium activates dendritic cells and enhances Th1 polarization, BMC Microbiol. 10 (2010) 263, http://dx.doi.org/10.1186/1471-2180-10-263.
[80] L.P. Randall, M.J. Woodward, The multiple antibiotic resistance (*mar*) locus and its significance, Res. Vet. Sci. 72 (2002) 87–93, http://dx.doi.org/10.1053/rvsc.2001.0537.
[81] T. Ferenci, Maintaining a healthy SPANC balance through regulatory and mutational adaptation, Mol. Microbiol. 57 (2005) 1–8, http://dx.doi.org/10.1111/j.1365-2958.2005.04649.x.
[82] M. Castillo-Keller, P. Vuong, R. Misra, Novel mechanism of *Escherichia coli* porin regulation, J. Bacteriol. 188 (2006) 576–586, http://dx.doi.org/10.1128/JB.188.2.576-586.2006.
[83] S.-Y. Qi, Y. Li, A. Szyroki, I.G. Giles, A. Moir, C.D. O'Connor, *Salmonella* Typhimurium responses to a bactericidal protein from human neutrophils, Mol. Microbiol. 17 (1995) 523–531, http://dx.doi.org/10.1111/j.1365-2958.1995.mmi_17030523.x.
[84] A. Van Parys, F. Boyen, B. Leyman, E. Verbrugghe, F. Haesebrouck, F. Pasmans, Tissue-specific *Salmonella* Typhimurium gene expression during persistence in pigs, PLoS One 6 (2011) e24120, http://dx.doi.org/10.1371/journal.pone.0024120.
[85] Y. Nakada, Y. Jiang, T. Nishijyo, Y. Itoh, C.D. Lu, Molecular characterization and regulation of the *aguBA* operon, responsible for agmatine utilization in *Pseudomonas aeruginosa* PAO1, J. Bacteriol. 183 (2001) 6517–6524, http://dx.doi.org/10.1128/JB.183.22.6517-6524.2001.
[86] J. Kieboom, T. Abee, Arginine-dependent acid resistance in *Salmonella enterica* serovar Typhimurium, J. Bacteriol. 188 (2006) 5650–5653, http://dx.doi.org/10.1128/JB.00323-06.
[87] R. Van Houdt, A. Aertsen, P. Moons, K. Vanoirbeek, C.W. Michiels, *N*-acyl-L-homoserine lactone signal interception by *Escherichia coli*, FEMS Microbiol. Lett. 256 (2006) 83–89, http://dx.doi.org/10.1111/j.1574-6968.2006.00103.x.
[88] C. Lévi-Meyrueis, V. Monteil, O. Sismeiro, M.A. Dillies, M. Monot, B. Jagla, et al., Expanding the RpoS/σ^S-network by RNA sequencing and identification of σ^S-controlled small RNAs in *Salmonella*, PLoS One 9 (2014) e96918, http://dx.doi.org/10.1371/journal.pone.0096918.
[89] L. Jelsbak, L.E. Thomsen, I. Wallrodt, P.R. Jensen, J.E. Olsen, Polyamines are required for virulence in *Salmonella enterica* serovar Typhimurium, PLoS One 7 (2012) 1–10, http://dx.doi.org/10.1371/journal.pone.0036149.
[90] C. Schroll, J.P. Christensen, H. Christensen, S.E. Pors, L. Thorndahl, P.R. Jensen, et al., Polyamines are essential for virulence in *Salmonella enterica* serovar Gallinarum despite evolutionary decay of polyamine biosynthesis genes, Vet. Microbiol. 170 (2014) 144–150, http://dx.doi.org/10.1016/j.vetmic.2014.01.034.
[91] L.L. Nesse, K. Berg, L.K. Vestby, Effects of norspermidine and spermidine on biofilm formation by potentially pathogenic *Escherichia coli* and *Salmonella enterica* wild-type strains, Appl. Environ. Microbiol. 81 (2015) 2226–2232, http://dx.doi.org/10.1128/AEM.03518-14.
[92] I.C. Espinel, P.R. Guerra, L. Jelsbak, Multiple roles of putrescine and spermidine in stress resistance and virulence of *Salmonella enterica* serovar Typhimurium, Microb. Pathog. 95 (2016) 117–123, http://dx.doi.org/10.1016/j.micpath.2016.03.008.
[93] S.D. Lawhon, R. Maurer, M. Suyemoto, C. Altier, Intestinal short-chain fatty acids alter *Salmonella* Typhimurium invasion gene expression and virulence through BarA/SirA, Mol. Microbiol. 46 (2002) 1451–1464, http://dx.doi.org/10.1046/j.1365-2958.2002.03268.x.
[94] Y. Huang, M. Suyemoto, C.D. Garner, K.M. Cicconi, C. Altier, Formate acts as a diffusible signal to induce *Salmonella* invasion, J. Bacteriol. 190 (2008) 4233–4241, http://dx.doi.org/10.1128/JB.00205-08.
[95] H.C. Barker, N. Kinsella, A. Jaspe, T. Friedrich, C.D. O'Connor, Formate protects stationary-phase *Escherichia coli* and *Salmonella* cells from killing by a cationic antimicrobial peptide, Mol. Microbiol. 35 (2000) 1518–1529, http://dx.doi.org/10.1046/j.1365-2958.2000.01820.x.
[96] M. McClelland, K.E. Sanderson, J. Spieth, S.W. Clifton, P. Latreille, L. Courtney, et al., Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2, Nature 413 (2001) 852–856, http://dx.doi.org/10.1038/35101614.