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11 1 Growth and Transcriptional Response of *Salmonella* Typhimurium LT2 to Glucose-
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13 2 Lysine-Based Maillard Reaction Products Generated Under Low Water Activity
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15 3 Conditions
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18 5 Vesela I. Chalova ^{1†}, Oswaldo Hernández-Hernández ², Arunachalam Muthaiyan ¹,
19
20 6 Sujata A. Sirsat¹, Senthil Natesan ³, María Luz Sanz ², F. Javier Moreno ⁴, Corliss A.
21
22 7 O'Bryan¹, Philip G. Crandall¹, Steven C. Ricke ^{1*}
23
24 8
25
26 9 ¹Center for Food Safety and Food Science Department, University of Arkansas,
27
28 10 Fayetteville, AR 72704
29
30 11 ²Instituto de Química Orgánica General (CSIC), Madrid, Spain
31
32 12 ³Albany College of Pharmacy and Health Sciences-Vermont Campus, Colchester, VT
33
34 13 ⁴Instituto de Fermentaciones Industriales (CSIC), Madrid, Spain
35
36 14 [†]Current address: Department of Biochemistry and Molecular Biology, University of
37
38 15 Food Technologies, Plovdiv, Bulgaria
39
40 16 *Corresponding author
41 17 Steven C. Ricke, Ph.D.
42 18 Department of Food Science
43 19 University of Arkansas
44 20 Fayetteville, AR 72704
45 21 Phone:479-575-4678
46 22 Fax: 479-575-6936
47 23 Email: sricke@uark.edu
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Abstract

This study reports the growth and transcriptional responses of *Salmonella* Typhimurium LT2 to Maillard reaction products (MRPs) generated in a simulation of reaction during food processing and storage. Maillard reactions between *N*- α -acetyl-lysine and glucose were stopped at 4, 23, or 143 h to generate sub-samples with prevailing Amadori compound, advanced glycation end products (AGEs), or melanoidins, respectively. When used as the only carbon source, MRPs disappearance occurred during *S.* Typhimurium secondary logarithmic growth phase after the glucose available in the MRP reaction mixtures was exhausted. Of the three MRPs sub-samples, the Amadori compound was the preferred carbon source (98% disappearance). Decreases in AGEs (37%) and melanoidins (15%) also occurred. Transcription profiles of cells grown on the MRPs revealed predominant up-regulation of genes associated with the functional groups of energy metabolism, fatty and phospholipid metabolism, cellular process, and regulatory functions, and general down-regulation of the genes in the groups of amino acid biosynthesis, protein synthesis and transcription, transport and binding proteins, and DNA metabolism.

1.0 Introduction

Maillard reaction products (MRPs) in foods and food components are formed due to a non-enzymatic reaction and are favored by elevated heat during food processing (Hurrell & Finot, 1983), although the reaction also occurs at low temperature during storage (Mastrocola & Munari, 2000). The reaction involves carbonyl substrates and the free amino groups of amino acids, peptides and proteins to form a variety of compounds (Ashoor & Zent, 1984). The first stage of this reaction involves the condensation of reducing sugars with amino groups, followed by the Amadori rearrangement and decomposition of ketose containing compounds to advanced glycation end products (AGEs), which further react to form an insoluble pigment, melanoidin (Friedman, 1999; Henle, 2005)

Salmonella is a foodborne enteropathogen causing an estimated 600 deaths and 1.4 million illnesses in the US annually (Mead et al., 1999). It is metabolically diverse and capable of assimilating a wide variety of carbon compounds and surviving under sub-optimal growth conditions (Ingraham & Marr, 1996; Lin, 1996). Regardless of the ubiquity of the Maillard reaction in nature and food processing (Wu & Monnier, 2003), the potential of MRPs to serve as a nutrient source for *Salmonella* has not been investigated. Most bacterial studies involving MRPs utilize model systems generated in liquid solutions (Kundinger, Zabala-Diaz, Chalova, & Ricke, 2008; Li & Ricke, 2002). To more closely simulate production during food processing, MRPs were produced for the current study at a lower water activity (0.44). The objective of this study was to determine the growth and transcriptional responses of *Salmonella* Typhimurium LT2 to

glucose-lysine-based MRPs, and identify the specific genes potentially associated with their metabolism.

2.0 Materials and methods

2.1 Generation and analytical characterization of glucose-lysine MRPs

Equimolar solutions of glucose (Sigma, St. Louis, MO) and *N*- α -acetyl-Lysine (Sigma, St. Louis, MO) in 0.1M sodium phosphate buffer, pH 7 (Merck, Darmstadt, Germany), were lyophilized and kept under vacuum for various periods of time in a dessicator at 40 °C; a water activity of 0.44 was achieved with a saturated K₂CO₃ solution. The samples were stored at -20 °C after incubation until analysis. Furosine (2-furoylmethyllysine) analysis was performed after acid hydrolysis as described in Moreno, López-Fandiño & Olano (2002). Filtered hydrolysate was applied to a previously activated Sep-Pak C₁₈ cartridge (Millipore Corp., Bedford, MA). Furosine was eluted with 3 mL of 3 N HCl, 50 μ L was used for injection, and analyses conducted via ion-pair RP-HPLC using a C₈ Alltech furosine-dedicated column (250 mm $\times$ 4.6 mm i.d.; Alltech, Nicholasville, KY) and a variable wavelength detector at 280 nm. Operating conditions were: column temperature, 35 °C; flow rate, 1.2 mL min⁻¹; solvent A, 0.4% HPLC-grade acetic acid in double-distilled water; solvent B, 0.3% KCl in solvent A. The elution followed the gradient as described by Resmini, Pellegrino & Batelli (1999), calibrated with using known concentrations of a commercial pure furosine standard from 0.52 to 5.2 mg L⁻¹. Amadori compound concentrations were calculated from furosine data using a constant factor of 2.2 as described by Krause, Knoll & Henle (2003).

Fluorescence, as an indicator of AGEs compounds production, was measured using a fluorometer (Beckman DU 70 spectrophotometer, Beckman Instruments, Inc.,

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11 94 Fullerton, CA) with $\lambda_{\text{ex}} = 340 \text{ nm}$ and $\lambda_{\text{em}} = 420 \text{ nm}$, with results expressed in
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13 95 fluorescence intensity (FI) corrected by dilution factors. Browning intensity of MRPs was
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15 96 determined by measuring the absorbance at 420 nm at room temperature, as an index
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17 97 of the melanoidins formed in more advanced stages of the Maillard reaction, and
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19 98 expressed in absorbance units corrected by dilution factors. Controls consisted of the
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21 99 incubation of glucose and N-acetyl-lysine alone under the same conditions as the
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23 100 mixtures, and no development of color was detected. Available *N*- α -acetyl-lysine was
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25 101 determined by combining Maillard reaction mixtures with 1 mL of ortho-phthalaldehyde
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27 102 (OPA) reagent, vortexing, and holding in dark at room temperature for 5 minutes
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29 103 (Church, Swaisgood, Poretr & Catignani, 1983). Fluorescence readings at $\lambda_{\text{ex}}=360 \text{ nm}$
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31 104 and $\lambda_{\text{em}}= 460 \text{ nm}$ were converted into free amino contents using a calibration curve
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33 105 obtained with *N*- α -acetyl-lysine (10 to 250 μM) as a standard.

34 106 Free glucose was determined colorimetrically with anthrone-sulfuric acid
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36 107 (Laurentin & Edwards, 2003) in 96-well microtitration plates (Microtest Plate 96-Well,
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38 108 Sarstedt, Inc., Newton, NC). Briefly, 40 μL of water (blank), standard (0.05-1.0 g L^{-1}
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40 109 glucose), or MRPs was added to individual wells and maintained in an ice bath at 4°C
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42 110 throughout the reaction. The standard glucose solutions were prepared in 0.1M sodium
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44 111 phosphate buffer, pH 7. An aliquot of 0.1 mL anthrone solution (2 g L^{-1} anthrone
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46 112 solution in concentrated sulfuric acid), freshly prepared, was added to each well. The
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48 113 plate was vortex-mixed gently but thoroughly, and incubated at 92 °C in a nonshaking
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50 114 water bath. After 3 min, the plate was transferred to a nonshaking water bath at room
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52 115 temperature for 5 min to stop the reaction and then placed into a 45 °C oven for 15 min
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11 116 to dry. Absorbance at 630 nm was read using a PowerWave XS microplate
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13 117 spectrophotometer (Bio-Tek, Winooski, VT). All analyses were carried out in duplicate.
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15 118 **2.2 Growth of *S. Typhimurium* LT2 with glucose-lysine MRPs**
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17 119 *S. Typhimurium* LT2 ATCC 19585 was purchased from the American Type
18 120 Culture Collection (Manassas, VA) and used throughout the experiments. Cells were
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20 121 grown overnight in 5 ml of Luria-Bertani broth (LB; BD Diagnostic Systems, Franklin
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22 122 Lakes, NJ) at 37 °C in a shaking water bath (Lab-Line, Melrose Park, IL), then collected
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24 123 by centrifugation (Eppendorf, Hamburg, Germany), washed three times with decreasing
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26 124 aliquots (5, 3, and 2 mL) of saline solution (0.85% NaCl), and used to inoculate
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28 125 previously described defined medium (Ames, 1964; Broach, Neumann & Kustu, 1976).
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30 126 The defined medium consisted of the following salts mixture: 1 g of K₂SO₄; 17.7 g of
31 127 K₂HPO₄ · 3H₂O; 4.7 g of KH₂PO₄; 0.1g of MgSO₄ · 7H₂O; and 2.5 g of NaCl per liter.
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33 128 Defined medium was supplemented with either glucose-lysine MRPs or a combination
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35 129 of 0.4% glucose and glucose-lysine MRPs. MRPs were added in increasing
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37 130 concentrations from 0.078 mg mL⁻¹ to 5 mg mL⁻¹, with the experiments performed in
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39 131 transparent round 96-well microtiter plates (VWR, West Chester, PA) at 37°C. Cell
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41 132 growth was evaluated by changes in the optical density (OD) of the bacterial cultures at
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43 133 λ_{600nm} by a microtiter plate reader (Infinite M200, Tecan US, Inc., Durham, NC) for 12 h.
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45 134 Growth in defined medium containing glucose was used as a control.

46 135 **2.3 RNA preparation**

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48 136 For genomic experiments, overnight grown and washed *S. Typhimurium* LT2
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50 137 cells (10⁵ CFU mL⁻¹) were used to inoculate 5 ml defined medium in glass culture tubes
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52 138 (16x100) containing only glu-lys1 (0.6 mg mL⁻¹), glu-lys2 (0.6 mg mL⁻¹), or glu-lys3 (3

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11 139 mg mL⁻¹) mixtures as carbon sources, with medium containing glucose as the control.
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13 140 The cells were grown at 37 °C in a shaking water bath (Lab-Line, Melrose Park, IL).
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15 141 After the second hour, the ODs ($\lambda_{600\text{ nm}}$) of the bacterial cultures were monitored in 30-
16 142 min intervals with a spectrophotometer (Milton Roy Spectronic 20D, Rockford, IL). Cells
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18 143 were collected by centrifugation (10 min at 3,000 rpm) in the mid-logarithmic phase
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20 144 (Heptinstall, 2000), and treated with RNA protect Bacteria Reagent (Qiagen Inc.,
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22 145 Valencia, CA) and were either stored at -80 °C or immediately used to extract RNA.
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24 146 Cells were broken by using FastPrep system (Qbiogene, Irvine, CA) and RNA was
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26 147 extracted and purified with RNeasy (Qiagen Inc.) minikit and RNase-Free DNase Set
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28 148 (Qiagen Inc.) respectively by following the manufacturer's procedure. RNA was
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30 149 quantified by using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA)
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32 150 and stored at -80 °C for later use. Supernatants were collected and filter-sterilized
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34 151 (cellulose acetate, 0.45 μm) and subjected to analyses for determination of Amadori
35 152 compounds, AGEs, and melanoidins. Tubes containing the same concentrations of the
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37 153 MRPs but without *Salmonella* cells incubated under the same conditions, were used as
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39 154 controls.

40 41 155 **2.4 Microarray hybridization and processing**

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43 156 Microarray hybridization was performed as described by Muthaiyan , Silverman,
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45 157 Jayaswal & Wilkinson (2008). Briefly, cDNA was generated via reverse transcription
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47 158 with random hexamers (Invitrogen, Carlsbad, CA) as primers. The primers were
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49 159 annealed to total RNA (2.5 μg) at 70°C for 10 min. The extension was performed with
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51 160 SuperScript II reverse transcriptase (Invitrogen) and the respective deoxynucleotides in
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53 161 the presence of 5-(3 aminoallyl)-dUTP (Amersham Pharmacia Biotech, Piscataway,
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11 162 NJ). The labelled cDNA was purified (Qiagen PCR purification kit) and hybridized with
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13 163 S. Typhimurium/Typhi version 5 genome microarrays obtained from Pathogen
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15 164 Functional Genomics Resource Center (National Institute of Allergy and Infectious
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17 165 Diseases). The full description of the microarray is available at
18 166 http://pfgrc.jcvi.org/index.php/microarray/array_description/salmonella_typhimurium/vers
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20 167 ion5.html. Hybridization signals were scanned by using Axon4000B scanner (Molecular
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22 168 Devices, Sunnyvale, CA). The resulting TIFF images were subsequently analyzed with
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24 169 Spotfinder software. The data set was normalized ($\log_2$ ration of test to control signal)
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26 170 by applying the LOWESS algorithm of MIDAS. Both Spotfinder and MIDAS analysis
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28 171 software applications were obtained from The Institute of Genomic Research (TIGR)
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30 172 TM4 microarray analysis software suite (Saeed et al., 2003).

31 173 **2.5 Quantitative real time polymerase chain reaction**

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33 174 The expression of six genes total per glucose-lysine fraction was evaluated by
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35 175 the qRT-PCR technique for validation purposes. The genes and specific primer pairs
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37 176 generated by the SciTools of Integrated DNA Technologies (www.idtdna.com) are listed
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39 177 in Table 1. The expression of the genes in test samples was estimated as relative to
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41 178 their respective expression in control samples by using QuantiTect SYBR Green RT-
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43 179 PCR kit (Qiagen Inc.) and Relative Quantification analysis module of Mastercycler EP
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45 180 Realplex 4 (Eppendorf) by following manufacturers' instructions. The gene expression
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47 181 was normalized against 16S RNA (*rsmC*, Kunding et al., 2007). The built in
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49 182 calculation instrument function based on $\Delta\Delta C_t$ method (Livak & Schmittgen, 2001) was
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51 183 used to calculate the relative expression (fold) of gene targets. To prevent false positive
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53 184 results, controls omitting either an RNA template or reverse transcriptase were included
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11 185 in the experiments. The microarray data were deposited in NCBI's Gene Expression
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13 186 Omnibus (Edgar, Domrachev & Lash, 2002) and are accessible through GEO Series
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15 187 accession number GSE18950
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17 188 <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE18950>.

18 189 **2.6 Statistical analysis**

20 190 All growth and qRT-PCR experiments were performed at least twice with two
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22 191 wells or tubes per replication and [data isare](#) presented as average means $\pm$ standard
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24 192 deviations where appropriate. Microarray data are average values of three independent
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26 193 experiments. To minimize technical and experimental errors, each RNA preparation was
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28 194 used to produce probes for at least two separate arrays for which the incorporated dye
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30 195 was reversed. In addition, each open reading frame (ORF) was present in triplicate on a
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32 196 single microarray array slide. Significant changes in gene expression in responses to
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34 197 glu-lys1, glu-lys2, or glu-lys3 MRP mixtures as compared to the control cells grown in
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36 198 glucose were identified with Significance Analysis of Microarrays (SAM; Tusher,
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38 199 Tibshirani & Chu, 2008) software using one class mode. A score was assigned to each
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40 200 gene on the basis of a change in gene expression relative to the standard deviation of
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42 201 repeated measurements. For genes with scores greater than an adjustable threshold,
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44 202 SAM used permutations of the repeated measurements to estimate the percentage of
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46 203 genes identified by chance, the false discovery rate (FDR). For each gene, the program
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48 204 calculated a q -value which is a positive FDR analogue of a p -value and a practical
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50 205 approach that eliminated the need to set the error rate beforehand as is traditionally
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52 206 done (Chu, Narasimhan, Tibshirani & Tusher, 2002). Expression alternations were
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54 207 considered significant at $q < 0.0001$. The genes with significant changes in expression

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($q < 0.0001$) were further classified according to the functional categories described in the comprehensive microbial resource of TIGR (<http://cmr.tigr.org/tigr-scripts/CMR/shared/Genomes.cgi>) by using in-house Gene Sorter software. Genes with at least 2-fold gene induction or repression were presented with negative values assumed to indicate repression.

3.0 Results and discussion

3.1 Generation and analytical characterization of glucose-lysine MRPs

Considering that the ϵ -amino group of lysine represents the primary target for an attack by carbohydrates in the case of protein-containing foods (Henle, 2005), a simple model containing *N*- α -acetyl-lysine rather than lysine was selected in order to understand the effect of MRPs on growth and transcriptional response of *S. Typhimurium* but remaining as close as possible to protein glycation conditions. The use of lysine would give rise to the additional formation of MRPs derived from the α -amino group which are not formed following protein glycation, as this primary amino-group is involved in the formation of peptide bonds. Cessation of Maillard reactions between glucose and *N*- α -acetyl-lysine at time 4 h, 23 h, and 143 h resulted in generation of three sub-samples, glu-lys1, glu-lys2, and glu-lys3, which differed in chemical composition as described by Friedman (1996). A gradual decrease in free *N*- α -acetyl-lysine and glucose was observed related to incubation time, indicating the progress of the Maillard reaction (Table 2). Given that the determination of glucose by the anthrone-sulfuric acid method could be subjected to interferences such as furfuraldehyde derivatives (Laurentin & Edwards, 2003), some selected samples were also analyzed by GC-MS using a two-step derivatization procedure (Sanz et al., 2004) (data not shown).

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Glucose levels obtained by both methods were consistent and, consequently, the anthrone-sulfuric acid method was employed to measure the glucose content in all samples as it is a much less laborious and time-consuming method.

Glu-lys1 samples consisted primarily of Amadori compounds, glu-lys2 samples were rich in AGEs and Amadori compounds, while melanoidins were prominent in the glu-lys3 fractions (Table 2). The moderate levels of AGEs detected in glu-lys1 could be attributed to the fact that the degradation of the Amadori compounds derived from glucose can be relatively accelerated at pH 7 (Blank et al., 1998) and/or the alternative pathways for formation of AGEs from glucose via dicarbonyl sugars (Degenhardt et al., 1998).

3.2 Growth responses of *S. Typhimurium* LT2 to glucose-lysine MRPs

The growth rate and maximal OD ~~responses~~ of *Salmonella* cells were unchanged by the addition of up to 5 mg mL⁻¹ reaction mixtures when compared to the control containing no glucose-lysine-based MRPs (Fig.1). Stecchini, Giavedoni, Sarais & Lericci (1991) also observed no differences in growth patterns of *S. Typhimurium* II 505 and *S. Enteritidis* with glucose-glycine-based MRPs. All three Maillard reaction mixtures used in our study supported growth of *Salmonella* cells as the only carbon sources (Fig 2A, 2B and 2C). After disappearance of available glucose in the reaction mixtures occurred (glu-lys1 and glu-lys2) with a cell growth rate of 0.479 h⁻¹, MRPs disappearance was observed during secondary logarithmic growth with reduced growth rates of 0.063 h⁻¹ and 0.072 h⁻¹ respectively (Fig. 2a and 2b 3A and 3B). The glu-lys3 sub-sample containing the highest amount of melanoidins also supported *S. Typhimurium* LT2

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11 254 growth (0.368 h^{-1} , Fig 2C). The glucose concentration (0.08 mg mL^{-1}) in the highest
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13 255 level of glu-lys3 mixture (5 mg mL^{-1}) was not sufficient to support apparent logarithmic
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15 256 growth of the bacterial cells (data not shown). *N*- α -acetyl-lysine failed to serve as a
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17 257 carbon source for *S. Typhimurium* LT2 (Fig. 2A, B and C).

18 258 Analysis of supernatants after the incubation of *S. Typhimurium* LT2 with glu-
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20 259 lys1, glu-lys2, and glu-lys3 revealed a high level of Amadori compound disappearance
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22 260 (98%, 98% and 87% respectively) compared to controls containing no bacterial cells
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24 261 (Table 3). Although with lower incremental decreases in AGEs (37.5% and 12.2%) and
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26 262 melanoidins (15.5% and 22.8%) in glu-lys2 and glu-lys3, concomitant increases of cell
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28 263 growth compared to the respective controls were observed. While this is the first report
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30 264 on potential fructosyllysine (Amadori product) utilization by *Salmonella*, previous reports
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32 265 indicated the existence and functionality of deglycating enzymes with bacterial or fungal
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34 266 origin (Monnier, 2005). *Escherichia coli* can also use fructosyllysine to sustain growth
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36 267 and as an energy substrate via a pathway involving the degradation of the product to
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38 268 glucose 6-phosphate and lysine (Wiame, Lamosa, Santos & van Schaftingen, 2005).

39 269 The disappearance of AGEs and melanoidins with concomitant *Salmonella*
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41 270 growth was more unexpected, as these compounds form as the Maillard reaction
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43 271 progresses and are characterized by more complex structure and molecular weights
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45 272 than Amadori compounds. This, together with the current analytical limitations for
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47 273 analysis of these compounds, may explain the limited reports on their susceptibility to
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49 274 breakdown by prokaryotes or eukaryotes. However, metabolism of melanoidins derived
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51 275 from an aqueous glucose-lysine model system by fecal microorganisms including
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53 276 bacteroides, clostridia, bifidobacteria, and lactobacilli has been demonstrated by both

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11 277 plate culture and fluorescent *in situ* hybridization technology (Ames, Wynne, Hofmann,
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13 278 Plos & Gibson, 1999).

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15 279 **3.3 Transcriptional responses of *S. Typhimurium* LT2 to glucose-lysine MRPs**

16 280 The results obtained from microarray assays demonstrated that the
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18 281 ~~T~~ranscriptional profiles of *Salmonella* cells grown on glucose-lysine derived MRPs
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20 282 significantly differed from glucose grown cells, with the most pronounced gene
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22 283 differential expression observed in energy metabolism (Table 4). This agreed with a
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24 284 previous report of differential expression of genes involved mainly in metabolism and
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26 285 less in replication, transcription, and translation in *E. coli* grown on a poor carbon source
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28 286 (Oh, Rohlin, Kao & Liao, 2002). The gene expression analysis in our study
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30 287 demonstrated specific activation of the glyoxylate pathway for carbon utilization in
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32 288 *Salmonella* which is designed to prevent carbon dissipation throughout the TCA cycle
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34 289 under carbon-limiting conditions. A data subset of All genes with an absolute value of
35 290 expression higher than 2 and $q < 0.0001$ are listed is presented in Table 5. aA
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37 291 Supplementary Table with a data sub-set presented in Table 5 containing all genes with
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39 292 an absolute value of expression higher than 2 and $q < 0.0001$ has been submitted. The
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41 293 glyoxylate shunt is considered essential for growth on poor carbon substrates such as
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43 294 acetate and fatty acids and is dysfunctional in the presence of preferred carbon sources
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45 295 including glucose or pyruvate (Cronan & LaPorte, 1996). Although the actual inducers of
46 296 the glyoxylate pathway in our study are not known, the high expression of the genes
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48 297 from the *aceBAK* operon implies that the major utilization of the glucose-lysine MRPs
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50 298 carbon occurred via this specific bypass. Although expressed to a lesser extent, the
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52 299 induction of the genes in the TCA cycle up to 10 fold suggested that it continued to be

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11 300 an important pathway for *Salmonella* carbon utilization and energy generation when
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13 301 grown on glucose-lysine MRPs. Assimilation of glucose-lysine MRPs via the glyoxylate
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15 302 pathway was also supported by the high induction of *acs* (acetyl-CoA synthetase) of up
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17 303 to 50-fold in *glu-lys3*. In addition to the acceleration of acetate assimilation and biomass
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19 304 increases (Kumari et al., 2000; Lin, Castro, Bennett & San, 2006), it appears that *acs*
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21 305 may act as a scavenger of extracellular acetate to maintain the intracellular
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23 306 concentration of acetyl-CoA (Rahman, Hasan, & Shimizu, 2008) which may explain the
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25 307 concomitant induction of the gene with *aceBAK* operon. Although the glyoxylate
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27 308 pathway has been considered only for acetate or fatty acids (Cronan & LaPorte, 1996),
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29 309 a more recent study indicated that during growth on mixed, low energy carbon
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31 310 substrates, the shunt may confer more metabolic flexibility to the cells (Ihssen & Egli,
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31 311 2005).

33 312 The adaptation of *Salmonella* cells to growth on glucose-lysine MRPs revealed a
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35 313 distinctive transcription pattern of various uptake systems. According to Franchini & Egli
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37 314 (2006), the growth of *E. coli* under glucose-limiting conditions causes a general
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39 315 induction of ABC transporters due to increase of cAMP cell concentrations. In our study,
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41 316 however, it appears that the induction of the transporters was substrate rather than
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43 317 transport specific. For example, the expression of *rbsB*, *rbsC*, and *livJ*, *livF* was highly
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45 318 up-regulated, while the uptake systems for glutamine, arginine, or sulfate, which also
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47 319 belong to ABC type transporters, were down-regulated. Interestingly, both high-affinity
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49 320 specific permease (*lysP*) and the biosynthetic pathway for lysine were repressed
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51 321 implying that this amino acid is of less importance for *Salmonella* cells growing under
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52 322 carbon-limiting conditions. Lysine is important not only for protein synthesis but also for

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11 323 cell wall construction (Cooper & Metzger, 1986; Grundy, Lehman & Henkin, 2003) and
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13 324 such concomitant repression of genes providing lysine availability in the cells appears
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15 325 surprising. However, according to Liu et al. (2005) and Ihssen and Egli (2005), under
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17 326 carbon starvation conditions, bacterial cells develop a foraging strategy that maximizes
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19 327 energy conservation at the expense of reduced growth rate. In addition, some lysine
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21 328 may replenish the cell amino acid pool due the degradation of fructosyllysine which after
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23 329 diffusing into the cells is degraded to glucose-6-phosphate and lysine (Wiame et al.,
24 330 2002).

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26 331 The repression of the *ptsG* up to -9.51 in *Salmonella* cells grown on glucose-
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28 332 lysine MRPs observed in our study agrees with previous reports of glucose availability
29
30 333 being a major regulator of *ptsG* expression (Aboulwafa, chung, Wai & Saier, 2003; Shin,
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32 334 cho, Heu & Ryu, 2003). Microarray data were confirmed by qRT-PCR analyses (Table
33
34 335 6) where higher absolute values could probably be explained by the higher sensitivity of
35
36 336 the qRT-PCR technique to lower amounts of RNA (Franchini & Egli, 2006). By analyzing
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38 337 the metabolic flux of *E. coli* under glucose-limiting conditions, Lemuth et al. (2008)
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40 338 observed that glucose concentrations as low as 0.05 g/L was not enough to alter *ptsG*
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42 339 expression. Growth on acetate, however, down-regulated *ptsG* 2 expression 4 fold (Oh
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44 340 et al., 2002). Furthermore, Kimata, Tanaka, Inada & Aiba (2001) demonstrated that the
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46 341 induction of *ptsG* is highly dependent on glucose availability and the glycolytic pathway
47
48 342 if malfunctioned markedly reduces the stability of *ptsG* mRNA.

49 343 The growth of *Salmonella* cells on glucose-lysine MRPs is typical for a growth
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51 344 under carbon-limiting conditions (Franchini & Egli, 2006; Oh et al., 2002; Lemuth et al.,
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53 345 2008). The highly induced expression of *cstA* and *dps* up to 17.52 and 24.77 fold

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11 346 agrees with previous reports indicating these specific genes are involved in nutrient
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13 347 scavenging and stress responses (Blum et al., 1990; Dubey et al., 2003). Predominant
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15 348 down-regulation of the genes in the groups of amino acid biosynthesis, protein
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17 349 synthesis and transcription, and DNA metabolism fits with the reduced *Salmonella*
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19 350 growth rates on the MRPs when compared to the growth on glucose since many of the
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21 351 genes (*arg*, *dap*, *lysA*, *rpmB*) belonging to these categories are known to be correlated
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23 352 with the growth rates of the cells (Keener & Noruma, 1996).

24 353 **4.0 Conclusions**

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27 354 *Salmonella* is a facultative anaerobic microorganism with diverse metabolic
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29 355 capacity and stress adaptive mechanisms which determine its persistency under
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31 356 conditions unfavorable for growth and multiplication. Significant amounts of food derived
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33 357 –MRPs may be ingested on a daily basis and due to their low gastrointestinal
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35 358 digestibility, MRPs remain available to various intestinal microorganisms including
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37 359 *Salmonella* for further metabolic modification. This study demonstrated the capability of
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39 360 *S. Typhimurium* LT2 to grow on glucose-lysine MRPs generated under low water
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41 361 activity conditions as a carbon source. Although occurring at reduced growth rates, the
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43 362 mixtures of different ratios of Amadori products, AGEs, and melanoidins (glu-lys1, glu-
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45 363 lys2, and glu-lys3) were able to sustain the growth of the bacterial cells. Transcriptional
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47 364 profiles of the cells grown in the presence of MRPs revealed predominant up-regulation
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49 365 of genes associated with energy metabolism, fatty acid and phospholipid metabolism,
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51 366 cell envelope, and transport binding proteins, and general down-regulation of the genes
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53 367 involved in amino acid biosynthesis, protein synthesis and transcription, and DNA
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55 368 metabolism. The glyoxylate cycle appeared to play an important role in the assimilation

of glucose-lysine-derived MRPs. However, additional research involving specific tracking of more refined glucose-lysine MRPs need to be conducted to elucidate the specific mechanism of their assimilation.

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Figure1: Growth of *S. Typhimurium* LT2 in defined medium containing 0.4% glucose and MRP reaction mixtures (5 mg mL⁻¹).Growth of *S. Typhimurium* LT2 in defined medium containing 0.4% glucose as the only carbon source was used as a control. Data are average of two independent experiments with two wells per repetition. Error bars represent standard deviation from the mean.

Figure 2: Growth of *S. Typhimurium* LT2 in defined medium containing glu-lys1 (A), glu-lys2 (B) or glu-lys3 (C) Maillard reaction mixtures as a carbon source. Numbers in brackets correspond to glucose concentrations (mg/ml) in mixture supplementations. Data are average of two independent experiments with two wells per repetition. Error

bars represent standard deviation from the mean. No growth is observed with no carbon source and with N α -Acetyl-lys.

Fig 3A and 3B represent data sub-set from Fig. 2A and 2B demonstrating bi-phasal growth of *S. Typhimurium* LT2 on glu-lys1 and glu-lys2 reaction mixtures respectively. Data are average of two independent experiments with two wells per repetition. Error bars represent standard deviation from the mean.

Table 1. Primer sets for genes used in qRT-PCR

Gene name	Primer sequence, 5' to 3'	
	Forward primer	Reverse primer
<i>aceA</i>	ATCGTTGTACCCGGCAAACCTCTGT	ATCAGCAACGATCGGCAGGAAGTA
<i>aceB</i>	AGGTGGTAACGATGGACAAACCGT	GCAAGGACCTGATTGTTGCGTTCA
<i>livJ</i>	TACTTGATGCCGTCGTTCACT	TTGCCGATATCAATGCGAAAGGCG
<i>ptsG</i>	TATTCACGCGATTCTGGCAGGTCT	AGCCACAATTTGCTGCTGTTACCG
<i>rpmB</i>	TGAGAGTGCAGGTTAGGCAGGAAA	ACATGTCCCGAGTCTGCCAAGTTA
<i>yfiD</i>	TCACGGTATTCAATCTCGCCCAGT	ACGACGACCTGCTGAATTCCTTCT
<i>acs</i>	TTCAATGGCTTTATCGCCTTCCGC	ACCTTAATGTTTGAAGGCGTGCCG
<i>cstA</i>	TGCCAATGAAGTCGGCGAACAATC	AGTGATACCAGAACGCCACATCCA
<i>rbsB</i>	AACGTGCAGGATTTAACCGTTCGC	TGATAACCGGAATCTTCGCCTGGT
<i>argG</i>	GCGATGAAAGAAGACGGCGTGAAT	TCGGTATCCAGCCACGGTTTGTAA
<i>rsmC</i>	ACGTCCTTTGACTTCCGAAAAG	GTTCTGGTAATCGCCGTTTCG

Table 2. Analytical data of Maillard reaction mixtures, glu-lys1, glu-lys2, and glu-lys3, generated during 4, 23, and 143 h, respectively^a

Sample	Incubation (h)	Furosine (mg 100mg ⁻¹ <i>N</i> - α -acetyl-lys)	Amadori compound (mg 100 mg ⁻¹ <i>N</i> - α -acetyl-lys)	AGEs (FI)	Melanoidins (AU)	Free <i>N</i> - α -acetyl-lys (%)	Free glucose (%)
glu-lys1	4	3.63 $\pm$ 0.28	10.99 $\pm$ 0.84	93.80 $\pm$ 3.60	0.07 $\pm$ 0.01	59.41 $\pm$ 1.81	84.39 $\pm$ 1.39
glu-lys2	23	6.68 $\pm$ 0.13	20.25 $\pm$ 0.41	349.65 $\pm$ 17.27	0.69 $\pm$ 0.07	57.32 $\pm$ 2.23	55.22 $\pm$ 3.00
glu-lys3	143	5.53 $\pm$ 0.30	16.76 $\pm$ 0.91	7.60 $\pm$ 1.45	3.70 $\pm$ 0.44	38.59 $\pm$ 0.37	24.29 $\pm$ 0.60

^aData are average of two independent experiments $\pm$ standard deviation of the mean.

Table 3. Analytical characteristics of glu-lys1, glu-lys2, and glu-lys3 Maillard reaction mixtures after incubation with *S. Typhimurium* LT2

Maillard compounds	Maillard reaction mixtures								
	Glu-lys1			Glu-lys2			Glu-lys3		
	No	With	Decrease	No	With	Decrease	No	With	Decrease
	Salmonella ¹	Salmonella ²	(%)	Salmonella ¹	Salmonella ²	(%)	Salmonella ¹	Salmonella ²	(%)
Amadori compound, (mg 100 mg ⁻¹ <i>N</i> -acetyl-lys)	9.15	0.16	98.19±0.54	22.48	0.34	98.51±0.66	20.72	2.61	87.43±1.26
AGEs, (FI)	73.35	133.49	0	312.36	195.16	37.52±8.33	9.36	8.22	12.18±0.15
Melanoidins, (AU)	ND	ND	ND	0.58	0.49	15.52±4.88	3.34	2.58	22.75±3.39

ND indicates not detected. ¹ Data are from one trial. ² Data are average values generated in two independent trials.

Table 4. Numbers and percentages (in brackets) of up- and down-regulated genes in each functional group for glu-lys1, glu-lys2, and glu-lys3

Functional groups	Glu-lys1			Glu-lys2			Glu-lys3		
	Up	Down	TN ¹	Up	Down	TN ¹	Up	Down	TN ¹
Amino acid biosynthesis	11(28.95)	27(71.05)	38	6(18.75)	26(81.25)	32	11(26.19)	31(73.81)	42
Cell envelope	19(48.72)	20(51.28)	39	18(50.00)	18(50.00)	36	21(60.00)	14(40.00)	35
Cellular processes	21(84.00)	4(16.00)	25	26(74.29)	9(25.71)	35	26(68.42)	12(31.58)	38
Central intermediary metabolism	7(33.33)	14(66.67)	21	11(44.00)	14(56.00)	25	9(34.62)	17(65.38)	26
DNA metabolism	2(13.33)	13(86.67)	15	2(14.29)	12(85.71)	14	4(21.05)	15(78.95)	19
Energy metabolism	38(55.07)	31(44.93)	69	46(60.53)	30(39.47)	76	54(72.00)	21(28.00)	75
Fatty acid and phospholipid metabolism	7(58.33)	5(41.67)	12	5(62.50)	3(37.50)	8	7(53.85)	6(46.15)	13
Mobile and extrachromosomal element functions	10(47.62)	11(52.38)	21	10(52.63)	9(47.37)	19	6(40.00)	9(60.00)	15
Protein synthesis	19(24.68)	58(75.32)	77	19(24.68)	58(75.32)	77	22(24.72)	67(75.28)	89
Regulatory functions	12(85.71)	2(14.29)	14	15(78.95)	4(21.05)	19	14(73.68)	5(26.32)	19
Transcription	3(18.75)	13(81.25)	16	3(20.00)	12(80.00)	15	2(12.50)	14(87.50)	16
Transport and binding proteins	35(44.30)	44(55.70)	79	41(48.24)	44(51.76)	85	44(47.83)	48(52.17)	92
Hypothetical proteins	15(45.45)	18(54.55)	33	17(54.84)	14(45.16)	31	19(67.86)	9(32.14)	28
Unknown function	52(61.90)	32(38.10)	84	62(66.67)	31(33.33)	93	58(68.24)	27(31.76)	85
Unclassified	7(36.84)	12(63.16)	19	8(42.11)	11(57.89)	19	10(43.48)	13(56.52)	23

TN¹ Total numbers of differentially expressed genes. Percentages are calculated based on the total number of the differentially expressed genes.

Table 5. *S. Typhimurium* LT2 key gene expression response to glucose-lysine-based MRPs

Locus ID	Gene symbol	Gene/Protein name	Sub-functional category	Gene expression (fold)		
				Glu-lys1	Glu-lys2	Glu-lys3
Amino acid biosynthesis						
STM3795	<i>ilvN</i>	acetolactate synthase I, small subunit	Pyruvate family	7.45	5.38	NC
STM3796	<i>ilvB</i>	acetolactate synthase I, large subunit, valine sensitive	Pyruvate family	7.11	2.58	NC
STM1795	<i>gdhA</i>	glutamate dehydrogenase	Glutamate family	5.65	5.85	20.55
STM3965	<i>metE</i>	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Aspartate family	-10.99	-12.76	-14.7
STM3013	<i>lysA</i>	diaminopimelate decarboxylase	Aspartate family	-9.15	-8.39	-3.91
STM0064	<i>dapB</i>	dihydrodipicolinate reductase	Aspartate family	-4.65	-4.78	-4.71
STM0213	<i>dapD</i>	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	Aspartate family	-2.44	-2.39	-2.21
STM4182	<i>metA</i>	homoserine transsuccinylase	Aspartate family	-3.87	-3	-4.23
STM4100	<i>metB</i>	cystathionine gamma-synthase	Aspartate family	-3.12	-4.89	-5.28
STM3161	<i>metC</i>	cystathionine beta-lyase (beta-cystathionase)	Aspartate family	-2.3	-2.62	-3.63
STM4105	<i>metF</i>	5,10-methylenetetrahydrofolate reductase	Aspartate family	-5.94	-7.19	-15.51
STM4101	<i>metL</i>	aspartokinase II in bifunctional enzyme: aspartokinase II	Aspartate family	-3.76	-4.2	-4.88
STM3062	<i>serA</i>	D-3-phosphoglycerate dehydrogenase	Serine family	-4.38	-4.42	-2.69
STM2440	<i>cysM</i>	cysteine synthase B (O-acetylserine sulfhydrylase B)	Serine family	-3.88	-3.4	-5.16
STM2430	<i>cysK</i>	subunit of cysteine synthase A and O-acetylserine	Serine family	NC	-2.35	-5.17
STM4007	<i>glnA</i>	glutamine synthetase	Glutamate family	-2.09	-2.7	-2.53
STM1299	<i>gdhA</i>	glutamate dehydrogenase, NADP-specific	Glutamate family	-3.66	-3.08	-2.58
STM4122	<i>argB</i>	acetylglutamate kinase	Glutamate family	-4.75	-5.84	-6.07
STM4121	<i>argC</i>	N-acetyl-gamma-glutamylphosphate reductase	Glutamate family	-4.18	-4.63	-6.77
STM3290	<i>argG</i>	argininosuccinate synthetase	Glutamate family	-3.13	-10.74	-15.34
STM4123	<i>argH</i>	argininosuccinate lyase	Glutamate family	-2.55	-2.68	-2.92
STM4469	<i>argI</i>	ornithine carbamoyltransferase 1	Glutamate family	-2.9	-11.77	NC
Cell envelope						
STM4079	<i>yneC</i>	putative inner membrane protein	Other	16.71	14.68	42.15
STM2802	<i>ygaM</i>	putative inner membrane protein	Other	13.65	12.35	7.94
STM0814	<i>ybhQ</i>	putative inner membrane protein	Other	8.15	9.99	8.02
STM2311	<i>elaB</i>	putative inner membrane protein	Other	8.1	8.9	9.43
STM4379	<i>yjfO</i>	putative lipoprotein	Other	6.4	11.17	22.79
STM0465	<i>ybaY</i>	glycoprotein/polysaccharide metabolism	Other	5.88	6.51	4.93
STM0378	<i>yaiY</i>	putative inner membrane protein	Other	4.03	6.36	5.33
STY3481	<i>NA</i>	putative membrane protein	Other	-5.17	-4.5	-5.26
STM0743	<i>ybgE</i>	putative inner membrane lipoprotein	Other	-5.14	-5.16	NC
STM2932	<i>ygbE</i>	putative inner membrane protein	Other	-4.64	-4.9	-4.91
STM0510	<i>sfbA</i>	putative ABC-type transport system ATPase component/cell division	Other	-4.01	-3.48	NC
STY4655	<i>vexA</i>	Vi polysaccharide export protein	Biosynthesis and degradation of surface polysaccharides and lipopolysaccharides	-3.08	-3.49	-6.46
Cellular processes						
STM0943	<i>cspD</i>	similar to CspA but not cold shock	Adaptations to atypical conditions	7.98	9.93	10.26
STM1688	<i>pspC</i>	phage shock protein regulatory gene, activates expression	Adaptations to atypical conditions	5.03	4.88	NC

STM1705	<i>osmB</i>	osmotically inducible lipoprotein putative resistance protein, osmotically inducible	Adaptations to atypical conditions	4.25	5.96	5.7
STM1563	<i>osmC</i>	small heat shock protein	Other	19.46	16.75	16.15
STM3808	<i>ibpB</i>	phage shock protein	Adaptations to atypical conditions	3.2	2.99	NC
STM1686	<i>pspE</i>	mechanosensitive channel	Adaptations to atypical conditions	2.33	3.01	5.18
STM3410	<i>mscL</i>	copper/zinc superoxide dismutase	Detoxification	2.9	3.79	5.09
STM1440	<i>sodC</i>	putative glutathione S-transferase	Detoxification	8.68	7	5.84
STM3684	<i>yibF</i>	superoxide dismutase, manganese	Detoxification	4.68	3.85	3.37
STM4055	<i>sodA</i>	PhoP regulated: reduced macrophage survival	Detoxification	4.35	5.21	NC
STM1246	<i>pagC</i>	Pathogenicity island encoded protein: SPI3	Pathogenesis	3.07	3.11	NC
STM1087	<i>pipA</i>	Pathogenicity island encoded protein: homologous to ipgE	Pathogenesis	NC	NC	4.09
STM1090	<i>pipC</i>		Pathogenesis	NC	NC	4.22
Central intermediary metabolism						
STM0309	<i>yafH</i>	putative acyl-CoA dehydrogenase	Other	NC	7.43	34.58
STM0384	<i>psiF</i>	induced by phosphate starvation	Phosphorus compounds	4.07	5.43	9.15
STM2915	<i>ygbM</i>	putative endonuclease	Other	3.91	6.82	3.36
STM2500	<i>purN</i>	polyphosphate kinase, component of RNA degradosome	Phosphorus compounds	-7.13	-8.4	-9.69
STM2935	<i>cysD</i>	ATP-sulfurylase, subunit 1	Sulfur metabolism	-6.1	-8.64	-13.81
STM2946	<i>cysH</i>	3-phosphoadenosine 5-phosphosulfate sulfotransferase	Sulfur metabolism	-6	-6.46	-9.77
STM3090	<i>metK</i>	methionine adenosyltransferase 1	Other	-5.92	-6.16	-10.21
STM2934	<i>cysN</i>	ATP-sulfurylase, subunit 1	Sulfur metabolism	-5.89	-6.34	-11.68
STM2946	<i>cysH</i>	3-phosphoadenosine 5-phosphosulfate reductase	Sulfur metabolism	-5.82	-6.02	-12.62
STM0603	<i>ybdL</i>	putative aminotransferase	Other	-5.32	-5.5	-9.86
STM0166	<i>speE</i>	spermidine synthase	Polyamine biosynthesis	-5.3	-3.98	-6.75
STM2933	<i>cysC</i>	adenosine 5-phosphosulfate kinase	Sulfur metabolism	-4.88	-6.04	-6.77
STM2338	<i>pta</i>	phosphotransacetylase	Other	-4.34	-4.19	-5.37
DNA metabolism						
STM3124	NA	putative response regulators	DNA replication	6.18	4.6	5
STM4237	<i>lexA</i>	SOS response regulator, transcriptional repressor	DNA replication	2.17	NC	3.52
STM3385	<i>fis</i>	site-specific DNA inversion stimulation factor	DNA replication	-5.69	-5.17	-9.16
STM4392	<i>priB</i>	primosomal replication protein N	DNA replication	-4.85	-4.41	-10.53
STM2385	<i>yfcB</i>	putative methylase	DNA replication	-3.09	-2.49	-5.55
STM3484	<i>dam</i>	DNA adenine methylase	DNA replication	-2.44	-2.19	-3.66
Energy metabolism						
STM4184	<i>aceA</i>	isocitrate lyase	Glyoxylate cycle	54.5	36.08	3.00
STM4183	<i>aceB</i>	malate synthase A	Glyoxylate cycle	3.15*	40.38	42.76
STM4185	<i>aceK</i>	isocitrate dehydrogenase	Glyoxylate cycle	5.75	5.34	4.02
STM0730	<i>gltA</i>	kinase/phosphatase	TCA cycle	7.9	8.13	9.63
STM1712	<i>acnA</i>	citrate synthase	TCA cycle	6.93	6.79	7.13
STM0158	<i>acnB</i>	aconitate hydratase 1	TCA cycle	4.54	3.49	2.68
STM1238	<i>icdA</i>	aconitate hydratase 2	TCA cycle	NC	2.53	2.51
STM0736	<i>sucA</i>	isocitrate dehydrogenase	TCA cycle	10.21	10.57	8.63
STM0737	<i>sucB</i>	2-oxoglutarate dehydrogenase	TCA cycle	7.35	7.29	12.95
STM0738	<i>sucC</i>	2-oxoglutarate dehydrogenase	TCA cycle	10.27	7.14	10.8
STM0739	<i>sucD</i>	succinyl-CoA synthetase, beta subunit	TCA cycle	6.77	5.79	7.23
STM0734	<i>sdhA</i>	succinyl-CoA synthetase, alpha subunit	TCA cycle	10.26	8.36	8.89
STM0735	<i>sdhB</i>	succinate dehydrogenase, Fe-S protein	TCA cycle	12.7	9.15	7.32
STM0732	<i>sdhC</i>	succinate dehydrogenase, cytochrome b556	TCA cycle	5.48	6.53	13.89
STM0733	<i>sdhD</i>	succinate dehydrogenase, hydrophobic subunit	TCA cycle	6.62	7.5	15.09

STM1468	<i>fumA</i>	fumarase A (fumarate hydratase class I), aerobic	TCA cycle	8.17	7.59	11.27
STM1469	<i>fumC</i>	fumarase C (fumarate hydratase Class II)	TCA cycle	7.71	8.49	11.47
STM3359	<i>mdh</i>	malate dehydrogenase	TCA cycle	5.87	4.81	7.14
STM4080	NA	putative ribulose-5-phosphate 3-epimerase	Pentose phosphate pathway	11.63	9.72	16.53
STM3500	<i>pckA</i>	phosphoenolpyruvate carboxykinase	Glycolysis-gluconeogenesis	10.3	7.45	7.47
STM4415	<i>fbp</i>	fructose-bisphosphatase	Glycolysis-gluconeogenesis	5.2	4.79	7.45
STM1349	<i>pps</i>	phosphoenolpyruvate synthase	Glycolysis-gluconeogenesis	4.12	4.84	4.45
STM4126	<i>udhA</i>	soluble pyridine nucleotide transhydrogenase	Pyruvate dehydrogenase	3.97	4.26	5.88
STM2646	<i>yfiD</i>	putative formate acetyltransferase	Anaerobic	-23.97	-27	-21.47
STM2850	<i>hycD</i>	hydrogenase 3, membrane subunit (part of FHL)	Fermentation	-6.77	-9.2	-4.04
STM2948	<i>cysJ</i>	sulfite reductase, beta (flavoprotein) subunit	Electron transport	-6.01	-7.89	-12.61
STM0973	<i>pflB</i>	pyruvate formate lyase I, induced anaerobically	Fermentation	-4.84	-4.2	-4.54
STM1749	<i>adhE</i>	iron-dependent alcohol dehydrogenase of the multifunctional alcohol	Fermentation	-4.52	-3.27	-3.19
STM3704	NA	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	Glycolysis-gluconeogenesis	-4.02	-3.18	-3.6
STM3384	<i>yhdG</i>	putative TIM-barrel enzyme, possibly dehydrogenase	Amino acids and amines	-3.58	-2.94	-6.48
STM2337	<i>ackA</i>	acetate kinase A (propionate kinase 2)	Fermentation	-3.46	-4.52	-4.45
Fatty acid and phospholipid metabolism						
STM4275	<i>acs</i>	acetyl-CoA synthetase	Biosynthesis	33.62	37.89	50.85
STM1818	<i>fadD</i>	acyl-CoA synthetase (long-chain-fatty-acid--CoA ligase)	Biosynthesis	3.95	3.61	15.84
STM3983	<i>fadB</i>	3-hydroxyacyl-coA dehydrogenase (EC 1.1.1.35) of 4-enzyme FadB	Degradation	6.87	4.01	6.12
STM3019	<i>yqeF</i>	putative acetyl-CoA acetyltransferase	Other	4.67	3.94	6.83
STM1193	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase III acetylCoA ACP transacylase	Biosynthesis	-3.04	-2.88	-5.51
STM1192	<i>plsX</i>	putative fatty acid/phospholipid synthesis protein	Biosynthesis	-2.6	-2.47	-4.78
Mobile and extrachromosomal element functions						
STM1690	<i>pspA</i>	phage shock protein negative regulatory gene for	Prophage functions	9.07	6.13	NC
STM1513	NA	putative cytoplasmic protein	Prophage functions	4.84	5.6	6.44
STM2854	<i>hypA</i>	guanine-nucleotide binding protein in formate-hydrogenlyase system, functions	Protein modification and repair	-4.87	-4.3	-4.23
STM3420	<i>secY</i>	preprotein translocase of IISp family, membrane subunit	Protein and peptide secretion and trafficking	-3.06	-4.51	-9.73
Protein synthesis						
STM0359	NA	putative cytoplasmic protein	tRNA aminoacylation	7.98	10.68	14.34
STM4240	<i>yjbJ</i>	putative cytoplasmic protein	tRNA aminoacylation	6.46	10.66	8.6
STM1810	NA	putative cytoplasmic protein	tRNA aminoacylation	5.52	6.28	8.18
STM0327	NA	putative cytoplasmic protein	tRNA aminoacylation	3.01	4.42	10.26
STM0043	<i>rpsT</i>	30S ribosomal subunit protein S20	Ribosomal proteins	-9.62	-5.55	-3.84
STM3728	<i>rpmB</i>	50S ribosomal subunit protein L28	Ribosomal proteins	-8.06	-7.76	-8.58
STM3283	<i>rpsO</i>	30S ribosomal subunit protein S15	Ribosomal proteins	-6.4	-4.15	-4.92
STM3344	<i>rpsI</i>	30S ribosomal subunit protein S9	Ribosomal proteins	-5.98	-6.35	-15.46
STM3431	<i>rpsQ</i>	30S ribosomal subunit protein S17	Ribosomal proteins	-5.94	-4.22	-9.11
STM1701	<i>yciW</i>	putative cytoplasmic protein	tRNA aminoacylation	-5.85	-7.26	-14.29
STM3441	<i>rpsJ</i>	30S ribosomal subunit protein S10	Ribosomal proteins	-5.47	-4.87	-14.38
STM3440	<i>rplC</i>	50S ribosomal subunit protein L3	Ribosomal proteins	-4.98	-4.91	-10.83
STM3436	<i>rpsS</i>	30S ribosomal subunit protein S19	Ribosomal proteins	-4.71	-6.47	-11.92
STM4151	<i>rplJ</i>	50S ribosomal subunit protein L10	Ribosomal proteins	-4.68	-6.23	-11.05

STM3432	<i>rpmC</i>	50S ribosomal subunit protein L29	Ribosomal proteins	-4.64	-4.5	-9.74
STM3433	<i>rplP</i>	50S ribosomal subunit protein L16	Ribosomal proteins	-4.41	-5.15	-13.58
STM4393	<i>rpsR</i>	30S ribosomal subunit protein S18	Ribosomal proteins	-4.33	-3.93	-11.15
STM3435	<i>rplV</i>	50S ribosomal subunit protein L22	Ribosomal proteins	-4.17	-5.66	-14.07
STM3434	<i>rpsC</i>	30S ribosomal subunit protein S3	Ribosomal proteins	-3.44	-4.39	-11.19
STM2674	<i>trmD</i>	tRNA (guanine-7-)-methyltransferase	tRNA and rRNA base modification	-3.42	-3.75	-13.15
Regulatory functions						
STM0831	<i>dps</i>	stress response DNA-binding protein starvation induced resistance	Other	16.56	18.47	24.77
STM0600	<i>cstA</i>	carbon starvation protein	Other	12.72	12.82	17.52
STM1311	<i>osmE</i>	transcriptional activator of ntrL gene	Other	5.09	4.56	4.26
STM4165	<i>rsd</i>	regulator of sigma D, has binding activity	Other	4.88	5.51	15.33
STM4006	<i>glnL</i>	sensory kinase (phosphatase) in two- component regulatory system	Other	-6.32	-7.39	-5.91
STM0151	<i>pdhR</i>	transcriptional repressor for pyruvate dehydrogenase complex (GntR	Other	-3.18	-4.4	-4.63
Signal transduction						
STM4537	NA	putative PTS permease	PTS	NC	NC	5.25
Transcription						
STM2784	<i>tctE</i>	tricarboxylic transport: regulatory protein	Transcription factors	7.46	5.87	7.5
STM2665	<i>yfiA</i>	ribosome associated factor, stabilizes ribosomes against dissociation	Transcription factors	3.69	5.05	9.88
STM2924	<i>rpoS</i>	sigma S (sigma 38) factor of RNA	Transcription factors	3.43	2.26	NC
STM3280	<i>deaD</i>	cysteine sulfinatase desulfinate	Transcription factors	-4.14	-4.19	-3.03
STM0820	<i>rhlE</i>	putative ATP-dependent RNA helicase	Other	-3.88	-5.04	-4.53
STM3734	<i>rph</i>	RNase PH	Degradation of RNA	-3.79	-2.04	-5.6
STM3415	<i>rpoA</i>	RNA polymerase, alpha subunit	DNA-dependent RNA polymerase	-2.98	-3.48	-5.03
STM4153	<i>rpoB</i>	RNA polymerase, beta subunit	DNA-dependent RNA polymerase	NC	-2.06	-3.92
STM4154	<i>rpoC</i>	RNA polymerase, beta prime subunit	DNA-dependent RNA polymerase	-2.58	-3.35	-3.62
STM3741	<i>rpoZ</i>	RNA polymerase, omega subunit	DNA-dependent RNA polymerase	-2.09	- NC	-2.68
Transport and binding proteins						
STM3692	<i>lldP</i>	LctP transporter, L-lactate permease	Carbohydrates, organic alcohols, and acids	19.17	3.85	NC
STM3567	<i>livJ</i>	ABC branched-chain amino acid transporter	Amino acids, peptides and amines	18.63	16.18	NC
STM3562	<i>livM</i>	ABC branched-chain amino acid transporter	Amino acids, peptides and amines	3.88	2.62	NC
STM3560	<i>livF</i>	ABC branched-chain amino acid transporter	Amino acids, peptides and amines	2.97	2.92	NC
STM1126	<i>phoH</i>	PhoB-dependent, ATP-binding pho regulon component	Amino acids, peptides and amines	13.32	11.94	52.04
STM2796	<i>yqaE</i>	putative YqaE family transport protein	Amino acids, peptides and amines	12.31	12.59	7.23
STM4273	<i>yjcG</i>	putative SSS family transport protein	Other	8.67	7.87	5.57
STM3614	<i>dctA</i>	DAACS family, C4-dicarboxylic acids transport protein	Carbohydrates, organic alcohols, and acids	5.53	4.83	9.31
STM3884	<i>rbsB</i>	ABC superfamily D-ribose transport protein	Carbohydrates, organic alcohols, and acids	4.73	4.72	7.2
STM3883	<i>rbsC</i>	ABC superfamily D-ribose high-affinity transport protein	Carbohydrates, organic alcohols, and acids	2.32	3.02	3.16
STM3881	<i>rbsD</i>	D-ribose high-affinity transport system membrane-associated protein	Carbohydrates, organic alcohols, and acids	2.22	4.06	4.52
STM0828	<i>glnQ</i>	ABC superfamily (atp_bind), glutamine high-affinity transporter	Amino acids, peptides and amines	-2.15	-2.72	-3.27
STM0829	<i>glnP</i>	ABC superfamily (membrane), glutamine high-affinity transporter	Glutamate family	-3.59	-4.17	-4.44
STM0887	<i>artJ</i>	ABC superfamily (bind_prot), arginine 3rd transport system	Amino acids, peptides and amines	-7.65	-8.91	-18.81

STM0888	<i>artM</i>	ABC superfamily (membrane), arginine 3rd transport system	Amino acids, peptides and amines	-4.83	-4	-5.43
STM0891	<i>artP</i>	ABC superfamily (atp&memb), arginine transport system	Amino acids, peptides and amines	-3.01	-3.03	-6.45
STM0889	<i>artQ</i>	ABC superfamily (membrane), arginine 3rd transport system	Amino acids, peptides and amines	-2.34	-3.27	-5.26
STM2200	<i>lysP</i>	APC family, lysine-specific permease	Amino acids, peptides and amines	-4.87	-3.85	-5.23
STM2441	<i>cysA</i>	ABC superfamily (atp_bind), sulfate permease A protein	Anions	-3.56	-3.68	-5.72
STM2444	<i>cysP</i>	ABC superfamily (bind_prot), thiosulfate transport protein	Anions	-7.2	-9.44	-7.51
STY2680	<i>cysU</i>	sulphate transport system permease protein CysT	Anions	-3.67	-5.74	-11.16
STM2443	<i>cysU</i>	ABC superfamily (membrane), thiosulfate transport protein	Anions	-2.87	-3.85	-7.35
STM2442	<i>cysW</i>	ABC superfamily (membrane), thiosulfate permease W protein	Anions	-4.35	-5.44	-13.03
STM1203	<i>ptsG</i>	Sugar Specific PTS family, glucose-specific IIBCcomponent	Restriction modification	-6.62	-6.86	-9.51
STM1831	<i>manY</i>	Sugar Specific PTS family, mannose-specific enzyme IIC	Carbohydrates, organic alcohols, and acids	-2.29	-2.64	-5.65
Hypothetical proteins						
STM4336	<i>ecnB</i>	putative entericidin B precursor	Hypothetical proteins	26.88	29.64	46.92
STM4562	NA	putative inner membrane protein	Hypothetical proteins	9.76	9.53	10.52
STM1121	<i>ymdF</i>	putative cytoplasmic protein	Hypothetical proteins	6.78	7.12	6.79
STM1292	<i>yeaC</i>	putative cytoplasmic protein	Conserved hypothetical proteins	5.29	4.84	4.69
STY4259	NA	hypothetical protein	Conserved hypothetical proteins	4.31	5.59	4.33
STM0454	<i>ybaW</i>	putative esterase	Conserved hypothetical proteins	3.98	4.49	7.03
STM1161	<i>yceP</i>	putative cytoplasmic protein	Conserved hypothetical proteins	2.36	3.96	12.95
STY1876	NA	hypothetical protein	Hypothetical proteins	2.17	3.73	15.02
STY4674	NA	hypothetical protein	Hypothetical proteins	-5.33	-4.47	-5.66
STM3841	NA	putative inner membrane protein	Conserved hypothetical proteins	-3.96	-4.09	-7.83
Unknown function						
STM4274	<i>yjch</i>	putative inner membrane protein	General	26.13	16.98	8.89
STM2983	<i>ygdI</i>	putative lipoprotein	General	15.28	17.33	13.52
STM1851	NA	putative cytoplasmic protein	General	14.57	12.02	17.66
STM0366	<i>yahO</i>	putative periplasmic protein	General	13.91	14.2	12.48
STM2795	<i>ygaU</i>	putative LysM domain	General	10.55	14.29	12.67
STM1284	<i>yeaH</i>	putative cytoplasmic protein	General	7.39	6.9	12.98
STM2553	<i>csiE</i>	stationary phase inducible protein	General	7.29	7.01	6.59
STM0601	<i>ybdD</i>	putative cytoplasmic protein	General	7.27	8.99	12.4
STM1984	<i>yodD</i>	putative cytoplasmic protein	General	7.14	8.95	12.05
STM4378	<i>yjfiN</i>	putative inner membrane protein	General	6.88	8.6	7.4
STM1652	<i>ynaF</i>	putative universal stress protein	General	5.87	7.11	12.69
STM1376	<i>lppB</i>	putative methyl-accepting chemotaxis protein	General	-6.16	-5.84	-3.33
STM3087	<i>yqgB</i>	putative inner membrane protein	General	-6.05	-5.11	NC
STM1527	NA	putative inner membrane protein	General	-4.23	-2.99	NC
STM0975	<i>ycaO</i>	putative cytoplasmic protein	General	-3.89	-3.69	-4.72
STM1936	<i>yecH</i>	putative cytoplasmic protein	General	-3.83	-5.21	-4.55
STM2576	<i>yfhL</i>	putative ferredoxin	General	-3.72	NC	NC
STM2951	<i>ygcF</i>	putative Organic radical activating enzymes	General	-3.71	NC	NC
STM1489	<i>ynfK</i>	putative dethiobiotin synthase	General	-3.69	-4.25	-9.66
STM3851	<i>yieG</i>	putative xanthine/uracil permeases family	General	-3.54	-3.57	-2.84
STM3168	<i>ygiR</i>	putative Fe-S oxidoreductase family 2	General	-3.32	-2.75	-4.71

Unclassified						
STM0933	<i>ybjT</i>	putative nucleoside-diphosphate-sugar epimerase	Role category not yet assigned	3.2	2.57	2.87
STM1867	<i>pagK</i>	PhoPQ-activated gene	Role category not yet assigned	2.85	3.07	3.92
STM2947	<i>cysI</i>	sulfite reductase, alpha subunit, NADPH dependent	Role category not yet assigned	-7.39	-8.79	-14.42
STM2676	<i>rpsP</i>	30S ribosomal subunit protein S16	Role category not yet assigned	-5.17	-3.26	-7.37
STM1965	<i>yedE</i>	paral putative membrane component of transport system	Role category not yet assigned	-4.02	-3.34	-3.83

Data are average of three independent experiments with $q < 0.0001$.
NC indicates no change in expression level. Negative values represent repression. * $q = 0.01$.

Table 6. Confirmatory gene expression analysis by quantitative RT-PCR

MRP fraction	Gene name	Gene expression, fold	
		qRT-PCR	Microarray
		analysis	analysis
Glu-lys1	<i>aceA</i>	35.40±9.934	54.50
	<i>aceB</i>	7.15±1.01	3.15
	<i>livJ</i>	18.05±1.60	18.63
	<i>ptsG</i>	-29.87±4.99	-6.62
	<i>rpmB</i>	-19.58±6.81	-8.06
	<i>yfiD</i>	-40.69±11.43	-23.97
Glu-lys2	<i>aceA</i>	23.11±6.26	36.08
	<i>acs</i>	42.39±8.66	37.89
	<i>cstA</i>	11.27±7.88	12.82
	<i>ptsG</i>	-17.67±10.99	-6.86
	<i>rpmB</i>	-26.54±8.82	-7.76
	<i>yfiD</i>	-35.15±13.65	-27.00
Glu-lys3	<i>acs</i>	64.74±7.84	50.85
	<i>aceB</i>	59.23±10.05	42.76
	<i>rbsB</i>	9.73±2.92	7.20
	<i>argG</i>	-32.78±11.43	-4.44
	<i>ptsG</i>	-16.95±5.57	-9.51
	<i>rpmB</i>	-16.88±2.86	-8.58

Supplementary Data
[Click here to download Supplementary Data: Supplementary table.doc](#)