Stress response, amino acid biosynthesis and pathogenesis genes expressed in *Salmonella enterica* colonizing tomato shoot and root surfaces

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**Abstract**

*Salmonella enterica* can colonize all parts of the tomato plant. Tomatoes have been frequently implicated in salmonellosis outbreaks. In agricultural settings, *Salmonella* must overcome stress, nutritional and competition barriers to become established on plant surfaces. Knowledge of the genetic mechanisms underlying *Salmonella*-plant associations is limited, especially when growing epiphytically. A genome-wide transcriptomic analysis of *Salmonella Typhimurium* (SeT) was conducted with RNA-Seq to elucidate strategies for epiphytic growth on live, intact tomato shoot and root surfaces. Six plasmid-encoded and 123 chromosomal genes were significantly (using Benjamini-Hochberg adjusted p-values) up-regulated; 54 and 110 detected in SeT on shoots and roots, respectively, with 35 common to both. Key signals included NsrR regulon genes needed to mitigate nitrosative stress, oxidative stress genes and host adaptation genes, including environmental stress, heat shock and acid-inducible genes. Several amino acid biosynthesis genes and genes indicative of sulphur metabolism and anaerobic respiration were up-regulated. Some Type III secretion system (T3SS) effector protein genes and their chaperones from pathogenicity island-2 were expressed mostly in SeT on roots. Gene expression in SeT was validated against SeT and also the tomato outbreak strain *Salmonella Newport* with a high correlation ($R^2 = 0.813$ and 0.874, respectively; both $p < 0.001$). Oxidative and nitrosative stress response genes, TSSS2 genes and amino acid biosynthesis may be needed for *Salmonella* to successfully colonize tomato shoot and root surfaces.

1. Introduction

*Salmonella enterica* subsp. *enterica* has been implicated in numerous foodborne illness outbreaks associated with the consumption of tomatoes (Bennett et al., 2015). In the period between 1998 and 2016, close to 20% of salmonellosis in the United States were attributed to seeded fruit (IFSAC, 2018). It is widely accepted that 20% of salmonellosis in the United States were attributed to seeded fruit (IFSAC, 2018). It is widely accepted that

Studies have begun to unravel the genetic mechanisms involved in *S. enterica* colonization of plant surfaces and plant tissues, and specific patterns are beginning to emerge. Several amino acid biosynthesis pathways have been identified as necessary for colonization of plants, including on alfalfa sprouts and in tomato fruit wounds (Brankatschk et al., 2012; de Moraes et al., 2017; Kwan et al., 2015), as well as vitamins in cilantro, lettuce and sprouts, and fatty acid biosynthesis in sprouts and tomato fruit tissue (Goudeau et al., 2012; Kwan et al., 2018; Noel et al., 2010). Biofilm formation has also been implicated in *S. enterica* interactions with sprouts, spinach and grape tomatoes (Brankatschk et al., 2012; Salazar et al., 2013). For attachment to plant surfaces, involvement of surface membrane components such as curli fimbriae, and O-antigen capsule assembly and translocation, was recognized (Barak et al., 2005; Barak et al., 2007; de Moraes et al., 2017; Marvasi et al., 2013). Stress mitigation has also been identified when enteropathogens are inhabiting...
plant disease lesions and under chlorine treatment (Goudeau et al., 2012; Wang et al., 2010).

Despite these advances, the full scope of _S. enterica_ adaptations to plant surface colonization, the most likely type of initial encounter in the field, is not well understood. The phyllosphere is regarded as a harsh habitat for human enteric pathogens such as _S. enterica_. Plants appear to recognize and mount an immune response against _S. enterica_ (Garcia et al., 2014; Meng et al., 2013; Roy et al., 2013), but the impact of this biotic stress on the bacterium has not been investigated. Moreover, the enteric pathogen has to contend with abiotic stresses and restricted nutrient availability while competing with microbiota that have co-evolved with plants. The tomato fruit surface may be particularly harsh due to its smooth skin, lack of stomata and presence of antimicrobial compounds (Han and Micallef, 2016). Generally, tomato leaves and roots appear to be a more hospitable environment than fruit, with higher _S. enterica_ counts consistently retrieved from leaves both in experimental and field settings (Barak et al., 2011; Gu et al., 2018; Han and Micallef, 2014). While leaves are not consumed, contamination of leaves increases the risk of fruit colonization, both during growth and post-harvest handling (Barak et al., 2011; Bolten et al., 2019). In this regard, our understanding of the genetic mechanisms by which _S. enterica_ can mitigate biotic and abiotic stresses during epiphytic interaction with tomato roots and leaves, the plant niches in which _S. enterica_ appears to fare best, is limited. In this study, a _S. enterica_ genome-wide transcriptomic analysis was conducted to identify stress and plant niche adaptation responses that come into play when this enteric pathogen associates with tomato shoots and roots. Continuing to decipher the complexity of the _S. enterica_-plant interaction in a plant tissue specific manner will equip us with better knowledge to improve agricultural practices and manage food safety risk.

2. Materials and methods

2.1. _S. enterica_ inoculation of tomato shoots and roots

_Salmonella enterica_ Typhimurium LT2 (ATCC700720) (SeT) and _S. enterica_ Newport MDD314 (SeN), an isolate matching a tomato outbreak strain (Greene et al., 2008), were used in this study. Colonies from 18 h cultures on trypticase soy agar (TSA) (BD, Sparks, MD, USA) were suspended in sterile phosphate buffered saline (PBS) to OD_{600} 0.5 (~10^{9} CFU/ml).

Tomato seeds _Solanum lycopersicum_ cv. ‘Heinz-1706’, Tomato Genetics Resource Center (TGRC), U.C. Davis, Davis, CA, USA) were sterilized by soaking in half-strength household bleach for 30 min, followed by 6–7 rinses in sterile water. Seeds germinated in the dark were grown on 2/3 strength trypticase soy agar (TSA) plates for bacterial enumeration and checking for contamination. Bacterial cells were also retrieved in PBS for plating on TSA and XLT4 (BD) plates for bacterial enumeration and checking for contamination. The control consisted of SeT colonies grown for 18 h on Luria-Bertani agar (LB; BD) at 28 °C in triplicate, directly suspended in 1 ml of RNAProtect Cell Reagent and pelletized. Total RNA was extracted using RNeasy mini kit (Qiagen) and quantitated on NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and a Bioanalyzer (Agilent, Santa Clara, CA, USA). Ribosomal RNA was depleted using Ribo-Zero rRNA removal kit (Epicentre, Madison, WI, USA). All procedures hereafter followed the protocols of ScriptSeq v2 RNA-Seq Library Preparation kit (Epicentre). Purified rRNA-depleted mRNA was fragmented for cDNA synthesis using StarScript reverse transcriptase (Epicentre). The resultant cDNA fragments were ligated with 3'-terminal-tags (adapter) and purified using Agencourt AMPure XP System (Beckman Coulter). The prepared RNA-Seq libraries were checked for quality and quantity on the Bioanalyzer and sequenced on an Illumina HiSeq 1000 to obtain 100 bp paired-end reads, at the sequencing facility of the Institute for Bioscience and Biotechnology Research, University of Maryland.

2.3. Mapping and statistical analysis

Data cleanup and analysis was carried out on a high performance computing cluster at the University of Maryland. Multiplexed raw data obtained from sequencing were cleaned and trimmed of the adaptor and barcode sequences using Trimmomatic (Bolger et al., 2014). Differential gene expression was analyzed with the bash scripts provided in Trapnell et al. (2012) using the _S. Typhimurium_ LT2 reference chromosome: NC_003197.2/AE006468.2 and plasmid pSLT: NC_003277.2/AE006471.2 genomes, indexed for read alignments and mapping in Bowtie2 (Langmead et al., 2009). Sequence reads for each treatment condition was mapped to the reference genome with TopHat2. A transcriptome assembly for each treatment condition was generated with Cufflinks and assemblies merged with the Cuffmerge utility. The reads and the merged assembly were fed to Cuffdiff which normalizes read counts into FPKM (fragments per kilobase of transcript per million mapped fragments), calculates expression levels, and tests the statistical significance of observed changes in expression levels (Trapnell et al., 2012). Significance of differentially transcribed genes was corrected for multiple testing errors using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to calculate false discovery rate (FDR) adjusted p-values (q-values). The expected number of false positives was reduced from 223 with p-value ≤ 0.05, to 26 and 36 with FDR ≤ 0.15 and 0.10 for shoot and root data, respectively. An FDR ≤ 0.10 was used for plasmid-encoded differentially-transcribed genes which halved the expected number of false positives to an average of 2.75. The resultant q-values were q ≤ 0.006 and ≤ 0.009 for chromosome-encoded genes from SeT-shoots and -roots, respectively and q ≤ 0.03 for plasmid-encoded genes. Gene functions were classified according to Clusters of Orthologous Groups of proteins (COGs) using EggNOG 4.5.1 and further searched in UniProt. Genetic pathways were searched using the KEGG PATHWAY database. Raw and processed data generated in this study are deposited in the NCBI GenBank Gene Expression Omnibus (GEO) repository under series accession number GSE73192.

2.4. Quantitative reverse transcription-PCR verification using _S. Newport_

RNA-Seq was performed in _Salmonella_ LT2 for the availability of an annotated genome. A subset of genes was selected for q-RT-PCR validation of RNA-Seq data in SeT and a serotype relevant to food safety of _S. Newport_ (Greene et al., 2008). Primers were designed using _S. Typhimurium_ LT2 as a reference genome sequence with an amplicon size...
between 70 and 150 bp for each gene (Table 1). Total RNA was extracted in replicates of four with Verso cDNA synthesis kit (Thermo Scientific) from SeT colonizing tomato shoots and roots under the same conditions as before, and SeN colonizing tomato shoots after 6 h of incubation, with 4 plants pooled for one biological replicate. All qPCR reactions were done with PerfeCTa SYBR Green SuperMix (Quanta Biosciences, MD, USA). Amplification of gene transcripts of interest was performed on an ABI Step-One Plus (Applied Biosystems, Foster City CA). Briefly, each of real-time PCR reactions consisted of 10 μl SYBR Green, 0.4 μl forward and 0.4 μl reverse primers, 8 μl cDNA template, and 1.2 μl H2O. PCR reaction underwent 40 cycles of PCR (15 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C). Relative gene expression was calculated for each gene of interest relative to the endogenous control (rpoD) following the ΔΔCt method of Schmittgen and Livak (Schmittgen and Livak, 2008). For each gene, ΔΔCt values were used to determine differential gene expression against the control. Student’s t-test was performed on ΔΔCt values to identify any statistically significant difference at p ≤ 0.05. Log2 fold-change values from q-RT-PCR and RNA-Seq were fitted to a linear equation to assess consistency of methods in measuring gene expression and validate the applicability of SeT LT2 data to SeN. Statistical analyses were conducted using JMP Pro 14.1.0.

3. Results and discussion

3.1. Global SeT gene expression

S. Typhimurium populations were stable on both shoots and roots 3 days post-inoculation when cells were retrieved for RNA-Seq analysis, and up to 11 days after inoculation (Figure 1). Global analysis of the transcriptome of SeT epiphythetically colonizing tomato resulted in expression signals for 4,227 chromosomal genes in SeT colonizing shoots (SeT-shoots) and 4,306 genes in SeT colonizing roots (SeT-roots), indicating 92–94% coverage of the whole transcriptome of S. Typhimurium. Also detected were 98 transcripts of plasmid-encoded genes from SeT-shoots and 96 from SeT-roots. Of the chromosomal genes, 173 (4.1 %; q ≤ 0.006) and 347 (8.1 %; q ≤ 0.009) were differentially expressed on tomato shoots and roots, respectively, relative to growth in LB culture, 123 of which were up-regulated (≥1.0 log2 fold change; Tables 2 and 3). More chromosomal genes were detected as up-regulated in SeT-roots – 107 compared to 49 in shoots. Only 16 genes were unique to SeT-shoots, compared to 74 in SeT-roots (Figure 2). In addition, 6 plasmid-encoded genes were found to be up-regulated (q ≤ 0.03). Genes up-regulated in SeT associated with both plant structures point to a core set of genes that facilitate Salmoneal tomato colonization of tomato plants, while tissue-specific up-regulated genes point to shoot- or root-specific responses and the complex nature of the Salmonella-tomato association. The down-regulated genes detected comprised mainly of genes involved in metabolic and cellular processes which would be expected to be less active in an epiphytic lifestyle compared to growth in a nutrient-rich medium and were hence not explored further (data not shown).

Although overall, genes involved in carbohydrate transport and metabolism were significantly down-regulated in relation to growth in nutrient rich medium, several SeT genes that were up-regulated on tomato shoots relative to LB culture were indicative of active metabolism (Figure 3). Functional categories most represented included genes needed for energy production and conversion (5 genes in SeT-shoots and 16 in SeT-roots) and genes involved in the transport and metabolism of amino acids and inorganic ions (13 and 11 genes in SeT-shoots and SeT-roots, respectively) (Figure 3, Tables 2 and 3). Several genes involved in transcription, translation, post-translational modifications, cell envelope biogenesis and replication and repair were also up-regulated. The majority of up-regulated genes in SeT-shoots and -roots, however, remained uncharacterized or unclassified, encoding hypothetical proteins or proteins with unknown function, including several prophage genes (Figure 3, Table 4).

Table 1. Primers used in q-RT-PCR.

| Gene | Function | Forward Primer 5'-3' | Reverse Primer 5'-3' | Reference |
|------|----------|----------------------|----------------------|-----------|
| lamA | Maltoporin (maltose transport) | GTATGGCTGAGCGGGAAGGC | TGCCGCCTTTCACACACTTC | This Study |
| apsA | Class B acid phosphatase | AACGGCTGAGATGAGGTTA | GTCTGACATGACACCTGAC | This Study |
| magA | MalE ABC transporter substrate-binding protein MalE | ATTCGAGCTCTTCTTTCAC | ACAAGACCTGCTTCCGAAC | This Study |
| marA | DNA-binding transcriptional activator for antibiotic resistance operon MarRAB | TACGGCTGCGGATGTATTGG | CGAGGATAACCTGGAGTCGC | This Study |
| nmpC | Outer membrane porin protein | GTCTGGTCACTGCTTACCTG | GCTTGCTGGAATGCTGTC | This Study |
| rpoD | RNA polymerase σ-factor | GTGAAATGGGGCCACGTCGACCTG | TTTCCAGCAGATAGTAATGCGGTC | Karlinsky et al. (2012) |
| sacA | Redox-sensitive transcriptional activator | ATGTTAAGGCAAGCTTCTACG | TACACGGCTCAGTTCCTACG | This Study |
| trpD | Tryptophan biosynthesis protein | GTCTGATCGAACACACAGG | AATCGGCTGGAGGTTGTCG | This Study |
| trpE | Tryptophan biosynthesis protein | GCCGCTGAGGAAGATGTCG | AAAGCCGTGGATGTCGACAT | This Study |
| trrA | Tetrathionate reductase subunit A | TCCATGAGCCAGGGTGGGC | GCTGCCGGATAGATTGTTG | This Study |
| ycrB | Outer membrane protein | ACCGCAAGAGCTCAACAGAA | GGGGGCGTAACAGGTTA | This Study |
| ydaA | Universal stress protein E | GCCACCCCTGCTTTCTCTG | GGACAGATTGGCGGACAC | This Study |
| yhgA | Cytoplasmic protein in NsrR regulon | GTGGGGCCAGCTGCTTCTCAG | GCGTGGCTAAAGCATGCTG | This Study |
| yhE | Outer membrane protein | GCGTTCAGCCCGAATGTCG | CTCATGGGCTAAGTGGTCG | This Study |
Table 2. Differentially up-regulated *Salmonella enterica* Typhimurium LT2 genes (>1.5 log₂ fold change) on tomato shoots, roots, or both shoots and roots, compared to growth in LB medium as obtained by genome-wide RNA-Seq analysis. Only genes with statistically significant q-values (Benjamini-Hochberg adjusted p-values) are shown.

| Gene                | NCBI tag | Annotation                                                                 | Shoots | q-value | Roots | log₂ fold change | q-value |
|---------------------|----------|-----------------------------------------------------------------------------|--------|---------|-------|-----------------|---------|
| **CELLULAR PROCESSES AND SIGNALING**                          |          |                                                                             |        |         |       |                 |         |
| [M] Cell wall/membrane/envelope biogenesis                     |          |                                                                             |        |         |       |                 |         |
| ybdV                | STM3392  | outer membrane lipoprotein                                                  | 1.7    | 0.002   | 3.7   | 0.003          |         |
| ycfR                | STM1214  | Reduces the permeability of the outer membrane to copper. Seems to be involved in the regulation of biofilm formation. May decrease biofilm formation by repressing cell-cell interaction and cell surface interaction | 6.9    | 0.006   | 6.6   | 0.011*         |         |
| ycfJ                | STM1212  | surface antigen; putative outer membrane lipoprotein                       | 3.8    | 0.01*   |       |                 |         |
| STM0908             | STM0908  | hypothetical protein                                                        | 2.0    |         | 0.005 |                 |         |
| STM1530             | STM1530  | putative outer membrane protein                                             | 1.6    |         | 0.003 |                 |         |
| STM1540             | STM1540  | hydrolase                                                                   | 2.7    |         | 0.003 |                 |         |
| **O** Post-translational modification, protein turnover, and chaperones |          |                                                                             |        |         |       |                 |         |
| lbpB                | STM3808LS| heat shock protein LbpB                                                      | 5.3    |         | 0.007 |                 |         |
| STM0912             | STM0912  | ATP-dependent Clp protease proteolytic subunit                              | 2.0    |         | 0.003 |                 |         |
| STM1251             | STM1251  | molecular chaperone                                                         | 1.0    | 0.005*  | 2.2   | 0.003          |         |
| STM1791             | STM1791  | hydrogenase expression                                                      | 1.5    |         | 0.008 |                 |         |
| **INFORMATION STORAGE AND PROCESSING**                         |          |                                                                             |        |         |       |                 |         |
| [K] Transcription                                            |          |                                                                             |        |         |       |                 |         |
| marA                | STM1519S | DNA-binding transcriptional activator MarA                                 | 4.8    | 0.002   | 2.5   | 0.003          |         |
| marR                | STM1520  | DNA-binding transcriptional represor MarR                                   | 3.7    | 0.002   | 1.6   | 0.003          |         |
| soxR                | STM4266  | redox-sensitive transcriptional activator SoxR                             | 3.1    | 0.002   | 1.9   | 0.003          |         |
| STM0898A            | STM0898A | hypothetical protein                                                        | 2.2    |         | 0.003 |                 |         |
| **L** Replication, recombination and repair                   |          |                                                                             |        |         |       |                 |         |
| deaD                | STM3280S | ATP-dependent RNA helicase DeaD                                             | 2.1    | 0.002   | 1.0   | 0.008          |         |
| **METABOLISM**                                               |          |                                                                             |        |         |       |                 |         |
| [C] Energy production and conversion                         |          |                                                                             |        |         |       |                 |         |
| hycG                | STM2847  | hydrogenase                                                                 | 1.7    |         | 0.007 |                 |         |
| yqhD                | STM3164  | alcohol dehydrogenase                                                        | 4.3    |         | 0.007 |                 |         |
| ymeI                | STM1524  | succinate semialdehyde dehydrogenase                                         | 1.4    | 0.002   | 1.6   | 0.003          |         |
| narI                | STM1761  | respiratory nitrate reductase                                                | 4.3    |         | 0.009 |                 |         |
| **D** Cell cycle control, cell division, chromosome partitioning |          |                                                                             |        |         |       |                 |         |
| yifE                | STM4399  | Di-iron-containing protein involved in the repair of iron-sulfur clusters damaged by oxidative and nitrosative stress conditions; NsrR regulon | 3.7    | 0.016*  | 2.9   | 0.017*         |         |
| **E** Amino acid transport and metabolism                     |          |                                                                             |        |         |       |                 |         |
| mtr                  | STM3279  | HAAAP family tryptophan-specific transport protein                          | 1.9    | 0.002   | 1.2   | 0.004          |         |
| trpB                 | STM1726  | tryptophan synthase subunit β                                               | 1.8    | 0.004   |       |                 |         |
| trpC                 | STM1725  | bifunctional indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase | 2.0    | 0.002   | 1.0   | 0.006          |         |
| trpD                 | STM1724  | bifunctional glutamine amidotransferase/anthranilate phosphoribosyltransferase | 3.0    | 0.002   | 1.8   | 0.005          |         |
| trpE                 | STM1723  | anthranilate synthase component I                                           | 3.1    | 0.002   | 2.0   | 0.003          |         |
| ilvA                 | STM3905  | Threonine dehydratase                                                        | 3.4    |         | 0.008 |                 |         |
| **F** Inorganic ion transport and metabolism                  |          |                                                                             |        |         |       |                 |         |
| cysD                 | STM2935  | sulfate adenylyltransferase subunit 2                                       | 3.0    | 0.002   | 2.5   | 0.003          |         |
| cysN                 | STM2934  | sulfate adenylyltransferase subunit 1                                       | 2.7    | 0.002   | 2.2   | 0.003          |         |
| marB                 | STM1518  | multiple antibiotic resistance protein MarB                                  | 3.7    | 0.002   | 1.7   | 0.005          |         |
| **Q** Secondary metabolites biosynthesis, transport, and catabolism |          |                                                                             |        |         |       |                 |         |
| basS                 | STM4291  | Member of the two-component regulatory system BasS/BtnR. Autophosphorylates and activates BasR by phosphorylation. Plays a role in the adaptation of the organism to the host environment (neutrophils) | 2.2    |         | 0.009 |                 |         |
| **U** Intracellular trafficking, secretion, and vesicular transport |          |                                                                             |        |         |       |                 |         |
| staC                 | STM1394  | Type III secretion system apparatus protein                                 | 1.4    | 0.010*  |       |                 |         |
| staH                 | STM1407  | Type III secretion system apparatus protein                                 | 5.0    |         | 0.009 |                 |         |
| staS                 | STM1420  | Type III secretion system apparatus protein                                 | 4.4    |         | 0.011* |                 |         |
| **FUNCTION UNKNOWN OR POORLY CHARACTERIZED**                  |          |                                                                             |        |         |       |                 |         |
| phnX                 | STM0432  | phosphonoacetaldehyde hydrolase, involved in phosphonate degradation         | 1.5    |         | 0.004 |                 |         |
| sseE                 | STM1396  | secretion system effector SseE                                             | 1.9    |         | 0.003 |                 |         |
| sseB                 | STM1398  | secreted effector protein SseB, enhanced serine sensitivity protein SseB  | 6.2    |         | 0.007 |                 |         |
| yeaK                 | STM1282  | YbaK 30 kDa-hydroxymethylbutyrate synthetase, YbaK, hydrolase                |       |         |       |                 |         |
| ygbA                 | STM2860  | Nitrous oxide-stimulated promoter; NsrR regulon                            | 3.5    | 0.002   | 3.5   | 0.003          |         |

(continued on next page)
3.2. Stress response and plant host adaptation

Several genes known to respond to environmental stresses were strongly induced in the S. T-shoots or S. T-roots. This 2-component system is induced in acidic conditions (Perez and Groisman, 2007) and regulates the synthesis of proteins that mediate increased resistance to antimicrobial peptides, common in the host environment, and the antibiotic polymyxin B (Gunn et al., 2000). The gene STM3030 was also markedly up-regulated in S. T-roots (5.7-fold increase, q = 0.008; Table 5). This gene is known to play a role in cephalothin and cefoxitin resistance in S. Typhimuroidum (Lin et al., 2019). STM1530, a gene that confers resistance to ceftriaxone, was upregulated in S. T-shoots and S. T-roots (Tables 2, 3, and 5). Moreover, the acid shock response of marA, a transcription factor that controls multidrug efflux and porin synthesis (Cohen et al., 1993), was also elicited, as shown by the genes STM1485, an acid shock protein and STM1513, a stress-induced acidophilic repeat motif (Table 2).

3.3. Nitrosative and oxidative stress

Genes in the nitrosative stress regulator regulon NsrR (ygbA, ytfE, yoaG in S. T-shoots and -roots and STM1808 S. T-roots) were markedly up-regulated (Table 5). The gene yfhH was also expressed in both S. T-shoots and -roots. This gene appears to offer an NsrR binding site in E. coli (Browning et al., 2010; Partridge et al., 2009). The NsrR regulon is needed for nitric oxide (NO) detoxification, nitrosative stress resistance and virulence (Karlinsky et al., 2012). Salmonella is exposed to both NO and reactive nitrogen species (RNS) at infection sites inside animal hosts and has developed mechanisms to detoxify NO and repair damage induced by RNS (Renard and Vazquez-Torres, 2011). NO is also an important plant signalling molecule involved in several processes including abiotic stresses, defence against pathogens and stomatal closure (Mur et al., 2013). Lipopolysaccharides induced an NO burst in S. Typhimurium (Zeidler et al., 2004). Further, NO was generated in response to abscisic acid and needed for abscisic acid-induced stomatal closure (Neill et al., 2002). The enteric pathogen E. coli O 157:H7 and, to a lesser degree, Salmonella SL1344, induced stomatal closure in Arabidopsis (Melotto et al., 2006; Roy et al., 2013).
Table 3. Differentially up-regulated *Salmonella enterica* Typhimurium LT2 genes (1.0-1.5 log₂ fold change) on tomato shoots, roots, or both shoots and roots, compared to growth in LB medium as obtained by genome-wide RNA-Seq analysis. Only genes with statistically significant q-values (Benjamini-Hochberg adjusted p-values) are shown.

| Gene | NCBI tag | Annotation | Shoots log₂ fold change | q-value | Roots log₂ fold change | q-value |
|------|----------|------------|-------------------------|---------|------------------------|---------|
| **CELLULAR PROCESSES AND SIGNALING** | | | | | |
| [M] Cell wall/membrane/envelope biogenesis | | | | | |
| dgkA | STM4236 | diacylglycerol kinase | 1.0 | 0.006 | | |
| **[N] Cell motility** | | | | | |
| iap | STM2936 | alkaline phosphatase isozyme conversion | 1.3 | 0.003 | | |
| **[O] Post-translational modification, protein turnover, and chaperones** | | | | | |
| hslU | STM1648 | heat-inducible protein HslU | 1.0 | 0.005 | 1.1 | 0.005 |
| **INFORMATION STORAGE AND PROCESSING** | | | | | |
| [J] Translation, ribosomal structure and biogenesis | | | | | |
| rsaA | STM2222 | rRNA small subunit pseudouridine synthase A | 1.0 | 0.005 | | |
| trpS2 | STM4508 | tryptophanyl-tRNA synthetase II | 1.1 | 0.004 | 1.0 | 0.006 |
| [K] Transcription | | | | | |
| ptsJ | STM2436 | transcriptional regulator PtsJ | 1.1 | 0.004 | | |
| yfhH | STM2572 | DNA-binding transcriptional regulator | 1.0 | 0.005 | 1.2 | 0.005 |
| yncJ | STM1523 | LysR family transcriptional regulator | 1.0 | 0.005 | 1.2 | 0.004 |
| **METABOLISM** | | | | | |
| [C] Energy production and conversion | | | | | |
| asrC | STM2550 | anaerobic sulfite reductase subunit C | 1.2 | 0.004 | | |
| hpaC | STM1098 | 4-hydroxophenylacetate 3-monoxygenase reductase subunit | 1.0 | 0.007 | | |
| hycC | STM2851 | hydrogenase 3 membrane subunit | 1.3 | 0.004 | 1.4 | 0.005 |
| hycD | STM2850 | hydrogenase 3 membrane subunit | 1.0 | 0.005 | 1.0 | 0.007 |
| hycE | STM2849 | hydrogenase 3 large subunit | 1.2 | 0.004 | | |
| pflF | STM0843 | pyruvate formate lyase | 1.2 | 0.004 | | |
| trpA | STM1383 | tetrathionate reductase subunit A | 1.3 | 0.003 | | |
| trpB | STM1385 | tetrathionate reductase subunit B | 1.2 | 0.005 | | |
| ydQ | STM1354 | Electron transfer flavoprotein; may play a role in electron transport between the anaerobic fatty acid oxidation pathway and the respiratory chain | 1.0 | 0.008 | | |
| STM1253 | | cytochrome b561 | 1.4 | 0.003 | | |
| STM1787 | | hydrogenase 1 large subunit | 1.1 | 0.006 | 1.2 | 0.006 |
| STM1792 | | putative cytochrome oxidase subunit I | 1.2 | 0.004 | | |
| STM1793 | | putative cytochrome oxidase subunit II | 1.0 | 0.006 | | |
| [E] Amino acid transport and metabolism | | | | | |
| asuF | STM2670 | phospho-2-dehydro-3-deoxyheptonate aldolase | 1.0 | 0.004 | 1.4 | 0.003 |
| hisG | STM2071 | ATP phosphoribosyltransferase | 1.2 | 0.002 | 1.0 | 0.005 |
| metA | STM4182 | homoserine O-succinyltransferase | 1.4 | 0.002 | - | |
| sreA | STM3062 | D-3-phosphoglycerate dehydrogenase | 1.2 | 0.004 | | |
| [G] Carbohydrate transport and metabolism | | | | | |
| fruF | STM2206 | bifunctional PTS system fructose-specific transporter subunit IIA HPr protein | 1.0 | 0.006 | - | |
| ydeA | STM1522 | sugar efflux transporter | 1.1 | 0.003 | - | |
| yicJ | STM3749 | α-xylulose isomerase | 1.1 | 0.005 | | |
| STM2757 | | cytoplasmic protein | 1.1 | 0.007 | | |
| STM0885 | | phosphotransferase system | 1.0 | 0.008 | | |
| [I] Lipid transport and metabolism | | | | | |
| ybG | STM0865 | undecaprenyl pyrophosphate phosphatase | 1.3 | 0.004 | | |
| ydfS | STM1257S | acetyl-CoA/acyetoacetyl-CoA transferase subunit β | 1.0 | 0.006 | | |
| [P] Inorganic ion transport and metabolism | | | | | |
| fhuF | STM4550 | ferric hydroximate transport ferric iron reductase | 1.0 | 0.004 | | |
| smvA | STM1574 | methyl viologen resistance protein SmvA | 1.0 | 0.004 | | |
| [Q] Secondary metabolites biosynthesis, transport, and catabolism | | | | | |
| hpaB | STM1099 | 4-hydroxophenylacetate 3-monoxygenase oxygenase subunit | 1.2 | 0.004 | | |
| FUNCTION UNKNOWN OR POORLY CHARACTERIZED | | | | | |
| sseI | STM1051 | secreted effector protein SseI | 1.2 | 0.004 | | |
| sshH2 | STM2241 | Effector protein, E3 ubiquitin ligase | 1.4 | 0.004 | | |
| yqA | STM3049 | hemolysin III | 1.2 | 0.004 | | |
| ydbI | STM1646 | Dicarboxylate transport | 1.2 | 0.004 | | |
| yebG | STM1882 | DNA damage-inducible protein | 1.3 | 0.005 | | |
The roles NsrR regulon gene products may be playing in the SeT-tomato interaction are not clear. A role in virulence has been identified for the NsrR-regulated STM1808 and ytfE (Karlinsey et al., 2012). While the NsrR regulon is needed for NO detoxification, the main gene used for this function under aerobic conditions, hmp (Bang et al., 2006; Karlinsey et al., 2012), was not detected in our study.

The redox-sensitive transcriptional activator soxR was up-regulated in shoots (3.1-fold increase, \( q = 0.002 \)) and roots (1.9-fold increase, \( q = 0.003 \) (Table 5)). SeT could be responding to oxidative stress induced in the plant. *Salmonella* flagellin 22 has been reported to induce reactive oxygen species (ROS) production in tomato and *Nicotiana benthamiana* (Meng et al., 2013). Interestingly, it was suggested that *S. Typhimurium*

### Table 3 (continued)

| Gene     | NCBI tag | Annotation                                      | Shoots | Roots |
|----------|----------|-------------------------------------------------|--------|-------|
|          |          |                                                 | \( \log_2 \) fold change | \( q \)-value | \( \log_2 \) fold change | \( q \)-value |
| yfcC     | STM2339  | e4-dicarboxylate anaerobic carrier               | 1.1    | 0.005 |
| ygaC     | STM2801  | hypothetical protein                            | 1.0    | 0.007 |
| yjH      | STM4225  | outer membrane lipoprotein                      | 1.2    | 0.006 |
| ykJ      | STM4263  | inner membrane protein                          | 1.1    | 0.008 |
| STM1650  | STM1650  | putative reverse transcriptase                   | 1.4    | 0.004 |
| STM1585  | STM1585  | outer membrane lipoprotein                      | 1.4    | 0.003 |
| STM2240  | STM2240  | Protein of unknown function (DUP968)            | 1.1    | 0.006 |
| STM1869A | STM1869A | glycoside hydrolase, family 19, chitinase       | 1.1    | 0.007 |
| STM1870  | STM1870  | RecE-like protein                                | 1.2    | 0.006 |

### Figure 2.

Distribution of significantly differentially up-regulated chromosomal and plasmid genes in *Salmonella* Typhimurium LT2 associating with tomato shoots and roots, altered in expression at least 1.0-fold.

### Figure 3.

Percentage of genes significantly differentially up-regulated, altered in expression by at least 1.0-fold, in *Salmonella* Typhimurium LT2 associating with tomato shoots and roots. Functions of genes of interest were classified according to the Clusters of Orthologous Groups of proteins (COGs) (EggNOG 4.5.1).
Table 4. Differentially expressed *Salmonella enterica* Typhimurium LT2 prophage genes on tomato shoots and roots, compared to growth in LB medium. Only genes with statistically significant q-values (Benjamini-Hochberg adjusted p-values) are shown.

| Prophage Genes | Shoots | Roots |
|----------------|--------|-------|
| **Gene ID** | **Function** | **log2 FC** | **q-value** | **log2 FC** | **q-value** |
| STM0894 | Putative Fels-1 prophage excisionase | 1.7 | 0.003 | | |
| STM0895 | Fels-1 prophage protein | 1.8 | 0.003 | | |
| STM0896 | Fels-1 prophage protein | 1.7 | 0.003 | | |
| STM0897 | Fels-1 prophage protein | 2 | 0.003 | | |
| STM0899 | Fels-1 prophage protein | 1.6 | 0.003 | | |
| STM0900 | DNA primase; Putative Fels-1 prophage DNA or RNA helicases of superfamily II | 1.8 | 0.009 | | |
| STM0904 | Fels-1 prophage protein | 1.1 | 0.004 | | |
| STM0906 | Phage holin, lambda family | 1.5 | 0.003 | | |
| STM0907 | Lytic enzyme, chitinase | 1.7 | 0.003 | | |
| STM0909 | Hypothetical protein; Fels-1 prophage protein | 2.1 | 0.006 | | |
| STM0911 | Phage portal protein, lambda family | 1.9 | 0.007 | | |
| STM1008.S | RecT protein; Gifsy-2 prophage protein | 1.1 | 0.007 | | |
| STM1010 | Gifsy-2 prophage protein | 1 | 0.008 | | |
| STM1011 | Gifsy-2 prophage protein | 1.8 | 0.006 | | |
| STM1868A | Lytic enzyme | 3.8 | 0.006 | 2.9 | 0.009 |
| STM1869 | Phage-tail assembly-like protein; head-tail joining protein | 1.4 | 0.004 | 1.3 | 0.005 |
| STM2237 | Phage holin, lambda family | 1.4 | 0.005 | | |
| STM2243 | Putative tail fiber protein of phage | 1 | 0.006 | | |
| STM2601 | Minor capsid protein FI | 1.1 | 0.006 | | |
| STM2604 | Phage head-like protein | 1.4 | 0.005 | | |
| STM2617 | Gifsy-1 prophage protein antitermination protein Q | 1.2 | 0.006 | | |

Table 5. Stress response and host adaptation genes up-regulated in *Salmonella* Typhimurium LT2 colonizing tomato shoots and roots compared to growth in LB, represented by log2 fold change with statistically significant q-values (Benjamini-Hochberg adjusted p-values; refer to Tables 2 and 3). The gene annotations and functions were searched in Clusters of Orthologous Groups of proteins (COGs) using EggNOG 4.5.1 and further searched in UniProt.

| Gene | Annotation/function | Shoots | Roots |
|------|---------------------|--------|-------|
| **YqR** | Reduces the permeability of the outer membrane to copper. Seems to be involved in the regulation of biofilm formation. May decrease biofilm formation by repressing cell-cell and cell-surface interaction | 6.9 | 6.6* |
| **YfE** | Di-iron-containing protein involved in the repair of iron-sulfur clusters damaged by oxidative and nitrosative stress conditions; NsrR regulon | 3.7* | 2.9* |
| **yoaG** | DUF1869 domain-containing protein; NsrR regulon gene | † | † |
| **ygbA** | Nitrous oxide-stimulated promoter; NsrR regulon | 3.5 | 3.5 |
| **STM1808** | Putative cytoplasmic protein; NsrR regulon | - | - |
| **yfhH** | DNA-binding transcriptional regulator; possible NsrR binding site in *E. coli* | 1.0 | 1.2 |
| **sosR** | Redox-sensitive transcriptional activator SoxR | 3.1 | 1.9 |
| **yhpD** | Alcohol dehydrogenase; induced under chlorine-based oxidative stress | - | - |
| **yhcN** | Putative outer membrane protein; induced under chlorine-based oxidative stress | † | † |
| **yhaK** | Pirin domain protein-oxidative stress sensor | 2.1 | 1.4 |
| **yjeE** | Exopolysaccharide production protein YjbE | 3.0 | 5.9 |
| **YqR** | Surface antigen; putative outer membrane lipoprotein | 3.8* | - |
| **IbpB** | Heat shock protein IbpB | - | 5.3 |
| **haJ** | Heat-inducible protein HaJ | 1.0 | 1.1 |
| **STM1485** | Acid shock protein | 5.7* | 8.0 |
| **STM1513** | Stress-induced bacterial acidophilic repeat motif | 3.3 | - |
| **yhhW** | Quecerin 2,3-dioxygenase | 3.5* | - |
| **basS** | Member of the 2-component regulatory system BasS/BasR. Autophosphorylates and activates BasR by phosphorylation. Plays a role in the adaptation of host environment (neutrophils) | - | 2.2 |
| **marA** | DNA-binding transcriptional activator MarA | 4.8 | 2.5 |
| **marB** | Multiple antibiotic resistance protein MarB | 3.7 | 1.7 |
| **marR** | DNA-binding transcriptional repressor MarR | 3.7 | 1.6 |
| **STM3030** | YhdX protein family; cephalosporin resistance in S. Typhimurium | - | 5.7 |

*Weakly significant.
†Expression not detected in LB but detected at high level in SeT-shoots or SeT-roots.
was able to suppress the oxidative burst in *N. tabacum* as only heat inactivated, but not live, *S. Typhimurium* induced an oxidative burst (Shirron and Yaron, 2011). Response to oxidative stress was identified in *S. infantis* internalized in lettuce leaves (Zhang et al., 2014). NO is also a known activator of SoxR in *E. coli* (Nanoshiba et al., 1993). Moreover, the genes *yhcN* and *yqhD*, which were previously induced under chlorine-based oxidative stress in *Salmonella* (Wang et al., 2010), were strongly up-regulated in *SeT*-shoots and *SeT*-roots, respectively (Table 5). Expression of the gene *yhaK*, which has been postulated to serve as a sensor for oxidative conditions in enterobacteria (Gurmu et al., 2009), was also detected in *SeT*-shoots and -roots. This gene was strongly up-regulated in *Salmonella* inoculated into macerated cilantro and lettuce leaves (Goudeau et al., 2012). Taken together, these results suggest that *SeT* is responding to nitrosative and oxidative stress when associating with the tomato leaf and root surface.

### 3.4. Energy production and conversion

Several hydrogenase- and dehydrogenase-encoding genes (*hycC, hycD, hycE, hycG, STM1787, yqhD, yneI*), oxidases and reductases were up-regulated in *SeT* associated with both shoots and roots (Tables 2 and 3). The up-regulated gene STM1253 encodes a cytochrome b, and genes STM1792 and STM1793 putatively encode cytochrome oxidase subunits, pointing to aerobic respiration. However, genes involved in anaerobic respiration - *asrC, trrA, trrB, yfcC* in *SeT*-roots, *nar* in *SeT*-shoots were also detected, suggesting that *SeT* may be employing anaerobic respiration on parts of the plant surface. The gene *nar* was the most strongly up-regulated (Table 2). The gene encoding succinate semialdehyde dehydrogenase, *yneI*, had increased levels of transcription in both shoot- and root-associated *SeT*. Aldehyde dehydrogenases are known to play an important role in the metabolic conversion of carbohydrates, as well as the detoxification of endogenous and exogenous aldehydes (Zheng et al., 2013) (Table 2).

### 3.5. Amino acid transport and metabolism

Biosynthesis of amino acids has been identified as an important process for successful colonization of tomato wounds (de Moraes et al., 2017). In this study assessing *SeT* epiphytic growth habit on the tomato plant surface, several genes known to be involved in various amino acid metabolism or biosynthesis pathways were also up-regulated (Tables 2 and 3). Most notable were genes involved in tryptophan biosynthesis, *trpB, trpC, trpD, trpE, aroF* and transport (*mtr*). The gene *tyrA* was also significantly differentially expressed but only 0.9-fold. A role for typhopain biosynthesis in *SeT* biofilm formation has been identified (Hamilton et al., 2009). By contrast, *ycfR*, a gene that encodes a putative outer membrane protein known to decrease biofilm formation (Zhang et al., 2007) was strongly expressed in *SeT*-shoots (6.9-fold increase, q = 0.006) and -roots (6.6-fold increase, q = 0.011) (Table 5). In *S. Typhimurium* LT2, *ycfR* deletion decreased cell attachment to spinach leaves and grape tomatoes (Salazar et al., 2013), but deletion of *ycfR* in *S. Typhimurium* 14028 enhanced attachment to cabbage leaves (Kim and Yoon, 2019). In addition to typhopain, other amino acid synthesis genes were detected; *aroE, hisG, tyrA* and *meta* play a role in the phenylalanine, tyrosine and methionine biosynthesis pathways. *S. Typhimurium* 14028 auxotrophs of several amino acids, including tryptophan, serine, methionine and threonine exhibited reduced fitness inside tomato fruit wound environments (de Moraes et al., 2017), and genes involved in the biosynthesis of all these amino acids were found to be up-regulated in *SeT* colonizing tomato shoot and/or root surfaces in this study (Tables 2 and 3). Cysteine biosynthesis genes *cysP* and *cysN*, encoding genes needed for sulphate reduction, were markedly more transcribed in both *SeT*-shoots and -roots compared to LB culture. Up-regulation of genes involved in cysteine biosynthesis has been previously reported in *Salmonella* colonizing sprouts (Brankatschk et al., 2012; Kwan et al., 2018) and in tomato fruit wounds (de Moraes et al., 2018). A cyst*E* mutant was impaired in a *Salmonella*-alfalfa system compared to wild type (Kwan et al., 2018).

### 3.6. Carbohydrate, lipid and inorganic ion metabolism and efflux transporters

Carbohydrate and lipid metabolism genes were up-regulated on roots, possibly reflecting nutrient limitations on shoots. The highest increase in transcription (4.4-fold) was detected in gntK (STM3542), which encodes a gluconokinase, specifically an ATP:D-glucosate 6-phosphotransferase in the pentose phosphate pathway, but this value was not significant (q = 0.02). Tomato colonization induced *SeT* gene expression of a number of efflux/transporter genes, *ydeA, smmA* and STM2757. The gene *smmA* in *S. Typhimurium* encodes an efflux pump shown to export acriflavin and other quaternary ammonium compounds (Villagra et al., 2008). In shoot-associated *SeT*, the ion transport protein *fluU* exhibited higher transcriptional levels than growth in LB (Table 3).

### Table 6. Pathogenesis-related genes up-regulated in *Salmonella* Typhimurium LT2 colonizing tomato shoots and roots compared to growth in LB, represented by log2 fold change with statistically significant q-values (Benjamini-Hochberg adjusted p-values; refer to Tables 2 and 3). The gene annotations and functions were searched in Clusters of Orthologous Groups of proteins (COGs) using EggNOG 4.5.1 and further searched in UniProt.

| Gene  | Annotation/function | Shoots | Roots | log2 Fold Change |
|-------|---------------------|--------|-------|-----------------|
| *sseB* | Secreted effector protein, enhanced serine sensitivity protein SseB | - | 6.2 |
| *ssaE* | Type III secretion system chaperone SsaE | - | 1.9 |
| *ssaC* | Type III secretion system apparatus protein | - | 1.4† |
| *ssaH* | Type III secretion system apparatus protein | - | 5.0 |
| *ssaS* | Type III secretion system apparatus protein | - | 4.4† |
| *ssel* | Secreted effector protein Ssel | - | 1.2 |
| *spH2* | Effector protein, E3 ubiquitin ligase | - | 1.4 |
| *yfaA* | Hemolysin III | - | 1.2 |
| *rck* | Resistance to complement killing; putative virulence related protein PagC | - | 1.0 |
| *sprA* | Salmonella plasmid virulence: outer membrane protein | 1.9 | 1.2 |
| *sprB* | Salmonella plasmid virulence protein | 1.9 | 1.5 |
| *sprC* | Virulence protein; secreted effector protein SprvC | 1.8 | - |
| *sprD* | SPI-2 type III secretion system effector cysteine hydrolase SprvD | 1.5 | - |

† Weakly significant.
3.7. Pathogenicity related genes

The gene \textit{sseB}, encoding a \textit{Salmonella} pathogenicity island 2 type III secretion system (T3SS2) effector protein, and its chaperone \textit{ssaE} required for \textit{sseB} secretion (Miki et al., 2009), were both up-regulated in \textit{SeT}-roots (Table 6). The genes \textit{ssaC}, \textit{ssaH} and \textit{ssaS} for secretion system apparatus proteins were upregulated in \textit{SeT}-roots. The gene \textit{ssaC} is induced under acidic conditions (Rappl et al., 2003) and \textit{ssaH}, which regulates the secretion of \textit{ssaI} (not detected in our study), was stabilized by \textit{ssaE} (Takaya et al., 2019). Transcription of effectors \textit{sseI} and \textit{sspH2}, together with a number of plasmid-encoded \textit{spv} genes was also detected. These genes are serving an unknown function in the plant niche. The \textit{spvABCD} genes comprise an operon whose expression is induced inside animal host cells, with \textit{spvB} and \textit{spvC} known to be involved in virulence by blocking host defence responses (Guiney and Fierer, 2011). When the phosphothreonine lyase SpvC was expressed in \textit{Arabidopsis} protoplasts, the protein also suppressed certain plant defence-related genes (Neumann et al., 2014).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Gene expression validation with select genes of (A) \textit{S. Typhimurium} and (B) \textit{S. Newport} colonizing tomato. The x-axis represents log$_2$ fold change in gene expression of \textit{S. Typhimurium} genes as measured by RNA-Seq and the y-axis represents log$_2$ fold change in gene expression of \textit{S. Typhimurium} or \textit{S. Newport} genes as measured by q-RT-PCR. The R$^2$ value of the regression line is denoted; $p < 0.001$.}
\end{figure}
Expression levels of select genes of SeT measured by q-RT-PCR in a repeated experiment correlated well with RNA-Seq results ($R^2 = 0.813, p < 0.001$; Figure 4A), validating the RNA-Seq results in SeT. In addition, expression levels of select genes were measured in SeN to determine whether an environmental strain of a serotype commonly involved in fruit and vegetable-borne illness outbreaks (Angelo et al., 2015; Greene et al., 2008) would show similar genetic responses to SeT. We assayed 10 genes and found high correlation between RNA-Seq of SeT and q-RT-PCR of SeN genes ($R^2 = 0.874, p < 0.001$; Figure 4B). As detected by q-RT-PCR, gene expression of the NsrR-regulated gene ygbA, the oxidative stress gene soxR, stress response/host adaptation genes ycfR, marR and yhbE and the amino acid biosynthesis genes tryD and tryE were significantly higher than the control ($p < 0.05$). In agreement with RNA-Seq analysis of SeT (data not shown), the genes lamb and nmpC were down-regulated in SeN ($p < 0.05$).

4. Conclusion

In this study, the gene expression profiles of S. enterica Typhimurium colonizing live, intact and non-diseased tomato shoot and root surfaces were assessed. This work adds to the body of knowledge generated from genome-wide screens to investigate the mechanisms used by S. enterica interacting with plants, which to date have targeted internal plant tissues such as tomato fruit wounds and fruit homogenates, lettuce leaf lysates and leaf lesions, and sprouts (Blankatsch et al., 2012; de Moraes et al., 2017, 2018; George et al., 2018; Goudeau et al., 2012; Kwan et al., 2018; Zarkani et al., 2019). The interaction of enteric pathogens with plants is confounding, as these microbes appear to neither behave as plant pathogens nor as enteric pathogens infecting their respective hosts. The present study provides clear evidence that S. enterica growth on tomato is highly responsive to the plant niche. The tomato surface appears to exert various stresses on S. enterica. Several genes known to be involved in host adaptation, and stress-related genes involved in multidrug resistance, heat and acid shock, nitrosative stress and oxidative stimuli were strongly induced in S. Typhimurium and S. Newport growing on tomato surfaces. Nitrosative and oxidative stress mitigating genes suggest the plant itself is responding to S. Typhimurium. Previous work has shown that S. enterica flagellin 22 can trigger pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) in Arabidopsis (Garcia et al., 2014). Here we provide evidence that S. enterica may be responding to the plant response, specifically invoking ROS and RNS mitigation. Although ROS bursts have been previously detected in flagellin 22-treated plants and dead Salmonella-plant interactions (Meng et al., 2013; Shirron and Yaron, 2011), the implication of NO in the Salmonella-tomato association is a novel discovery. At high levels, NO is itself bactericidal, and at lower levels serves as a signalling molecule modulating several plant processes, including defence against pathogens and stomatal aperture regulation (Muri et al., 2013). The up-regulation of genes involved in dealing with oxidative and nitrosative stress conditions suggests that Salmonella must respond to these stresses to successfully colonize the plant surface. Our research group continues to investigate this interaction and recently revealed that S. Newport can, in fact, elicit the release of NO and ROS in tomato plants (Ferelli et al., 2020).

Whilst most of the metabolic pathways were down-regulated in SeT colonizing tomato shoot and root surfaces in relation to growth in a nutrient-rich medium, biosynthesis of phenylalanine, tyrosine and tryptophan were up-regulated. Amino acid biosynthesis has been identified as a major strategy in S. enterica colonization of tomato wounds, tomato exudates and sprouts (de Moraes et al., 2018; Kwan et al., 2015; Zarkani et al., 2019). Interestingly, in this study, a subset of these amino acid pathway genes was involved in sulphur metabolism and/or anaerobic respiration (aarC, cysD, cysN, metA, narL, ttrA and ttrB). Evidence of anaerobic respiration was detected in S. Typhimurium colonizing lettuce and cilantro soft rot lesions caused by the plant pathogen Dickeya dadantii, where low oxygen tensions exist (Goudeau et al., 2012). In this study, S. enterica was colonizing the surfaces of intact tissues, suggesting that microaerophilic microsites may be present on the plant surface perhaps in leaf crevices or as a result of high bacterial population density. Additionally, Wang et al. (2010) postulated that the up-regulation of cysteine biosynthesis genes of Salmonella under chlorophyll-induced oxidative stress suggested the involvement of cysteine in the oxidative stress response, as a component of iron-sulphur clusters involved in redox reactions. Cysteine may have been similarly implicated in combating oxidative stress in SeT shoots and -roots in this study. The capability of Salmonella to thrive on tomato may in part be due to the dual competitive advantage conferred by switching to anaerobic respiration and the ability to attenuate oxidative stress.

The major difference in gene expression patterns between S. enterica colonizing shoots versus roots was in the expression of genes involved in T3SS2 on SeT-roots. So far, T3SSs have not been thought to play a major role in plant colonization, since S. enterica is unable to infect plant cells. However, the expression of effectors in plant surface-associated SeT in this study may point to a role of effectors in suppressing the immune system, as has been suggested to occur in some studies (Garcia et al., 2014; Neumann et al., 2014; Shirron and Yaron, 2011), or to an as yet unidentified alternate function for these genes.

In this study SeT LT2 was used in the Salmonella-tomato interaction since this strain has a fully annotated genome. Validation using an environmental strain of SeN demonstrated concordance in gene expression with SeT. This study therefore provides a valuable baseline for research in epiphytic Salmonella-plant associations. Future work assessing S. enterica surface colonization of plants should continue to decipher the interaction with the plant immune system triggered through enteropathogen recognition. Investigating where enteropathogens fit along the spectrum of plant-bacterial interactions, spanning plant pathogens to benign microorganisms, will moreover further reveal the mechanisms by which plants recognize and recruit commensal and beneficial microbes to their surface, while excluding perceived threats.

Declarations

Author contribution statement

S. Han: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
A. M. C. Ferelli: Performed the experiments; Analyzed and interpreted the data.
S.-S. Lin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
S. A. Micallef: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at NCBI GenBank Gene Expression Omnibus (GEO) under the accession number GSE73192.
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