Heliyon 6 (2020) e04952

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

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

Sanghyun Han [a,1,2], Angela Marie C. Ferelli [a], Shih-Shun Lin [b], Shirley A. Micallef [a,c,*,2]

[a] Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland, USA
[b] Institute of Biotechnology, National Taiwan University, Taipei, Taiwan
[c] Centre for Food Safety and Security Systems, University of Maryland, College Park, Maryland, USA

ARTICLE INFO

Keywords:
Agricultural science
Microbiology
Human pathogens on plants
Nitrosative stress
Oxidative stress
Type III secretion systems
NsrR regulon
Food safety

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 (*Se*T) 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 *Se*T 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 *Se*T on roots. Gene expression in *Se*T was validated against *Se*T 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, T3SS2 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 vegetables including tomato (IFSAC, 2018). It is widely accepted that enteric pathogens have evolved strategies to survive the transition between herbivorous hosts by successfully colonizing and persisting in plant-associated niches (Fletcher et al., 2013). Our previous work supports this notion; *S.* Typhimurium and *S.* Newport colonizing tomato shoot and fruit surfaces could proliferate in a serotype, cultivar and plant organ dependent manner (Han and Micallef, 2014) and responded to cultivar differences in the exometabolome of tomato roots, shoots and fruit (Han and Micallef, 2016).

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

* Corresponding author.
E-mail address: smicall@umd.edu (S.A. Micallef).
[1] Current Address: Rural Development Administration, Jeonju 54875, Republic of Korea.
[2] Authors made equal contribution.

https://doi.org/10.1016/j.heliyon.2020.e04952
Received 3 January 2020; Received in revised form 13 March 2020; Accepted 11 September 2020

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) (*Se*T) and *S. enterica* Newport MDD314 (*Se*N), 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 ($\sim 10^9$ 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 gnotobiotically in an upright position in 245 mm × 245 mm square culture dishes (Corning, Acton, MA, USA) containing Murashige and Skoog (MS) medium (MP Biomedicals LLC, Solon, OH, USA) supplemented with 2% sucrose and 1.2% agar. The culture dishes were kept at 26°C/18 °C (day/night) under a 16L:8D photoperiod. Inoculation was performed on leaves at the three-to four-true leaf stage or on roots on separate plants; 10 locations per plant on leaves or roots were spotted with 10 μl of $\sim 10^9$ CFU/ml *S.* Typhimurium or *S.* Newport, in triplicate. The culture dishes were re-incubated for 3 days at 28 °C under a 16L:8D photoperiod until *S. enterica* cell retrieval.

2.2. Total RNA isolation, rRNA removal, cDNA synthesis and RNA-Seq libraries

Inoculated plant shoots and roots (N = 3 each) were cut and placed in separate Whirl-Pak bags containing 30 ml of RNAprotect Cell Reagent (Qiagen, Valencia, CA, USA) and 15 ml of sterile PBS to stabilize microbial RNA. The bags were sonicated in Branson Ultrasonic Cleaner (Branson Ultarasonics Corperation, Danbury, CT, USA) for 2 min and hand-rubbed for another minute to dislodge attached *S. enterica* cells from the plant, and rinsates centrifuged for 1 h at 9,000 *g* at 4 °C. 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 *Se*T 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 (adaptor) and purified using Agencourt AMPure XP System (Beckman Coulter, Brea, CA, USA), followed by enrichment of cDNA in the library by performing PCR with two primers that specifically anneal to the ends of the adaptors. Index barcodes were incorporated during this step to replace the reverse primer. For each reaction, different barcodes were added. PCR products were purified using Agencourt AMPure XP System (Beckman Coulter). The prepared RNA-Seq libraries were checked for quality and quantity on the Bioanalyser and sequenced on an Illumina Hi-Seq 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 $q \leq 0.006$ and ≤ 0.009 for chromosome-encoded genes from *Se*T-shoots and -roots, respectively and $q \leq 0.03$ 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 *Se*T and a serotype relevant to food safety of tomato - *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 *Se*T colonizing tomato shoots and roots under the same conditions as before, and *Se*N 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 H_2O. 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 $p \leq 0.05$. Log_2 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 *Se*T LT2 data to *Se*N. 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 *Se*T epiphytically colonizing tomato resulted in expression signals for 4,227 chromosomal genes in *Se*T colonizing shoots (*Se*T-shoots) and 4,306 genes in *Se*T colonizing roots (*Se*T-roots), indicating 92–94% coverage of the whole transcriptome of *S*. Typhimurium. Also detected were 98 transcripts of plasmid-encoded genes from *Se*T-shoots and 96 from *Se*T-roots. Of the chromosomal genes, 173 (4.1 %; $q \leq 0.006$) and 347 (8.1%; $q \leq 0.009$) were differentially expressed on tomato shoots and roots, respectively, relative to growth in LB culture, 123 of which were up-regulated (≥1.0 log_2 fold change; Tables 2 and 3). More chromosomal genes were detected as up-regulated in *Se*T-roots – 107 compared to 49 in shoots. Only 16 genes were unique to *Se*T-shoots, compared to 74 in *Se*T-roots (Figure 2). In addition, 6 plasmid-encoded genes were found to be up-regulated ($q \leq 0.03$). Genes up-regulated in *Se*T associating with both plant structures point to a core set of genes that facilitate *Salmonella* 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).

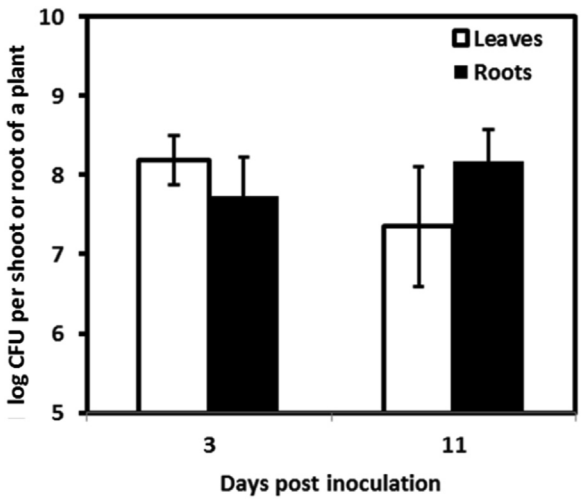

Figure 1. Log CFU of *Salmonella* LT2 retrieved from shoots and roots of separate tomato plants 3 and 11 days post-inoculation. Inoculum load was 8 log CFU of *S*. Typhimurium per shoot or root of plants. Error bars represent standard deviation, N = 6.

Although overall, genes involved in carbohydrate transport and metabolism were significantly down-regulated in relation to growth in nutrient rich medium, several *Se*T 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 *Se*T-shoots and 16 in *Se*T-roots) and genes involved in the transport and metabolism of amino acids and inorganic ions (13 and 11 genes in *Se*T-shoots and *Se*T-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 *Se*T-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
lamB	Maltoporin (maltose transport)	GTATTGGCTGGACGGGAAGC	TCGCCCTCTTTCCACACTTC	This Study
aphA	Class B acid phosphatase	AACGGCTGGGATGAGTTCAG	CGTCTGACTACGACCAGTGAC	This Study
malE	Maltose ABC transporter substrate-binding protein MalE	ATCGCCGACTTCCCTTTCAC	ACAAAGACCTCGTCCCGAAC	This Study
marA	DNA-binding transcriptional activator for antibiotic resistance operon MarRAB	TACGGCTGCGGATGTATTGG	CGAGGATAACCTGGAGTCGC	This Study
nmpC	Outer membrane porin protein	GTCCGTCCATCGCTTACCTG	GCTTTGGTGAAGTCGCTGTC	This Study
rpoD	RNA polymerase σ-factor	GTGAAATGGGCACTGTTGAACTG	TTCCAGCAGATAGGTAATGGCTTC	Karlinsey et al. (2012)
soxR	Redox-sensitive transcriptional activator	AGTGGAAGCAGCTCTCATCG	TACAACCGTCCAGCTCATCG	This Study
trpD	Tryptophan biosynthesis protein	GTCCATCCTGACGACACAGG	AATCGGCTGTAGGGTGTTGG	This Study
trpE	Tryptophan biosynthesis protein	CGCTTTTTCACCAGGTCTGC	AACGCCTGAATGGTGACAGT	This Study
ttrA	Tetrathionate reductase subunit A	TCCATTGAGACAGGTTGCCC	CGCTGGCGGATTACATTGTG	This Study
ycfR	Outer membrane protein	ACGCCAGAAGGTCAACAGAA	GGGCCGGTAACAGAGGTAA	This Study
ydaA	Universal stress protein E	GACCACCCTGCTTTCTCCTG	GGACGATTGTGCCAGACCAC	This Study
ygbA	Cytoplasmic protein in NsrR regulon	GTGGGCACTGGCTTTCATAC	GCCTGGTAAACGTATCGCTC	This Study
yjbE	Outer membrane protein	GCGTTAGCACCGTAAGTTCG	CTACTGGGTACTGGTGGTCG	This Study

Table 2. Differentially up-regulated *Salmonella enterica* Typhimurium LT2 genes (≥1.5 $\log_2$ 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		Roots	
			$\log_2$ fold change	*q*-value	$\log_2$ fold change	*q*-value
CELLULAR PROCESSES AND SIGNALING						
[M] Cell wall/membrane/envelope biogenesis						
yhdV	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						
ibpB	STM3808.S	heat shock protein IbpB			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	STM1519.S	DNA-binding transcriptional activator MarA	4.8	0.002	2.5	0.003
marR	STM1520	DNA-binding transcriptional repressor 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	STM3280.S	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
yneI	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						
ytfE	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	SSTM3905	Threonine dehydratase			3.4	0.008
[P] 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/BasR. 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						
ssaC	STM1394	Type III secretion system apparatus protein			1.4	0.010*
ssaH	STM1407	Type III secretion system apparatus protein			5.0	0.009
ssaS	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
ssaE	STM1396	secretion system effector SsaE			1.9	0.003
sseB	STM1398	secreted effector protein SseB, enhanced serine sensitivity protein SseB			6.2	0.007
yeaK	STM1282	YbaK prolyl-tRNA synthetase associated			‡	0.006
ygbA	STM2860	Nitrous oxide-stimulated promoter; NsrR regulon	3.5	0.002	3.5	0.003

(*continued on next page*)

Table 2 (*continued*)

Gene	NCBI tag	Annotation	Shoots		Roots	
			log_2 fold change	*q*-value	log_2 fold change	*q*-value
yoaG	STM1272	DUF1869 domain-containing protein; NsrR regulon gene	‡	0.004	‡	0.006
ygbE	STM2932	inner membrane protein	1.5	0.002	1.7	0.003
yhaK	STM3236	pirin domain protein	2.1	0.002	1.4	0.003
yhcN	STM3361	Putative outer membrane protein	‡	0.004		
STM3362	STM3362	Putative periplasmic protein	‡	0.004		
yjbE	STM4222.S	Exopolysaccharide production protein YjbE	3.0	0.003	5.9	0.003
yhhW	STM3544	quercetin 2,3-dioxygenase	3.5	0.009*		
STM0910	STM0910	terminase, large subunit	-		2.0	0.003
STM1485	STM1485	Acid shock protein	5.7	0.012*	8.0	0.008
STM1513	STM1513	Stress-induced bacterial acidophilic repeat motif	3.3	0.002		
STM1808	STM1808	Putative cytoplasmic protein; NsrR regulon			‡	0.008
STM1851	STM1851	hypothetical protein	1.3	0.003	1.6	0.003
STM05615	STM05615	hypothetical protein	2.2	0.002	2.0	0.003
STM4271	STM4271	murein hydrolase regulator LrgA	1.4	0.006	2.2	0.003
STM4552	STM4552	putative inner membrane protein	2.3	0.002		
STM1528	STM1528	putative outer membrane protein; calcium/calmodulin dependent protein kinase ii association			1.9	0.003
STM3030	STM3030	YfdX protein			5.7	0.008
STM04875	STM04875	hypothetical protein			1.6	0.005
STM04895	STM04895	hypothetical protein			1.5	0.007
PLASMID ENCODED						
spvA	PSLT040	plasmid virulence; outer membrane protein	1.9	0.014	1.2	0.030
spvB	PSLT039	plasmid virulence: hydrophilic protein	1.9	0.015	1.5	0.028
spvC	PSLT038	plasmid virulence: hydrophilic protein; secreted effector protein SpvC	1.8	0.016		
spvD	PSLT037	hydrophilic protein	1.5	0.026		

*Weakly significant.
‡Expression not detected in LB but detected at high level in *Se*T-shoots or *Se*T-roots.

3.2. Stress response and plant host adaptation

Several genes known to respond to environmental stresses were strongly induced in the *Se*T-tomato interaction, compared to *Se*T growing in LB. In both shoot- and root-associated *Se*T, genes in the multiple antibiotic resistance operon MarRAB (*marR*, *marA*, *marB*), known to confer resistance to multiple antibiotics, disinfectants and oxidizers in *Escherichia. coli* and *Salmonella* (Alekshun and Levy, 1999), were up-regulated (Tables 2 and 5). Salicylates, found in abundance in tomato, are known inducers of the marRAB operon by binding to MarR which represses expression of marRAB. Salicylate binding to MarR lifts repression of *marA*, a transcription factor that controls multidrug efflux and porin synthesis (Cohen et al., 1993). The gene *basS/pmrB* (part of the 2-component regulatory system PmrA/PmrB) was up-regulated in *Se*N--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 *Se*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*. Typhimurium (Lin et al., 2019). STM1530, a gene that confers resistance to ceftriaxone, was upregulated on roots (Hu et al., 2011) (Table 2). The gene *yhhW* which encodes quercetin 2,3-dioxygenase showed 3.5-fold ($q = 0.009$) higher expression in *Se*T-shoots (Table 5). This gene is known to break down the plant flavonoid quercetin in *E. coli* (Adams and Jia, 2005) and has been reported to be transcribed in *Salmonella* inhabiting cilantro and lettuce soft rot lesions (Goudeau et al., 2012) and in *E. coli* subjected to chlorine or hydrogen peroxide stress (Wang et al., 2009).

The gene of unknown function *yjbE* also exhibited markedly higher transcriptional levels in both *Se*T-shoots (3.0-fold increase; $q = 0.003$) and -roots (5.9-fold increase; $q = 0.003$). This gene was reported to facilitate growth inside red tomatoes (Marvasi et al., 2016). Together with *ycfJ*, a putative outer membrane lipoprotein, these two genes are responsive to acetyl phosphate and regulated by RscC, and possibly RscB which represses flagellar synthesis (Wolfe, 2005). Transcription of the heat shock protein *ibpB* was over 5-fold higher in root-associated *Se*T, while another heat shock protein, *hslJ*, was weakly induced in both shoots and roots (Tables 2, 3, and 5). Moreover, the acid shock response 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 *Se*T-shoots and –roots and STM1808 *Se*T-roots) were markedly up-regulated (Table 5). The gene *yfhH* was also expressed in both *Se*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 (Karlinsey 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 (Henard 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 suspension-grown *Arabidopsis* cells (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 $\log_2$ 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		Roots	
			$\log_2$ fold change	*q*-value	$\log_2$ 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						
hslJ	STM1648	heat-inducible protein HslJ	1.0	0.005	1.1	0.005
INFORMATION STORAGE AND PROCESSING						
[J] Translation, ribosomal structure and biogenesis						
rsuA	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
yneJ	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-hydroxyphenylacetate 3-monooxygenase 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
ttrA	STM1383	tetrathionate reductase subunit A			1.3	0.003
ttrB	STM1385	tetrathionate reductase subunit B			1.2	0.005
ydiQ	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	STM1253	cytochrome b561			1.4	0.003
STM1787	STM1787	hydrogenase 1 large subunit	1.1	0.006	1.2	0.006
STM1792	STM1792	putative cytochrome oxidase subunit I			1.2	0.004
STM1793	STM1793	putative cytochrome oxidase subunit II			1.0	0.006
[E] Amino acid transport and metabolism						
aroF	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	-	
serA	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	-	
yicI	STM3749	α-xylosidase			1.1	0.005
STM2757	STM2757	cytoplasmic protein			1.1	0.007
STM0885	STM0885	phosphotransferase system			1.0	0.008
[I] Lipid transport and metabolism						
ybjG	STM0865	undecaprenyl pyrophosphate phosphatase			1.3	0.004
ydiF	STM1357.S	acetyl-CoA/acetoacetyl-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-hydroxyphenylacetate 3-monooxygenase oxygenase subunit			1.2	0.004
FUNCTION UNKNOWN OR POORLY CHARACTERIZED						
sseI	STM1051	secreted effector protein SseI			1.2	0.004
sspH2	STM2241	Effector protein, E3 ubiquitin ligase			1.4	0.004
yqfA	STM3049	hemolysin III			1.2	0.004
ydbH	STM1646	Dicarboxylate transport			1.2	0.004
yebG	STM1882	DNA damage-inducible protein			1.3	0.005

(*continued on next page*)

Table 3 (*continued*)

Gene	NCBI tag	Annotation	Shoots		Roots	
			$\log_2$ fold change	q-value	$\log_2$ fold change	q-value
yfcC	STM2339	c4-dicarboxylate anaerobic carrier			1.1	0.005
ygaC	STM2801	hypothetical protein			1.0	0.007
yjbH	STM4225	outer membrane lipoprotein			1.2	0.006
yjcB	STM4263	inner membrane protein			1.1	0.008
STM1650	STM1650	putative reverse transcriptase	1.4	0.004	1.3	0.006
STM1585	STM1585	outer membrane lipoprotein			1.4	0.003
STM2240	STM2240	Protein of unknown function (DUF968)			1.1	0.006
STM1869A	STM1869A	glycoside hydrolase, family 19, chitinase			1.1	0.007
STM1870	STM1870	RecE-like protein			1.2	0.006
PLASMID ENCODED						
rck	PSLT040	plasmid virulence; outer membrane protein			1.0	0.03
PSLT062	PSLT062	putative cytoplasmic protein	1.0	0.023		

The roles NsrR regulon gene products may be playing in the *Se*T-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)). *Se*T 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

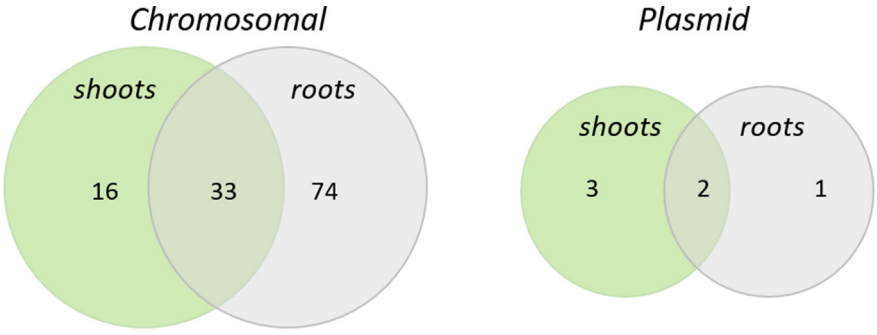

Figure 2. Distribution of significantly differentially up-regulated chromosomal and plasmid genes in *Salmonella* Typhimurium LT2 associating with tomato shoots and roots, altered in expression at least 1.0-fold.

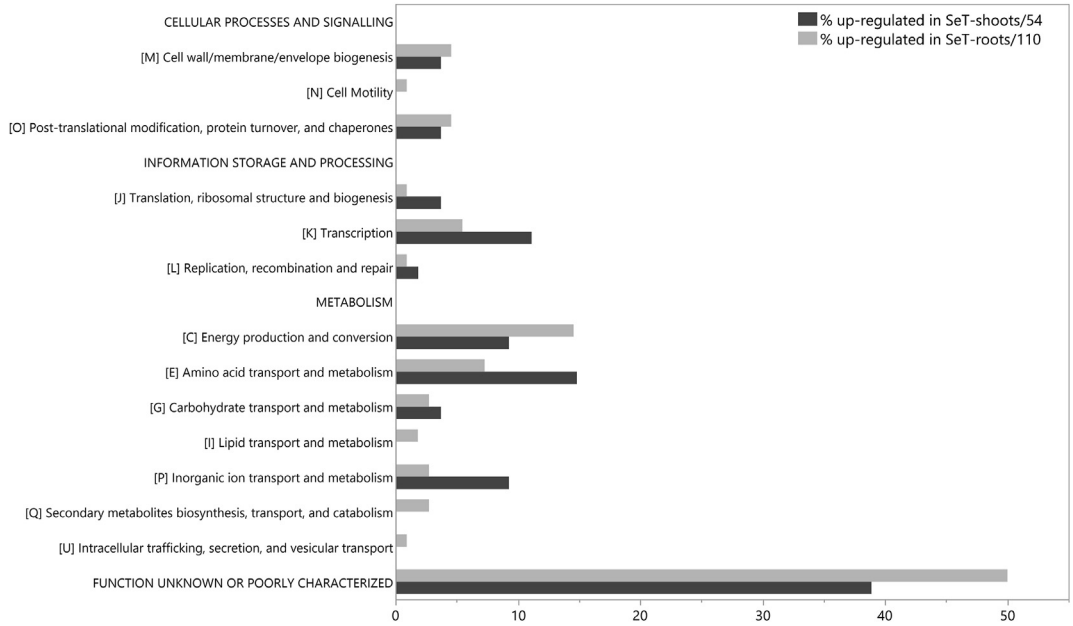

Figure 3. Percentage of genes significantly differentially up-regulated, altered in expression by at least 1.0-fold, in *Salmonella* Typhimurium LT2 associating with tomato shoots and roots. Functions of genes of interest were classified according to the Clusters of Orthologous Groups of proteins (COGs) (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	log_2 FC	*q*-value	log_2 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 FII			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 log_2 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
		log_2 Fold Change	
ycfR	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*
ytfE	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
soxR	Redox-sensitive transcriptional activator SoxR	3.1	1.9
yqhD	Alcohol dehydrogenase; induced under chlorine-based oxidative stress	-	4.3
yhcN	Putative outer membrane protein; induced under chlorine-based oxidative stress	‡	-
yhaK	Pirin domain protein-oxidative stress sensor	2.1	1.4
yjbE	Exopolysaccharide production protein YjbE	3.0	5.9
ycfJ	Surface antigen; putative outer membrane lipoprotein	3.8*	-
ibpB	Heat shock protein IbpB	-	5.3
hslJ	Heat-inducible protein HslJ	1.0	1.1
STM1485	Acid shock protein	5.7*	8.0
STM1513	Stress-induced bacterial acidophilic repeat motif	3.3	-
yhhW	quercetin 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	YfdX protein family; cephalosporin resistance in *S.* Typhimurium.	-	5.7

*Weakly significant.
‡Expression not detected in LB but detected at high level in *Se*T-shoots or *Se*T-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* (Nunoshiba 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 *Se*T-shoots and *Se*T-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 *Se*T-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 *Se*T 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 *Se*T 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*, *ttrA*, *ttrB, yfcC* in *Se*T-roots*, narI* in *Se*T-shoots were also detected, suggesting that *Se*T may be employing anaerobic respiration on parts of the plant surface. The gene *narI* 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 *Se*T. 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 *Se*T 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 tryptophan biosynthesis in *Se*T 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 *Se*T-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 tryptophan, other amino acid synthesis genes were detected; *aroF, 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 threonione 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 *Se*T colonizing tomato shoot and/or root surfaces in this study (Tables 2 and 3). Cysteine biosynthesis genes *cysD* and *cysN*, encoding genes needed for sulphate reduction, were markedly more transcribed in both *Se*T-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 *cysE metA* 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-gluconate 6-phosphotransferase in the pentose phosphate pathway, but this value was not significant ($q = 0.02$). Tomato colonization induced *Se*T gene expression of a number of efflux/transporter genes, *ydeA*, *smvA* and STM2757. The gene *smvA* in *S.* Typhimurium encodes an efflux pump shown to export acriflavine and other quaternary ammonium compounds (Villagra et al., 2008). In shoot-associated *Se*T, the ion transport protein *fhuF* 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 $\log_2$ 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
		$\log_2$ 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*
sseI	Secreted effector protein SseI	-	1.2
sspH2	Effector protein, E3 ubiquitin ligase	-	1.4
yqfA	Hemolysin III	-	1.2
rck	Resistance to complement killing; putative virulence related protein PagC	-	1.0
spvA	*Salmonella* plasmid virulence: outer membrane protein	1.9	1.2
spvB	*Salmonella* plasmid virulence protein	1.9	1.5
spvC	Virulence protein; secreted effector protein SpvC	1.8	-
spvD	SPI-2 type III secretion system effector cysteine hydrolase SpvD	1.5	-

* Weakly significant.

3.7. Pathogenicity related genes

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

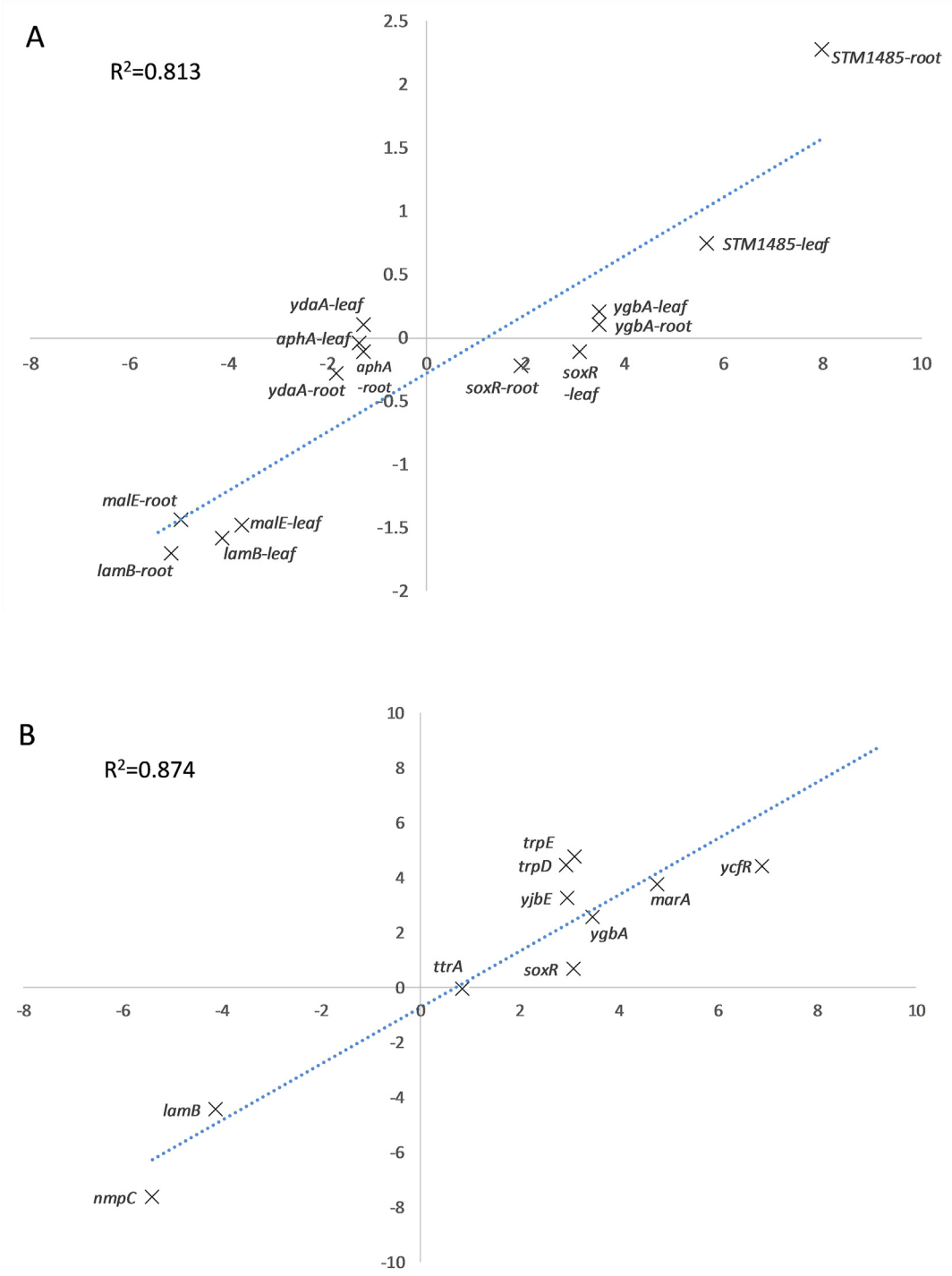

Figure 4. Gene expression validation with select genes of (A) *S.* Typhimurium and (B) *S.* Newport colonizing tomato. The *x*-axis represents $\log_2$ fold change in gene expression of *S.* Typhimurium genes as measured by RNA-Seq and the *y*-axis represents $\log_2$ fold change in gene expression of *S.* Typhimurium or *S.* Newport genes as measured by q-RT-PCR. The R^2 value of the regression line is denoted; $p < 0.001$.

3.8. Validation of transcriptional responses in Salmonella Newport

Expression levels of select genes of *Se*T 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 *Se*T. In addition, expression levels of select genes were measured in *Se*N 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 *Se*T. We assayed 10 genes and found high correlation between RNA-Seq of *Se*T and q-RT-PCR of *Se*N 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*, *marA* and *yjbE* and the amino acid biosynthesis genes *trpD* and *trpE* were significantly higher than the control ($p < 0.05$). In agreement with RNA-Seq analysis of *Se*T (data not shown), the genes *lamB* and *nmpC* were down-regulated in *Se*N ($p < 0.05$).

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 (Brankatschk 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. enterica* recognition. 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 (Mur 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 *Se*T 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 (*asrC*, *cysD*, *cysN*, *metA*, *narI*, *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 chlorine-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 *Se*T-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 *Se*T-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 *Se*T 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 *Se*T LT2 was used in the *Salmonella*-tomato interaction since this strain has a fully annotated genome. Validation using an environmental strain of *Se*N demonstrated concordance in gene expression with *Se*T. 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; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by a grant to SAM through the University of Maryland ADVANCE Program for Inclusive Excellence (HRD-1008117), funded by the National Science Foundation, USA, to the University of Maryland, College Park, USA.

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.

References

Adams, M., Jia, Z., 2005. Structural and biochemical analysis reveal pirins to possess quercetinase activity. J. Biol. Chem. 280 (31), 28675–28682.

Alekshun, M.N., Levy, S.B., 1999. The *mar* regulon: multiple resistance to antibiotics and other toxic chemicals. Trends Microbiol. 7 (10), 410–413.

Angelo, K.M., Chu, A., Anand, M., Nguyen, T.-A., Bottichio, L., Wise, M., Gieraltowski, L., 2015. Outbreak of *Salmonella* Newport infections linked to cucumbers — United States, 2014. MMWR 64 (6), 144–147.

Bang, I.-S., Liu, L., Vazquez-Torres, A., Crouch, M.-L., Stamler, J.S., Fang, F.C., 2006. Maintenance of nitric oxide and redox homeostasis by the *Salmonella* flavohemoglobin Hmp. J. Biol. Chem. 281 (38), 28039–28047.

Barak, J.D., Gorski, L., Naraghi-Arani, P., Charkowski, A.O., 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. Appl. Environ. Microbiol. 71 (10), 5685–5691.

Barak, J.D., Jahn, C.E., Gibson, D.L., Charkowski, A.O., 2007. The role of cellulose and *O*-antigen capsule in the colonization of plants by *Salmonella enterica*. Mol. Plant Microbe Interact. 20 (9), 1083–1091.

Barak, J.D., Kramer, L.C., Hao, L.-y., 2011. Colonization of tomato plants by *Salmonella enterica* is cultivar dependent, and type 1 trichomes are preferred colonization sites. Appl. Environ. Microbiol. 77 (2), 498–504.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. 57, 289–300.

Bennett, S.D., Littrell, K.W., Hill, T.A., Mahovic, M., Behravesh, C.B., 2015. Multistate foodborne disease outbreaks associated with raw tomatoes, United States, 1990–2010: a recurring public health problem. Epidemiol. Infect. 143 (7), 1352–1359.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.

Bolten, S., Gu, G., Luo, Y., Van Haute, S., Zhou, B., Millner, P.D., Micallef, S.A., Nou, X., 2019. *Salmonella* inactivation and cross-contamination on cherry and grape tomatoes under simulated wash conditions. Food Microbiol. 87, 103359. Available online 6 November 2019.

Brankatschk, K., Blom, J., Goesmann, A., Smits, T.H.M., Duffy, B., 2012. Comparative genomic analysis of *Salmonella enterica* subsp. *enterica* serovar Weltevreden foodborne strains with other serovars. Int. J. Food Microbiol. 155 (3), 247–256.

Browning, D.F., Lee, D.J., Spiro, S., Busby, S.J.W., 2010. Down-regulation of the *Escherichia coli* K-12 promoter by binding of the NsrR nitric oxide-sensing transcription repressor to an upstream site. J. Bacteriol. 192 (14), 3824.

Cohen, S.P., Levy, S.B., Foulds, J., Rosner, J.L., 1993. Salicylate induction of antibiotic resistance in *Escherichia coli*: activation of the *mar* operon and a *mar*-independent pathway. J. Bacteriol. 175 (24), 7856–7862.

de Moraes, M.H., Desai, P., Porwollik, S., Canals, R., Perez, D.R., Chu, W., Teplitski, M., 2017. *Salmonella* persistence in tomatoes requires a distinct set of metabolic functions identified by transposon insertion sequencing. Appl. Environ. Microbiol. 83 (5).

de Moraes, M.H., Soto, E.B., Salas González, I., Desai, P., Chu, W., Porwollik, S., Teplitski, M., 2018. Genome-wide comparative functional analyses reveal adaptations of *Salmonella* sv. Newport to a plant colonization lifestyle. Front. Microbiol. 9, 877.

Ferelli, A.M.C., Bolten, S., Szczesny, B., Micallef, S.A., 2020. *Salmonella enterica* elicits and is restricted by nitric oxide and reactive oxygen species on tomato. Front. Microbiol. 11, 391.

Fletcher, J., Leach, J.E., Eversole, K., Tauxe, R., 2013. Human pathogens on plants: designing a multidisciplinary strategy for research. Phytopathology 103 (4), 306–315.

Garcia, A.V., Charrier, A., Schikora, A., Bigeard, J., Pateyron, S., de Tauzia-Moreau, M.-L., Hirt, H., 2014. *Salmonella enterica* flagellin is recognized via FLS2 and activates PAMP-triggered immunity in *Arabidopsis thaliana*. Mol. Plant 7 (4), 657–674.

George, A.S., Cox, C.E., Desai, P., Porwollik, S., Chu, W., de Moraes, M.H., Teplitski, M., 2018. Interactions of *Salmonella enterica* serovar typhimurium and *Pectobacterium carotovorum* within a tomato soft rot. Appl. Environ. Microbiol. 84 (5) e01913-1917.

Goudeau, D.M., Parker, C.T., Zhou, Y., Sela, S., Kroupitski, Y., Brandl, M.T., 2012. The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. Appl. Environ. Microbiol.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Painter, J.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiol. Infect. 136 (2), 157–165.

Gu, G., Strawn, L.K., Oryang, D.O., Zheng, J., Reed, E.A., Ottesen, A.R., Rideout, S.L., 2018. Agricultural practices influence *Salmonella* contamination and survival in pre-harvest tomato production. Front. Microbiol. 9, 2451.

Guiney, D.G., Fierer, J., 2011. The role of the *spv* genes in *Salmonella* pathogenesis. Front. Microbiol. 2, 129-129.

Gunn, J.S., Ryan, S.S., Van Velkinburgh, J.C., Ernst, R.K., Miller, S.I., 2000. Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar Typhimurium. Infect. Immun. 68 (11), 6139–6146.

Gurmu, D., Lu, J., Johnson, K.A., Nordlund, P., Holmgren, A., Erlandsen, H., 2009. The crystal structure of the protein YhaK from *Escherichia coli* reveals a new subclass of redox sensitive enterobacterial bicupins. Proteins Struct. Funct. Bioinf. 74 (1), 18–31.

Hamilton, S., Bongaerts, R.J.M., Mulholland, F., Cochrane, B., Porter, J., Lucchini, S., Hinton, J.C.D., 2009. The transcriptional programme of *Salmonella enterica* serovar Typhimurium reveals a key role for tryptophan metabolism in biofilms. BMC Genom. 10 (1), 599.

Han, S., Micallef, S.A., 2014. *Salmonella* Newport and Typhimurium colonization of fruit differs from leaves in various tomato cultivars. J. Food Protect. 77 (11), 1844–1850.

Han, S., Micallef, S.A., 2016. Environmental metabolomics of the tomato plant surface provides insights on *Salmonella enterica* colonization. Appl. Environ. Microbiol. 82 (10), 3131–3142.

Henard, C., Vazquez-Torres, A., 2011. Nitric oxide and *Salmonella* pathogenesis. Front. Microbiol. 2 (84).

Hu, W.S., Chen, H.-W., Zhang, R.-Y., Huang, C.-Y., Shen, C.-F., 2011. The expression levels of outer membrane proteins STM1530 and OmpD, which are influenced by the CpxAR and BaeSR two-component systems, play important roles in the ceftriaxone resistance of *Salmonella enterica* Serovar Typhimurium. Antimicrob. Agents Chemother. 55 (8), 3829–3837.

IFSAC, 2018. Interagency Food Safety Analytics Collaboration. Foodborne Illness Source Attribution Estimates for 2016 for *Salmonella, Escherichia coli* O157, *Listeria Monocytogenes* and *Campylobacter* Using Multi-Year Outbreak Surveillance Data, United States. GA and D.C. U.S. Department of Health and Human Services, CDC, FDA, USDA-FSIS. https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2016-report-TriAgency-2508.pdf.

Karlinsey, J.E., Bang, I.-S., Becker, L.A., Frawley, E.R., Porwollik, S., Robbins, H.F., Fang, F.C., 2012. The NsrR regulon in nitrosative stress resistance of *Salmonella enterica* serovar Typhimurium. Mol. Microbiol. 85 (6), 1179–1193.

Kim, S.I., Yoon, H., 2019. Roles of YcfR in biofilm formation in *Salmonella* typhimurium ATCC 14028. Mol. Plant Microbe Interact. 32 (6), 708–716.

Kwan, G., Pisithkul, T., Amador-Noguez, D., Barak, J., 2015. *De Novo* amino acid biosynthesis contributes to *Salmonella enterica* growth in alfalfa seedling exudates. Appl. Environ. Microbiol. 81 (3), 861–873.

Kwan, G., Plagenz, B., Cowles, K., Pisithkul, T., Amador-Noguez, D., Barak, J.D., 2018. Few differences in metabolic network use found between *Salmonella enterica* colonization of plants and typhoidal mice. Front. Microbiol. 9 (695).

Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10 (3).

Lin, Y.-H., Yang, T.-C., Hu, W.S., 2019. Characterization of STM3030 and STM3031 genes of *Salmonella enterica* serovar Typhimurium in relation to cephalosporin resistance. J. Microbiol. Immunol. Infect. 52 (2), 282–288.

Marvasi, M., Cox, C.E., Xu, Y., Noel, J.T., Giovannoni, J.J., Teplitski, M., 2013. Differential regulation of *Salmonella* typhimurium genes involved in *O*-antigen capsule production and their role in persistence within tomato fruit. Mol. Plant Microbe Interact. 26 (7), 793–800.

Marvasi, M., de Moraes, M.H., Salas-Gonzalez, I., Porwollik, S., Farias, M., McClelland, M., Teplitski, M., 2016. Involvement of the Rcs regulon in the persistence of *Salmonella* Typhimurium in tomatoes. Environ. Microbiol. Rep. 8 (5), 928–935.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., He, S.Y., 2006. Plant stomata function in innate immunity against bacterial invasion. Cell 126 (5), 969–980.

Meng, F., Altier, C., Martin, G.B., 2013. *Salmonella* colonization activates the plant immune system and benefits from association with plant pathogenic bacteria. Environ. Microbiol. 15 (9), 2418–2430.

Miki, T., Shibagaki, Y., Danbara, H., Okada, N., 2009. Functional characterization of SsaE, a novel chaperone protein of the type III secretion system encoded by *Salmonella* pathogenicity island 2. J. Bacteriol. 191 (22), 6843.

Mur, Luis A.J., Mandon, Julien, Persijn, Stefan, Cristescu, Simona M., Moshkov, Igor E., Novikova, Galina V., Hall, Michael A., Harren, Frans J.M., Hebelstrup, Kim H., Gupta, Kapuganti J., 2013. Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plants 5.

Neill, S.J., Desikan, R., Clarke, A., Hancock, J.T., 2002. Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. Plant Physiol. 128 (1), 13.

Neumann, Christina, Fraiture, Malou, Hernàndez-Reyes, Casandra, Akum, Fidele N., Virlogeux-Payant, Isabelle, Chen, Ying, Pateyron, Stephanie, Colcombet, Jean, Kogel, Karl-Heinz, Hirt, Heribert, Brunner, Frédéric, Schikora, Adam, 2014. The *Salmonella* effector protein SpvC, a phosphothreonine lyase is functional in plant cells. Front. Microbiol. 5, 548.

Noel, J.T., Arrach, N., Alagely, A., McClelland, M., Teplitski, M., 2010. Specific responses of *Salmonella enterica* to tomato varieties and fruit ripeness identified by *in Vivo* expression technology. PloS One 5 (8), e12406.

Nunoshiba, T., deRojas-Walker, T., Wishnok, J.S., Tannenbaum, S.R., Demple, B., 1993. Activation by nitric oxide of an oxidative-stress response that defends *Escherichia coli* against activated macrophages. Proc. Natl. Acad. Sci. Unit. States Am. 90 (21), 9993.

Partridge, J.D., Bodenmiller, D.M., Humphrys, M.S., Spiro, S., 2009. NsrR targets in the *Escherichia coli* genome: new insights into DNA sequence requirements for binding and a role for NsrR in the regulation of motility. Mol. Microbiol. 73 (4), 680–694.

Perez, J.C., Groisman, E.A., 2007. Acid pH activation of the PmrA/PmrB two-component regulatory system of *Salmonella enterica*. Mol. Microbiol. 63 (1), 283–293.

Rappl, C., Deiwick, J., Hensel, M., 2003. Acidic pH is required for the functional assembly of the type III secretion system encoded by *Salmonella* pathogenicity island 2. FEMS Microbiol. Lett. 226 (2), 363–372.

Roy, D., Panchal, S., Rosa, B.A., Melotto, M., 2013. *Escherichia coli* O157:H7 induces stronger plant immunity than *Salmonella enterica* Typhimurium SL1344. Phytopathology.

Salazar, J.K., Deng, K., Tortorello, M.L., Brandl, M.T., Wang, H., Zhang, W., 2013. Genes *ycfR, sirA* and *yigG* contribute to the surface attachment of *Salmonella enterica* Typhimurium and Saintpaul to fresh produce. PloS One 8 (2), e57272.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3 (6), 1101–1108.

Shirron, N., Yaron, S., 2011. Active suppression of early immune response in tobacco by the human pathogen *Salmonella* Typhimurium. PloS One 6 (4), e18855.

Takaya, A., Takeda, H., Tashiro, S., Kawashima, H., Yamamoto, T., 2019. Chaperone-mediated secretion switching from early to middle substrates in the type III secretion system encoded by *Salmonella* pathogenicity island 2. J. Biol. Chem. 294 (10), 3783–3793.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pachter, L., 2012. Differential gene and transcript expression analysis of RNA-Seq experiments with TopHat and Cufflinks. Nat. Protoc. 7 (3), 562–578.

Villagra, N.A., Hidalgo, A.A., Santiviago, C.A., Saavedra, C.P., Mora, G.C., 2008. SmvA, and not AcrB, is the major efflux pump for acriflavine and related compounds in *Salmonella enterica* serovar Typhimurium. J. Antimicrob. Chemother. 62 (6), 1273–1276.

Wang, S., Deng, K., Zaremba, S., Deng, X., Lin, C., Wang, Q., Zhang, W., 2009. Transcriptomic response of *Escherichia coli* O157:H7 to oxidative stress. Appl. Environ. Microbiol. 75 (19), 6110.

Wang, S., Phillippy, A.M., Deng, K., Rui, X., Li, Z., Tortorello, M.L., Zhang, W., 2010. Transcriptomic responses of *Salmonella enterica* serovars Enteritidis and Typhimurium to chlorine-based oxidative stress. Appl. Environ. Microbiol. 76 (15), 5013.

Wolfe, A.J., 2005. The acetate switch. Microbiol. Mol. Biol. Rev. 69 (1), 12.

Zarkani, A.A., Schierstaedt, J., Becker, M., Krumwiede, J., Grimm, M., Grosch, R., Schikora, A., 2019. *Salmonella* adapts to plants and their environment during colonization of tomatoes. FEMS Microbiol. Ecol. 95 (11).

Zeidler, D., Zähringer, U., Gerber, I., Dubery, I., Hartung, T., Bors, W., Durner, J., 2004. Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc. Natl. Acad. Sci. U. S. A 101 (44), 15811–15816.

Zhang, X.-S., García-Contreras, R., Wood, T.K., 2007. YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity. J. Bacteriol. 189 (8), 3051.

Zhang, Y., Nandakumar, R., Bartelt-Hunt, S.L., Snow, D.D., Hodges, L., Li, X., 2014. Quantitative proteomic analysis of the *Salmonella*-lettuce interaction. Microbial. Biotechnol. 7 (6), 630–637.

Zheng, H., Beliavsky, A., Tchigvintsev, A., Brunzelle, J.S., Brown, G., Flick, R., Yakunin, A.F., 2013. Structure and activity of the NAD(P)+-dependent succinate semialdehyde dehydrogenase YneI from *Salmonella* typhimurium. Proteins Struct. Funct. Bioinformat. 81 (6), 1031–1041.