ABSTRACT

Title of Document: *SALMONELLA ENTERICA* INTERACTIONS WITH TOMATO: PLANT GENOTYPE EFFECTS AND *SALMONELLA* GENETIC RESPONSES

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Several outbreaks of *Salmonella enterica* infections have been linked to tomatoes. One cost-effective way to complement on-farm preventive Good Agricultural Practices would be to identify cultivars with inherent decreased susceptibility to *Salmonella* colonization. Various tomato cultivars with distinct phenotypes were screened to evaluate their susceptibility to *Salmonella* epiphytic colonization. The potential role of plant exudates, collected from the same cultivars, on the growth kinetics of *Salmonella* was examined. These investigations were supplemented with *Salmonella* genome-wide transcriptomics that showed bacterial responses to colonization of tomato shoots and roots. Epiphytic colonization of fruit by *S. enterica* was cultivar-dependent and serotype-specific, but did not correlate with leaf colonization. Fruit and leaves of the same cultivar differed in their ability to support *Salmonella* growth. Quantitative and qualitative analysis of tomato exudates

provided a possible explanation for the differential susceptibility to bacterial colonization among tomato cultivars. Tomato exudates alone were capable of supporting *Salmonella* growth, and the growth kinetics of *Salmonella* in tomato exudates differed by cultivar. Characterization of the chemical composition of primary and secondary metabolites in tomato exudates pointed to potential causes for the differential growth of *Salmonella* observed in the exudates of various tomato cultivars. Key transcriptomic signals that were down- and up-regulated in *Salmonella* upon interacting with tomato were identified, enabling us to elucidate the molecular mechanisms underlying this enteric pathogen-plant interaction. Overall, the identified signals lead to a proposed model that depicts the cellular processes needed to preserve cell viability when multiple abiotic stresses in conjunction with low nutrient availability are encountered, while simultaneously repressing unnecessary energy demands or maintaining them at a level equivalent to growth in a nutritious medium. These findings strongly support the hypothesis that plant-regulated mechanisms influence enteric pathogen colonization. It is clear that *Salmonella* can sense subtle environmental cues brought about by the genotype or physiological state of plants and can respond with distinct patterns of gene expression. Future work should focus on whether this bacterial behavior on plants results from an evolutionary adaptation to use plants as a vector to re-enter animal hosts.

SALMONELLA ENTERICA INTERACTIONS WITH TOMATO: PLANT GENOTYPE EFFECTS AND *SALMONELLA* GENETIC RESPONSES

By

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Fig. 1. Growth of *S.* Typhimurium in stem exudates; population densities measured at 6 hours (a) and 24 hours (b) post inoculation. Error bars indicate standard error of the mean; bars labeled with the same letter are not significantly different within the same time point measurement by Tukey's HSD test ($p<0.05$).

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Chapter 1: Introduction

There is an increasing consumer demand for safe food. While health and nutritional benefits will derive from consuming fruit and vegetables, the perishable nature of fresh products and the lack of sanitizing intervention measures during production could give rise to fresh produce with intrinsic food safety problems. Under even optimally controlled conditions, fresh produce could be contaminated with fecal matter entailing the potential presence of foodborne pathogens pre- and post-harvest through contaminated manure, irrigation water, excrement from wildlife animals, or improper personnel hygiene.

Traditionally, fresh fruits and vegetables were considered safe foods compared to meat and animal products because they, in theory, are not the hosts for zoonotic pathogens. Thus, until recently, relatively little attention had been paid to the microbial safety of fresh fruits and vegetables. Frequent and increasing foodborne illness outbreaks caused by consumption of fresh produce have questioned this notion. From 1998 to 2008, fresh produce was linked to more outbreaks than beef, pork, or poultry, which resulted in making fresh produce to be perceived by the public as the potentially riskiest food (DeWaal and Bhuiya, 2007; Batz et al., 2011; Painter et al., 2013). Among enteric pathogens involved in foodborne illness outbreaks, *Salmonella enterica* is the most common bacterial etiological agent responsible for produce-related outbreaks in the United States (Hanning et al., 2009). *Salmonella* on vine-stalk vegetables was the pathogen-commodity pair responsible for the highest number of outbreak-related illnesses in 2008 (CDC, 2011a). Tomatoes have been linked to at least 7 multistate outbreaks since 2002 (CDC, 2007b; Gupta et al., 2007;

Greene et al., 2008). Moreover, in 2005, a *Salmonella* Newport strain isolated from an irrigation pond on the Eastern Shore of Virginia matched a salmonellosis outbreak strain of that year (Greene et al., 2008), and was linked to the previous 2002 outbreak. Since then, the need to better understand and control pre-harvest contamination of tomatoes with *Salmonella* was highlighted, and collaborative multi-agency efforts were established to prevent contamination of tomatoes in the Mid-Atlantic region.

A key to augment microbial food safety of fresh produce is to elucidate the factors that influence the fate of enteric pathogens in association with plants, as well as to understand the physiological responses of enteric pathogens to the environment they encounter during plant colonization. However, in spite of the magnitude of the problem, relatively little is known about the traits and mechanisms that allow *Salmonella* to survive and persist outside animal hosts. To add to our knowledge regarding factors influencing the interaction of *Salmonella* with plants, as well as with the overall aim of investigating how *Salmonella* responds at the molecular level to the environment established by plants, the following studies were undertaken:

- *Salmonella enterica* Newport and Typhimurium Colonization of Fruit and Leaves in Various Tomato Cultivars[1]
- Potential Role of Plant Exudates on the Fate of *Salmonella enterica* Typhimurium in the Phyllosphere and Root System of Tomato Plants
- Genome-wide Transcriptional Profiling of *Salmonella enterica* Typhimurium Epiphytically Attaching and Colonizing Tomato Plants

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The overall goals of these studies were to test two hypotheses:

1) Various tomato cultivars exhibit a differential susceptibility to colonization by *Salmonella* as a result of cultivar differences in chemical composition.
2) *Salmonella* is adapted to inhabit the phyllosphere and root system of tomato plants, and will express a specific set of genes when interacting with tomato plants.

Thirteen cultivars with distinct phenotypes, including cultivars recommended for growth in the Mid-Atlantic region, were evaluated. The objectives and specific aims of the study were as follows:

Objective 1: Examine ability of *Salmonella* to epiphytically colonize different tomato plant organs

Specific aim 1-1: *S.* Typhimurium and *S.* Newport growth on seedlings of selected tomato cultivars, grown under sterile conditions when two true leaves fully emerged, was determined using culture methods.

Specific aim 1-2: *S.* Typhimurium and *S.* Newport growth on fruit of selected tomato cultivars, grown under greenhouse conditions, was determined using culture methods.

Objective 2: Evaluate leaf, root, stem, and fruit exudates at different growth stages, for their chemical composition and their effect on *Salmonella* growth.

Specific aim 2-1: *S.* Typhimurium growth was determined using culture methods over a 24 hour period in:

2-1a: leaf and root exudates collected from 3-week old seedlings grown under sterile conditions when two true leaves fully emerged.

2-1b: leaf and root exudates collected from 6-week old plants grown in the Research Greenhouse Complex of UMD when plants set flowers.

2-1c: stem exudates, collected from 15-week old plants grown in the Research Greenhouse Complex of UMD when plants set fruit.

2-1d: fruit exudates, collected from mature plants grown in the Research Greenhouse Complex of UMD.

2-1e: fruit exudates, collected from immature green and mature ripe tomato fruit of cv. 'Nyagous' plants grown in the Research Greenhouse Complex of UMD.

Specific aim 2-2: Variation in chemical composition of exudates of the different tomato cultivars were examined using GC-TOF-MS analysis.

Objective 3: Investigate genome-wide transcriptomes of *Salmonella* epiphytically attaching and colonizing tomato plants.

Specific aim 3-1: mRNA, isolated from *S*. Typhimurium epiphytically attaching and colonizing shoots and roots of tomato cultivar 'Heinz 1706', grown under sterile conditions in culture dishes for 6 weeks, was used to construct RNA-seq libraries for genome-wide transcriptome analysis.

Specific aim 3-2: Sequenced reads were mapped to the reference genome, aligned and merged for transcript assembly, and fed to Cuffdiff transcriptome analysis tool that calculates expression levels and tests statistical significance of observed changes in expression levels.

Specific aim 3-3: A pararrel experiment was performed to verify and confirm RNA-seq analysis data using quantitative reverse transcription-PCR methods on selected differentially expressed genes.

Chapter 2: Literature Review

1. *Salmonella* and salmonellosis

Salmonella spp. are rod-shaped, predominantly motile, Gram negative, and facultative anaerobic bacteria belonging to the family Enterobacteriaceae (Garrity et al., 2005). They are recognized as an important zoonotic bacterial pathogen of clinical as well as economic significance in animals and humans worldwide.

The genus *Salmonella* is currently divided into two species: *S. enterica* and *S. bongori* (formerly subspecies V) (Brenner et al., 2000; Tindall et al., 2005). *S. enterica* is further divided into six sub-species, with most zoonotic *Salmonella* belonging to the subspecies I (subsp. *enterica*). Six subspecies of *S. enterica* are *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). Among more than 2,500 known serotypes of *S. enterica*, about 100 of them account for ~98% of all clinical isolates from humans and domestic animals, and most of them belong to the subspecies I (CDC, 2011b). However, all *S. enterica* serovars are regarded as being capable of causing salmonellosis (Coburn et al., 2007; Grassl and Finlay, 2008). *S.* Typhimurium and *S.* Enteritidis are the two most prevalent *S. enterica* serotypes with broad host range, whilst a few others are predominantly found in one particular host (Uzzau et al., 2000). *S.* Typhi and *S.* Paratyphi are host-restricted serotypes, with exclusive expression of systemic disease in human hosts (Coburn et al., 2007).

Salmonella can grow within a wide range of temperature between 8 and 45°C and pH range between 4 and 8, with an optimal temperature of 37°C and pH of 6.5 to 7.5.

They catabolize D-glucose and other carbohydrates with the production of acid and hydrogen sulfide gas (D'Aoust and Maurer, 2013). Much of *Salmonella*'s virulence is associated with type III secretion systems (TTSS) located within *Salmonella* pathogenicity islands (SPI) (Collazo and Galán, 1997). These include TTSS-1 associated with intestinal invasion and encoded by SPI-1 as well as TTSS-2 associated with systemic spread of the organism and encoded by SPI-2.

Salmonella enterica is a major cause of salmonellosis in humans. Human salmonellosis is most often associated with consumption of contaminated food. Although early review showed that ingestion of less than 10^3 organisms can cause disease (Blaser and Newman, 1982), later evidence suggests that less than 10 cells can constitute a human infectious dose (Kapperud et al., 1990). Symptoms typically develop within 12 to 36 hours after consumption of contaminated food products (Benenson et al., 2001), and include in general one or more of the following: abdominal pain, nausea and vomiting, diarrhea and headache (Pelzer, 1989). There are four disease patterns recognized in humans: enteric fever (typhoid fever), gastroenteritis, bacteraemia, and chronic asymptomatic carriage (Coburn et al., 2007).

The systemic disease, enteric fever, caused by *S.* Typhi and *S.* Paratyphi, is often associated with poor hygienic conditions, and infection typically occurs due to the ingestion of food and water contaminated with human waste (Parry et al., 2002). *S.* Typhi along with non-typhoid *Salmonella* infections are endemic in many developing countries. In developed countries the leading cause of human salmonellosis are the non-typhoidal *Salmonella*, which generally cause gastroenteritis and are transmitted through the ingestion of food or water contaminated with animal waste (WHO, 2002).

The principle reservoir of *Salmonella* is the intestinal tract of a wide range of domesticated and wild animals from where they spread and persist for a period of time in food, water, soil, insects, and plants (D'Aoust and Maurer, 2013). The spread of *Salmonella* is in part favored by a massive commercial distribution of food products (CDC, 2011b).

Non-typhoidal salmonellosis (NTS) caused by non-typhoidal *Salmonella* is one of the leading causes of foodborne illness in the United States, posing a major public health burden and representing a significant economic impact (Mead et al., 1999; Lynch et al., 2009). It has been estimated that *Salmonella* causes 1.4 million cases of human salmonellosis in the U.S., resulting in 16,430 hospitalizations with almost 600 deaths each year. These numbers could be an underestimate, since for every *Salmonella* case reported, there are 29 cases that are not reported or diagnosed (Mead et al., 1999; Voetsch et al., 2004). *Salmonella* account for an estimated 27% of all foodborne illnesses caused by known bacterial agents with the majority of human salmonellosis cases related to the consumption of contaminated food products (Mead et al., 1999). The CDC's Foodborne Diseases Active Surveillance Network (FoodNet) estimated that the annual cost due absence from work, medical care, and lost productivity caused by foodborne salmonellosis in the United States ranges from $2.3 billion to $3.6 billion (Frenzen et al., 1999).

Traditionally, a wide range of food products, especially food of animal origin such as poultry products, beef and pork, have been implicated in foodborne illness attributable to human salmonellosis (WHO, 2002). In the past decade, disease transmission routes involving fresh produce such as fruit and vegetables have become

a major food safety concern in industrialized countries (Brandl et al., 2013; Hofmann et al., 2014; Martinez-Vaz et al., 2014). Annually, forty six percent of infections caused by foodborne illness in the United States have been attributed to fresh produce crops (Painter et al., 2013). Other sources of exposure to *Salmonella* include water, farm animals and pets, and human to human contact may be a source *Salmonella* infection (WHO, 2002).

Although the estimates of *Salmonella* harborage in livestock vary depending on the farms surveyed, approximately 1-10% of farm animals have been estimated to be *Salmonella* positive in the United States (Foley et al., 2008). *Salmonella* shed in feces of livestock and poultry is mainly responsible for *Salmonella* persistence in reservoirs (Baumler et al., 2000).

2. Salmonellosis incidence in association with consumption of fresh produce

Salmonella can infect numerous animal species including chickens, turkeys, cattle, pigs, sheep, horses, dogs, cats, reptiles, and humans. This ability to infect multiple hosts makes it a highly successful pathogen as well as a significant food safety risk.

In the last two decades, the contamination of fresh fruit and vegetables with *Salmonella* shifted concern to fresh produce as a vehicle of human salmonellosis. Repeated worldwide outbreaks of human salmonellosis from fresh tomatoes, lettuce, mixed salads, bean and alfalfa sprouts, raw almonds and cantaloupe assigned a challenge to the fresh produce industry and government regulatory agencies in implementing stringent on-farm pathogen control measures (Fan et al., 2009). Obviously, such outbreaks are destructive to consumer confidence in the safety of the fresh produce supply chain and have resulted in economic losses to farmers and fresh

produce industry (Ribera et al., 2012).

In the United States, while *Salmonella* prevalence on foods of animal origin has been well studied, resulting in considerable regulatory attention, relatively less is known about prevalence on fresh fruit and vegetables, although salmonellosis outbreaks linked to these nontraditional sources are continuously reported (CDC, 2006; DeWaal and Bhuiya, 2007; CDC, 2011a). Occurrence of pathogen presence on fresh produce is much more sporadic, does not have a specific point source, and is therefore much more problematic to track and control. Among many bacterial pathogens, *Salmonella* is the leading cause of fresh produce-related outbreaks in the United States (Hanning et al., 2009) and has been a frequent target pathogen in a number of studies to determine incidence on farms or retail produce (Mukherjee et al., 2004; Johnston et al., 2005; Johnston et al., 2006; Mukherjee et al., 2006; Abadias et al., 2008). *Salmonella* prevalence on mid-Atlantic produce farms has been estimated to be up to 2.2% (Micallef et al., 2012; Marine et al., 2015; Pagadala et al., 2015).

Since 2000, at least 25 multistate salmonellosis outbreaks have been traced back to fresh produce in the United States – CDC *Salmonella* Outbreakes (CDC) (Table 1). Among them, 7 outbreaks were associated with tomatoes, which is the highest number of salmonellosis outbreaks for a single commodity. The *Salmonella*-tomato pair is particularly problematic in the Mid-Atlantic region since a variety of *Salmonella* serotypes have been recovered from tomato production areas in this region (Micallef et al., 2012). *Salmonella* serovars isolated from tomato outbreaks are *S.* Newport, *S.* Javiana, *S.* Braenderup and *S.* Typhimurium.

Table 1. Multistate foodborne disease outbreaks of human salmonellosis from fresh fruit and vegetables

***Salmonella* Serovar**	**Year**	**Cases**	**Vehicle**	**Reference(s)**
S. Poona	2000-2002	58	Cantaloupe	CDC (2002)
S. Enteritidis	2000-2001	168	Almonds, raw	Isaacs et al. (2005)
S. Newport	2002	510	Tomatoes	Greene et al. (2008)
S. Enteritidis	2003-2004	29	Almonds, raw	CDC (2004)
S. Braenderup	2004	125	Tomatoes	Gupta et al. (2007)
S. Javiana and other serovars	2004	429	Tomatoes, presliced	Gupta et al. (2007)
S. Braenderup	2005	82	Tomatoes, prediced	CDC (2007a)
S. Newport	2005	72	Tomatoes	CDC (2007a), Greene et al. (2008)
S. Newport	2006	115	Tomatoes	CDC (2007a)
S. Typhimurium	2006	190	Tomatoes	CDC (2007a)
S. Litchfield	2008	51	Cantaloupe	CDC *Salmonella* Outbreaks
S. Saintpaul	2008	>1200	Peppers	CDC *Salmonella* Outbreaks
S. Saintpaul	2009	235	Alfalfa Sprouts	CDC *Salmonella* Outbreaks
S. Montevideo	2009-2010	272	Black and Red Pepper	CDC *Salmonella* Outbreaks
S. Newport	2010	44	Alfalfa Sprouts	CDC *Salmonella* Outbreaks
Salmonella serotype I 4,[5],12:i:-	2010-2011	140	Alfalfa Sprouts	CDC *Salmonella* Outbreaks
S. Panama	2011	20	Cantaloupe	CDC *Salmonella* Outbreaks
S. Enteriditis	2011	25	Alfalfa and Spicy Sprouts	CDC *Salmonella* Outbreaks

S. Agona	2011	106	Whole, Fresh Imported Papayas	CDC *Salmonella* Outbreaks
S. Enteriditis	2011	43	Turkish Pine Nuts	CDC *Salmonella* Outbreaks
S. Braenderup	2012	127	Mangoes	CDC *Salmonella* Outbreaks
S. Typhimurium and Newport	2012	261	Cantaloupe	CDC *Salmonella* Outbreaks
S. Saintpaul	2013	84	Cucumbers	CDC *Salmonella* Outbreaks
S. Newport	2014	275	Cucumbers	CDC *Salmonella* Outbreaks
S. Enteritidis	2014	115	Bean sprouts	CDC *Salmonella* Outbreaks

Unlike the meat supply chain, in the fresh produce sector it is often difficult to pinpoint the source of contamination. *Salmonella* contamination of fresh fruit and vegetables could arise from epiphytic colonization of fruit, entry of pathogens through scar tissue, natural uptake of pathogens through root systems or leaf hydathoes, from the surface contamination of flowers and subsequent entrapment of the pathogen in fruit or seeds, and from the transfer of contaminants onto edible plant tissues during slicing or minimal processing (Lin and Wei, 1997; Guo et al., 2001; Gu et al., 2013). In addition, the great variation which exists in farming and harvesting practices that also vary by commodity, hinders food safety surveillance and intervention efforts.

3. Survival and persistence of *Salmonella* in the environment

Salmonella can be disseminated in the natural environment such as water and soil. The possibility of *Salmonella* contaminating agricultural crops is mainly dependent

on their ability to survive and persist in the agricultural environment outside animal hosts. They can survive several weeks in water and several years in soil if conditions of temperature, humidity, and pH are favorable. For instance, *Salmonella* can be deposited in aquatic environments via various ways including untreated or partially treated wastewater effluent, agricultural run-off, and waste discharges from domestic and wild animals (Leclerc et al., 2002; Dechesne and Soyeux, 2007). Upon entering surface water systems, *Salmonella* has been shown to be capable of a long-term survival. *Salmonella* has been demonstrated to survive for approximately 56 days in freshwater (Fish and Pettibone, 1995). McEgan and her colleague showed that in Forida, where winter temperatures are mild, *Salmonella* could persist in various types of water for durations exceeding 6 months and their survival was enhanced when there was no background microflora population (McEgan, 2013). Another study showed that in freshwater *Salmonella* appeared to die off in 2-3 days, but when nutrients were supplemented to the water, *Salmonella* began to grow (Roszak et al., 1984). In river water which was used for irrigation, *S. enterica* was able to survive more than 45 days (Santo Domingo et al., 2000). One-year long monthly monitoring on *Salmonella* contamination in the surface water in Georgia yielded 57 samples of *Salmonella* detection out of 72 water samples (Haley et al., 2009).

During pre- and post-harvest stages of fresh produce production and handling, water can be a risk factor as a potential source of *Salmonella* contamination. In the cropping field, run-off from animal pastures and irrigation with contaminated water are considered primary sources of the pathogen (Islam et al., 2004a; Steele and Odumeru, 2004; Greene et al., 2008; Pagadala et al., 2015). Contaminated water is

also a concern in the post-harvest processing of fruit and vegetables. Washes with contaminated water were reported to be the cause of *Salmonella* outbreaks in mangoes (Sivapalasingam et al., 2003) and in cantaloupe (Gagliardi et al., 2003).

Since the potential of contaminating pre-harvest crops via soil also exists, numerous studies have investigated the survival and persistence of *Salmonella* in soil. *S. enterica* was reported to survive for more than 120 days in soil (Holley et al., 2006). Two different studies using manure compost amended soils also found that *S.* Typhimurium and *S.* Newport survived for 231 and 332 days, respectively (Islam et al., 2004a; You et al., 2006). Similar survival duration was reported for *S. enterica* in soil collected from a chicken farm (Davies and Breslin, 2003). In addition, Uesugi et al. (2007) reported isolating the same strain (*S.* Enteritidis PT30) from a single almond orchard for over a 5-year period, confirming the potential for years-long persistence of *Salmonella* in the agricultural environment. In contrast, shorter times of survival for *S. enterica* in soil and tomato crop debris mixtures have been reported (Barak and Liang, 2008). This discrepancy could be attributed to different experimental conditions such as soil type, temperature, moisture content, background microflora, as well as *Salmonella* strains used.

Livestock waste in the form of raw or composted manure is common fertilizer applied to crop soil. Although valuable as fertilizers, these wastes pose a food safety threat due to the high probability of containing human pathogenic bacteria (Mawdsley et al., 1995; Chadwick et al., 2008). Hutchison et al. (2004) reported that 5-18 % of fresh and stored manure samples collected from cattle, pig, poultry and sheep contained *Salmonella* with 10^3-10^5 CFU/g population levels. Once these wastes of fecal origin

are introduced into the environment, hydrological pathways such as run-off and preferential flow readily facilitate the dispersal of potential pathogens, while leaching can lead to groundwater contamination (Chalmers et al., 2000; Collins and Rutherford, 2004). Such risks are of particular concern in rural areas where groundwater is easily accessible for drinking, irrigation, and post-harvest produce handling.

Although many environmental factors can affect the survival and persistence of bacteria in water and soil, *Salmonella* can survive long enough to contaminate crops in the field and gain access to our food chains.

4. Survival and persistence of *Salmonella* on/in a non-host system, especially plants

Salmonella is able to survive outside animal hosts for extended periods of time, although its principal reservoir is the intestinal tract of a wide range of farmed and wild animals (Winfield and Groisman, 2003). While *Salmonella* manages stresses encountered through its journey after being excreted from the animal host, plants may be a valuable transitional refuge for this enteric pathogen, greatly augmenting the chances of re-entering herbivorous or omnivorous hosts. In fact, the animal-plant-animal cycle could well be the natural fecal-oral route for which *Salmonella* has evolved adaptive strategies.

Field studies revealed that *S.* Typhimurium was not only capable of persisting in manure-amended soil for up to 231 days, but also detectable on the above-ground parts of lettuce and parsley grown in the same soil for 2-3 months (Islam et al., 2004b). This strongly suggests that transmission from soil to plants, followed by

colonization of plant tissues, could be a potential survival strategy for this organism. Brandl and her colleagues showed that *S. enterica* was able to colonize, multiply, and form microcolonies in the phyllosphere of cilantro (Brandl and Mandrell, 2002) and lettuce (Brandl and Amundson, 2008). Guo et al. (2001) investigated the fate of *Salmonella* applied to tomato plants and concluded that *Salmonella* can survive in or on tomato fruit from the time of inoculation at flowering stage through fruit ripening. Van der Linden et al. (2013) reported that *S. enterica* was recovered from stored lettuce seeds two years after the initial inoculation. The germination of the stored contaminated seeds yielded seedlings that tested positive for the presence of *Salmonella*, indicating that the pathogen has the ability to persist on seeds and proliferate in the spermosphere and on germinating seedlings.

Traditionally, the phyllopshere, has long been noted as a hostile environment for bacterial colonists due to the rapid fluctuation in abiotic conditions such as solarization, temperature and relative humidity. In addition to these factors, the availability of nutrients on plants is a major determinant of successful epiphytic colonization (Mercier and Lindow, 2000). Some evidence has been put forward to show that plant-derived nutrients or exudates are actively metabolized by enteric pathogens and, therefore, could support their persistence in this niche. *Salmonella* moves toward lettuce root exudates, and sugar-based compounds in root exudates drive this chemotaxis (Klerks et al., 2007). The population sizes of *Salmonella* on the lettuce leaf surface are correlated with the availability of leaf exudates, especially total N content (Brandl and Amundson, 2008). *Salmonella* in the tomato phyllosphere preferentially colonizes type 1 trichomes which are thought to release

more exudates (Barak et al., 2011). The population sizes of *Salmonella* on cilantro and lettuce leaves increase when co-inoculated with a phytopathogen that can liberate nutrients from plant cells (Goudeau et al., 2013).

Although numerous laboratory studies have demonstrated that *Salmonella* is capable of colonizing plants through multiple routes including leaves, roots, seeds and flowers coming in contact with contaminated soil or water (Guo et al., 2001; Brandl and Mandrell, 2002; Cooley et al., 2003; Gu et al., 2013), the presence of this enteric pathogen in pre-harvest crop plants, is hardly detectable. For instance, Mukherjee et al. (2006) tested 2,029 pre-harvest produce samples for *Salmonella* presence all of which were negative for the pathogen. Other studies done by Gorski et al. (2011) and by Micallef et al. (2012) also found that none of the pre-harvest produce samples had detectable *Salmonella* out of 261 and 331 plant samples, respectively, whilst some environmental samples yielded *Salmonella* isolates (Micallef et al., 2012; Marine et al., 2015; Pagadala et al., 2015). This leads to a notion that other than plant factors providing a challenging niche for bacterial colonizers, there must be other external environmental factors affecting the frequency and prevalence of enteric pathogens in the field.

5. Factors influencing the fate of *Salmonella* on plants

It has been questioned whether genetic traits and/or environmental conditions make plants more susceptible to colonization by enteric pathogens. Some of the biotic factors proposed to influence plant colonization by enteric pathogens include plant

and bacterial genotypes, physiological state of plants, and interaction with indigenous microflora in the phyllosphere.

Assessment of the effects of plant genotype on the colonization of tomato plants with *Salmonella* have been conducted by enumerating *Salmonella* population levels on tomato leaves. Differences on tomato seedling leaves were more obvious between tomato (*Solanum lycopersicum*) and its closely related species (*Solanum pimpinelifolium*) (Barak et al., 2011), although cultuvar effects are also detectable (Han and Micallef , 2014; Chapter 3). Barak et al. (2011) reported that there was an approximately 100-fold difference in the phyllosphere populations of *Salmonella* between 4 tomato cultivars and its relative, *Solanum pimpinellifolium* WVa700, which supported the lowest level of bacteria. Barak et al. (2008) reported that *Salmonella* contamination incidence rates of soil-germinated tomato seedlings varied depending on the cultivar they screened, with the tomato cultivars 'Nyagous' and 'Yellow Pear' being less frequently contaminated. Internalization of *Salmonella* into plant tissues varies greatly among plant species (Jablasone et al., 2005). Gu et al. (2013) reported cultivar effects on the internalization and survival of *Salmonella* Typhimurium in tomato leaves. These observations suggest that specific genetic factors to microbial colonization or differences in phytochemicals such as the availability of assimilable nutrients determine the fate of enteric pathogens on plants. These findings also point to the potential of breeding for resistance or reduced susceptibility to colonization by the enteric pathogen.

A study by Brandl and Amundson (2008) demonstrated that enteric pathogens could multiply on lettuce leaves and that bacterial population sizes were strongly dependent

on leaf age. *Salmonella* populations were shown to be consistently larger in young lettuce leaves than in middle leaves harvested from mature lettuce heads. In addition, they found that the population sizes of *Salmonella* on lettuce leaf surface were correlated with the availability of leaf exudates, especially total N content (Brandl and Amundson, 2008).

While interest in understanding the role of plant genotypes is being addressed, the effects of bacterial genotypes remain less investigated. A few studies used cocktail inocula consisting of multiple *S. enterica* serovars although serovar-specific responses to plants were not examined (Barak et al., 2008; Beuchat and Mann, 2008; Barak et al., 2011). Zheng et al. (2013b) carried out a comparative study with two different *Salmonella* serotypes and found that *S.* Newport exhibited a higher survival rate on tomato leaves than *S.* Typhimurium following a *Salmonella* cocktail inoculation, although competition among serotypes could also be at play. Shi et al. (2007) inoculated tomato fruit with different *Salmonella* serovars individually and found that *S.* Enteritidis, *S.* Typhimurium, and *S.* Dublin were less adapted to grow on or in tomato fruit than *S.* Hadar, *S.* Montevideo, and *S.* Newport. A comparison of *S.* Typhimurium and *S.* Newport revealed *S.* Newport, a tomato outbreak strain, colonizes tomato fruit more efficiently than *S.* Typhimurium (Han and Micallef, 2014; Chapter 3). These discoveries indicate that the fitness of *Salmonella* on or in plant may differ among serotypes. For salmonellosis in animal hosts, host-adaptation is seen in *S.* Dublin and *S.* Choleraesuis, which is strongly associated with cattle and pigs, respectively, although they still can cause disease in other hosts and are highly pathogenic in humans (Coburn et al., 2007).

6. RNAseq as a new tool to quantify *Salmonella* responses to plants

When excreted on plants outside animal hosts, *Salmonella* must manage stresses ranging from differences in pH, osmolarity, and temperature to various types of oxidative stress and anti-microbial compounds encountered from the phyllosphere environment. The ability of bacteria to sense and respond to these changes in the environment is important for their survival (Foster and Spector, 1995). Under hostile environmental conditions, such as nutrient limitation, changes in pH, and temperature, bacteria activate stress responses that substantially improve their chances of survival in unfavorable environments.

One useful way to elucidate the mechanisms that allow *Salmonella* to manage stresses and survive on plants is to understand the global transcriptional responses triggered by the association with plant tissues. High-throughput sequencing technologies are now in common use in biology. These technologies produce millions of short sequence reads and are routinely being applied to genomic as well as transcriptomic studies. Sequencing steady-state RNA in a sample, known as RNA-seq, is free from many of the limitations of previous technologies such as the dependence on prior knowledge of the organism. However, the datasets produced are large and complex so that data analysis methodology is challenging.

Most RNA-seq experiments take a sample of purified RNA, fragment it, convert it to cDNA, and sequence on a high-throughput platform such as Illumina HiSeq/MiSeq, SOLiD, or Roche 454 (Shendure and Ji, 2008). This process generates millions of short reads taken from one or both end(s) of the cDNA fragments. The reads are then mapped to a reference genome or transcriptome. The mapped reads for each sample

are assembled into gene-level, exon-level, or transcript-level expression summaries. The summarized data are normalized, followed by statistical testing of differential gene expression, leading to a ranked list of genes with associated *p*-values and fold changes. Finally, interpretation on biological meanings can be obtained from these lists by performing functional genomics.

So far, one RNA-seq study analyzed the transcriptome of *Salmonella* grown on fresh produce (Brankatschk et al., 2014). The authors showed that genes encoding proteins involved in cellular attachment with curli, motility, and biofilim formation were induced when *S.* Weltevreden was cultured with alfalfa sprouts in comparison to M9-glucose medium. Relatively fewer stress-responsive genes were found up-regulated in their study than other comparable microarray studies, which might be because of the liquid culture conditions in their system. To date, RNA-seq approaches to evaluate genome-wide gene expression profiling of *Salmonella* associating with field crops have not been conducted.

Chapter 3: *Salmonella* Newport and Typhimurium Colonization of Fruit Differs from Leaves in Various Tomato Cultivars

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1. Introduction

While regular consumption of fruits and vegetables is encouraged owing to their nutritional value and potential in reducing risks associated with chronic diseases (Temple, 2000), the past decades have seen an increase in the number of foodborne illness outbreaks associated with the consumption of fresh produce (Lynch et al., 2006; DeWaal and Bhuiya, 2007; CDC, 2011a). Outbreaks are not only a risk to public health, but also frequently damage consumer confidence in the safety of the fresh produce supply chain, leading to substantial economic losses to produce growers and associated industries (Ribera et al., 2012).

Salmonella enterica is the most common bacterial etiological agent responsible for produce-related outbreaks in the U.S. (Hanning et al., 2009). *Salmonella* on vine-stalk vegetables was the pathogen-commodity pair responsible for the highest number of outbreak-related illnesses in 2008 (CDC, 2011a). Among those fresh produce commodities, tomatoes have been linked to at least 7 multistate outbreaks since 2002 (CDC, 2007a; Gupta et al., 2007). The *Salmonella*-tomato pair is particularly problematic in the Mid-Atlantic region. A variety of *Salmonella* serotypes have been recovered from tomato production areas (Micallef et al., 2012) and *S.* Newport isolated from irrigation ponds on the Eastern Shore of Virginia have been matched by

pulsed-field gel electrophoresis (PFGE) to the outbreak strain of 2002 and 2005 (Greene et al., 2008). The latest multistate outbreak caused by *S.* Newport associated with tomatoes occurred in 2011 and sickened 166 people (CDC FOOD).

Contamination of tomatoes may occur both pre- and post-harvest. Although the routes and mechanisms of contamination of fresh produce with *Salmonella* are still not fully understood, recent food safety efforts have focused on establishing preventive measures. On-farm Good Agricultural Practices (GAP) and Good Handling Practices (GHP) for tomatoes have been established, and the Food Safety Modernization Act (U.S. FDA, 2011), signed into law in 2011, continues to put the emphasis on prevention. While current GAP and GHP have done much to educate farmers on ways to reduce bacterial contamination of fresh produce, it appears that alone they are insufficient to completely eliminate tomato contamination since tomato-associated *Salmonella* illnesses continue to occur (CDC, 2011a).

One cost-effective way to complement primary on-farm preventative interventions to reduce contamination is to identify tomato cultivars with inherently decreased susceptibility to *Salmonella* contamination. The use of such cultivars could serve as a second tier control measure, by further minimizing the risk of tomato contamination in the event of on-farm presence of *Salmonella,* or sporadic introduction through random events such as wildlife or rain run-off. A number of studies have shown that leaves of different cultivars vary in their susceptibility to this enteric pathogen (Barak et al., 2008; Gu et al., 2013) and *Salmonella* genes required for colonization were differentially regulated in response to tomato cultivar (Noel et al., 2010), implying

that different plant genotypes impose different selective pressures on this human pathogen.

Although differential epiphytic colonization of tomato leaves with *Salmonella* has been reported, more pertinent data on *Salmonella* colonization of fruit is lacking. Adaptability of *Salmonella* strains isolated from tomato outbreaks has also not been assessed against a variety of cultivars. Most studies to date have assessed seedling or leaf colonization with *Salmonella* laboratory strains. To address this data gap, the objective of this study was to screen fruit and seedlings of thirteen tomato cultivars with distinct phenotypes. Their susceptibility to epiphytic colonization by *S. enterica* Typhimurium and *S. enterica* Newport, a tomato outbreak strain, was investigated.

2. Materials and Methods

2.1. Tomato cultivars and bacterial strains

Thirteen tomato (*Solanum lycopersicum*) cultivars were selected based on a range of distinct fruit phenotypes, including morphology ('California Red Cherry', 'Heinz-1706', and 'Micro-Tom'), pigment formation ('LA4013', 'Nyagous', and 'Virginia Sweets'), resistance to phytopathogens ('Florida 91 VFF', 'Mobox', 'Movione', and 'Rutgers VFA'), and suitability of cultivation in the Mid-Atlantic region of the U.S ('Moneymaker', 'Rutgers Select', and 'Plum Dandy VF'). 'Mobox' and 'Movione' are near isogenic lines (NILs) bred from the parent cultivar 'Moneymaker' for resistance to phytopathogens. These cultivars were included in this study with a specific purpose of answering a question regarding effects of phytopathogen resistance on the outbreak strain *S.* Newport. The 13 cultivars used in this study are listed in Table 1. Two *Salmonella enterica* subsp. *enterica* serotypes were selected -

S. Typhimurium LT2 (ATCC700720), a frequently used laboratory strain in food safety research, and *S.* Newport, an isolate recovered from a salmonellosis outbreak associated with tomato consumption (Greene et al., 2008), both adapted for rifampicin-resistance. Rifampicin-adapted strains were used in all inoculations except for experiments with tomato seedlings grown under sterile conditions in culture plates, in which *S.* Typhimurium LT2 lacking rifampicin resistance was used. These *Salmonella* strains were maintained at -80°C in Brucella broth (BD, Sparks, MD) containing 15% glycerol, and plated on trypticase soy agar (TSA) (BD, Sparks, MD) plates incubated at 35°C overnight, prior to experiments. For growth of rifampicin-resistant *Salmonella* strains, archiving and culture media were supplemented with 50 µg/ml rifampicin (Tokyo Chemical Industry Co. LTD., Japan).

Table 1. Tomato (*Solanum lycopersicum*) cultivars used in this study

Cultivar	Source	Note*
CA Red Cherry	Tomato Genetics Resource Center	Cherry variety
Heinz-1706		Genome sequenced by International Sequencing Project
Moneymaker		Suitable for Maryland
Nyagous		Black variety; Suitable for Maryland
LA4013		*hp-2* (High pigment-2) mutant in Moneymaker background
Mobox		Near isogenic line in Moneymaker background with R gene immunity to *Fusarium oxysporum* f. sp. *Lycopersici*
Movione		Near isogenic line in Moneymaker background with R gene immunity to *Pseudomonas syringae* pv. tomato
Micro-Tom		Miniaturized cultivar
Florida 91 VFF	Tomato Growers Supply Co.	VFF resistance; Recommended for Mid-Atlantic[1]
Rutgers Select		Recommended for Mid-Atlantic[2]
Rutgers VFA		VFA resistance; Recommended for Mid-Atlantic[2]
Virginia Sweets		Heirloom; Bi-color variety
Plum Dandy VF	Territorial Seed Co.	Recommended for Mid-Atlantic[1]

* V = Resistance to *Verticillium* wilt; F = Resistance to *Fusarium* wilt; A = *Alternaria* resistance; Double letters mean resistance to two or more strains of the disease.

[1] http://www.mdipm.umd.edu/state_resources/MD%20VEG%20REC%202009.pdf

[2] http://www.hgic.umd.edu/content/documents/HG70RecommendedVegetableCultivarsrevised2_2010.pdf.

2.2. *In vitro* tomato seedling growth

Tomato seeds were surface sterilized by soaking in 2.7% sodium hypochlorite for 30 min, followed by 6-7 rinses in sterile water, as recommended by the Tomato Genetics Resource Center (TGRC, UC Davis, CA). Seeds were germinated in the dark on Murashige and Skoog (MS) medium (MP Biomedicals LLC, Solon, OH)

supplemented with 2% sucrose and 1.2% agar. Germinated tomato seedlings were grown gnotobiotically in an upright position in 13 mm × 13 mm square culture plates at a 16L:8D photoperiod and at 26°C during the day and 18°C at night.

2.3. Tomato fruit harvesting and surface sterilization

To evaluate the epiphytic colonization on tomato fruit, 13 cultivars were grown at experimental field plots at the Wye Research and Education Center (WyeREC), University of Maryland (UMD). Tomato transplants were started at the Research Greenhouse Complex, UMD, and transplanted into the WyeREC field plots 3 weeks after germination. Plants were grown to fruit maturity under recommended irrigation and fertilization regimes. Pesticide application was discontinued one month prior to tomato harvesting. Ripe fruit were picked into sterile sampling bags avoiding direct contact with gloved hands, and the bags were transported in coolers on ice to a cold chamber at 4°C. Within 24 hours of sampling, tomato fruit were submerged in 2% household bleach for 10 min to sterilize the surface of fruit and then rinsed adequately with deionized water twice. Surface-sterilized fruit was dried in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) with the bag open for 1.5 days prior to *Salmonella* inoculation.

2.4. Preparation of *Salmonella* inocula

Overnight cultures of *Salmonella* grown on TSA at 35°C were suspended in sterile phosphate buffered saline (PBS) at an OD600 of 0.5, which yields ~10^9 CFU/ml. Further dilutions were made in sterile PBS. Actual cell concentrations of *Salmonella* suspension were enumerated on TSA plates. TSA culture plates containing 50 µg/ml

rifampicin were used to prepare and enumerate rifampicin-resistant *Salmonella* strains.

2.5. Tomato seedling and fruit inoculation

At 3 weeks post-germination, multiple locations on leaves were spotted with 100 µl of either 3.2×10^4 or 10^8 CFU/ml *S.* Typhimurium LT2, or 3.2×10^4 CFU/ml *S.* Newport, or sterile PBS. Square culture plates holding the inoculated seedlings were re-sealed with micropore tape (3M, St. Paul, MN) maintaining high relative humidity inside the plates but allowing aeration, and re-incubated. For surface-sterilized fruit, 50 µl of 6.4×10^3 CFU/ml rifampicin -adapted *S.* Typhimurium LT2 or *S.* Newport, or sterile PBS were spot-inoculated on intact areas of the fruit surface forming 5 droplets of 10 µl, spotted as tightly within a minimum diameter as possible. The inoculated fruit were incubated in sterile Whirl-Pak bags at room temperature. The bags were closed to maintain humid conditions, and care taken to avoid *Salmonella* inocula from contacting the sides of the bags during incubation. For fruit, inoculations were done in replicates of 5, except for 'Rutgers Select' and 'Rutgers VFA' with replicates of 3-4 for *S.* Newport, due to low fruit yields. For seedlings, inoculations were performed in replicates of 3-5. Data were pooled from separate experiments up to a total of 10 replicates.

2.6. *Salmonella* retrieval from inoculated tomato seedlings and fruit, and *Salmonella* enumeration

Three days after *Salmonella* inoculation, seedling leaves were aseptically cut and transferred to sterile 50 ml conical tubes containing 20 ml of PBS. The tubes were

sonicated in Branson Ultrasonic Cleaner (Branson Ultirasonics Corperation, Danbury, CT) for 2 min and vortexed briefly at maximum speed in order to dislodge attached *Salmonella* cells from the plant surface. For inoculated fruit 24 h post-inoculation, the fruit skin where the *Salmonella* inocula had been mounted was cut off using a sterile scalpel and transferred into sterile 1.5 ml microcentrifuge tubes containing 1 ml of PBS. Tubes were vortexed at maximum speed for 10 min. Serial dilutions were prepared from the rinsates, and plated on TSA for *S.* Typhimurium LT2 or TSA with 50 µg/ml rifampicin culture plates for rifampicin-adapted *S.* Typhimurium LT2 and *S.* Newport quantification.

2.7. Statistical analysis

Enumeration data in CFU/unit of sample were $\log_{10}$ transformed to satisfy the assumptions on normality of residuals and homogeneity of variances. Differences in log CFU/unit of sample detected between levels of treatments were tested for significance using one-way ANOVA and Tukey's HSD test. Student's t-test was performed when a comparison between only two levels of treatments was necessary. Specific interest comparing 'Movione' or 'Mobox' with its parent cultivar 'Moneymaker' was tested by a pre-planned comparison procedure, called contrasts. Statistical analyses were carried out using JMP Pro 10 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Epiphytic colonization of tomato fruit with *Salmonella* is cultivar-dependent and serotype-specific

Fruit of different cultivars, field-harvested then surface-sterilized, were inoculated with either *S.* Newport or *S.* Typhimurium. Growth of *Salmonella* populations was observed for both *Salmonella* serotypes on all cultivars screened but was generally higher for the former (Fig. 1). When each of the cultivars was initially loaded with 2.5 log CFU *S.* Newport per fruit, 1.4 to 3.1 log CFU increases in population density were observed one day post-inoculation, and was cultivar-dependent. 'Heinz-1706' was significantly less colonized per fruit than 'Nyagous' - 3.9 log CFU versus 5.6 log CFU, respectively (p=0.0139) (Fig. 1a). *S.* Newport populations on 'Micro-Tom' and 'Virginia Sweets', at 3.9 log CFU per fruit for both, were also less than those on 'Nyagous' (p=0.0930 and 0.0797, respectively), but not significant at p<0.05 level. The highest log CFU of *S.* Newport was retrieved from 'Nyagous', followed by 'LA4013' and 'Florida 91 VFF' (5.5 and 5.4 log CFU/fruit, respectively). For *S.* Typhimurium, 0.7 to 2.2 log CFU increases in population density were observed on fruit (Fig. 1b). The largest population of *S.* Typhimurium was recovered from 'LA4013' (4.7 log CFU/fruit) which was followed by 'Rutgers VFA' and 'Florida 91 VFF' (4.5 and 4.3 log CFU/fruit, respectively), while the smallest from 'Mobox', 'Heinz-1706', and 'Rutgers Select' (3.2, 3.4, and 3.4 log CFU/fruit, respectively).

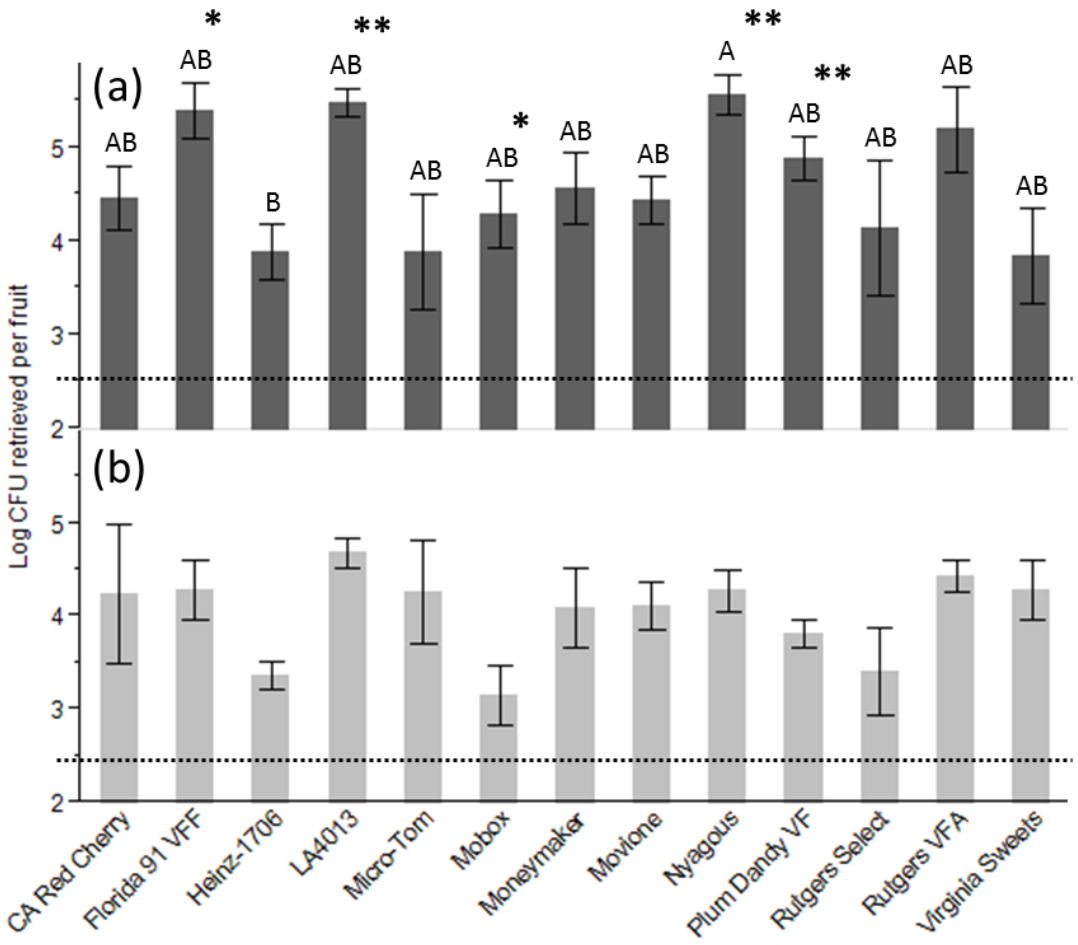


FIGURE 1. Cell density of rifampicin-adapted (a) *S.* Newport and (b) *S.* Typhimurium LT2 on fruit of various tomato cultivars, 24 h post-inoculation; 2.5 log CFU for all strains were loaded initially per fruit (dash line). Error bars indicate standard error of the mean. Asterisks denote the significance in side-by-side cultivar comparison between serotypes (a) and (b) at $p<0.05$ (*) or $p<0.01$ (**); bars labeled with the same uppercase letter are not significantly different within serotype treatment by Tukey's HSD test ($p<0.05$).

3.2. Cultivar-dependent and serotype-specific differential colonization by *Salmonella* was also observed on leaves of tomato seedlings, but the patterns differed from the fruit colonization data

Seedling leaves of seven different cultivars grown sterilely in culture plates for 3 weeks were inoculated with either *S*. Newport or *S*. Typhimurium. Consistent with fruit colonization, overall population growth for both serotypes was observed on all the cultivars (Fig. 2). For *S*. Newport, seedlings were initially loaded with 3.5 log CFU. Three days post-inoculation, 5.9 to 7.6 log CFU per seedling were recovered (Fig. 2a). 'Florida 91 VFF' and 'Movione' were the cultivars least susceptible to *S*. Newport colonization (5.9 and 6.4 log CFU/seedling, respectively), compared to the most colonized 'Virginia Sweets' (7.6 log CFU/seedling) ($p<0.05$). When inoculated with *S*. Typhimurium, 'Nyagous' and again 'Movione' and 'Florida 91 VFF' exhibited reduced susceptibility to *Salmonella* colonization (6.7, 6.7, and 6.8 log CFU/seedling, respectively) and were significantly different from 'Moneymaker' and 'Heinz-1706' (7.8 and 7.6 log CFU/seedling, respectively) ($p<0.05$) (Fig. 2b). These data contrast with counts obtained from fruit colonization experiments, where 'Heinz-1706' was the least colonized and 'Nyagous' the most colonized (Fig. 1a). Cultivar-dependent differential colonization of *S*. Typhimurium on tomato seedlings was observed in repeated experiments using a higher initial *S*. Typhimurium load (7.0 log CFU per seedling) on a subset of cultivars (Fig. 2c), significant at $p<0.1$ level. The discrepancies in CFU per unit of sample among the cultivars was more marked when the seedlings were loaded with a lower concentration of *Salmonella* (Fig. 2b and 2c). No apparent correlation pattern was observed when population increases of

Salmonella on fruit were plotted against those on seedling leaves (Fig. 3). This indicates that a tomato cultivar's susceptibility to leaf colonization with *Salmonella* is not necessarily indicative of fruit susceptibility.

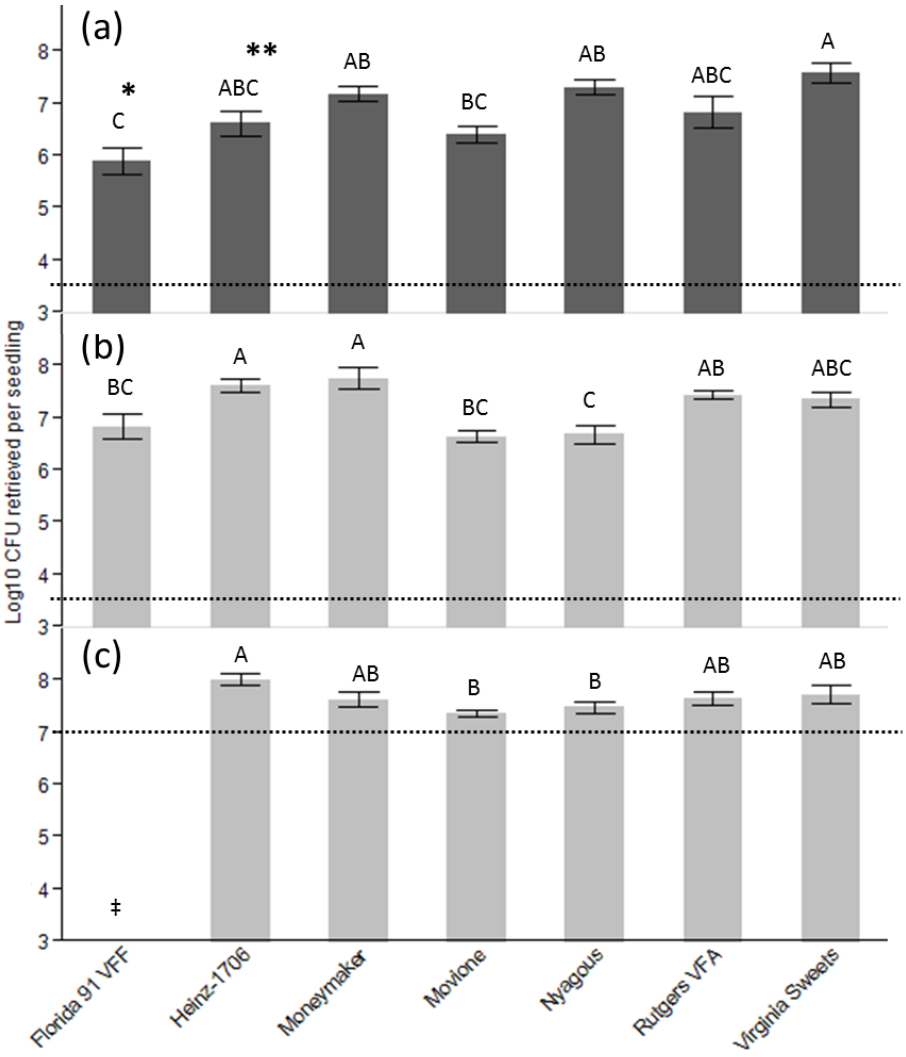


FIGURE 2. Cell density of (a) rifampicin-adapted *S.* Newport, and (b) and (c) *S.* Typhimurium LT2 on leaves of 3-week-old seedlings of various tomato cultivars, 3 days post-inoculation; (a) and (b) 3.5 log CFU, and (c) 7.0 log CFU were loaded initially per seedling (dash line). Error bars indicate standard error of the mean. Asterisks denote the significance in side-by-side cultivar comparison between

serotypes (a) and (b) at $p<0.05$ (*) or $p<0.01$ (**); bars labeled with the same uppercase letter are not significantly different within serotype treatment by Tukey's HSD test (a) and (b) at $p<0.05$, and (c) at $p<0.1$; ‡ on panel (c) denotes that seedling inoculation was not carried out for 'Florida 91 VFF'.

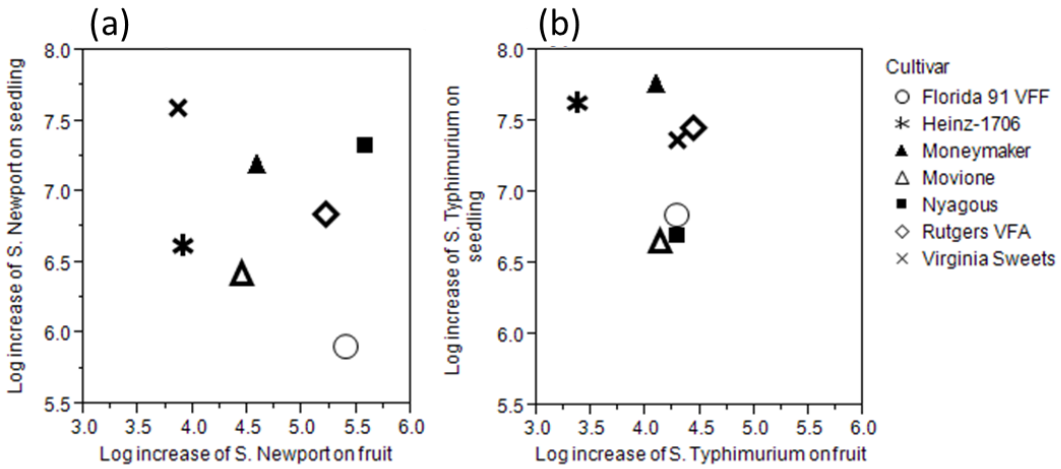


FIGURE 3. Association between population increases in log CFU/fruit or log CFU/seedling of (a) *S.* Newport and (b) *S.* Typhimurium, for each cultivar.

3.3. *S.* Newport, a tomato outbreak strain, colonizes tomato fruit more efficiently than *S.* Typhimurium

To determine whether *S.* Newport, a tomato outbreak strain, is better adapted to colonize and persist on tomato plants than *S.* Typhimurium, *Salmonella* colonization data on seedling leaves and fruit were combined by *Salmonella* strain and compared.

The population of *S.* Newport was significantly higher than that of *S.* Typhimurium on tomato fruit (Fig. 4a). By contrast, *S.* Newport was less able to colonize seedling leaves than *S.* Typhimurium (Fig. 4b). These differences were statistically supported ($p<0.05$). In pairwise comparisons between cultivars inoculated with the two *Salmonella* serotypes, significant differences in cell counts were observed on fruit of 'Florida 91 VFF', 'LA4013', 'Mobox', 'Nyagous', and 'Plum Dandy VF', with higher counts recovered for *S.* Newport compared to *S.* Typhimurium (Fig. 1). On leaves, higher cell counts were recovered from 'Florida 91 VFF' and 'Heinz-1706' for *S.* Typhimurium comparing to *S.* Newport (Fig. 2). Cell counts from the other 5 cultivars were not statistically different between the serotypes.

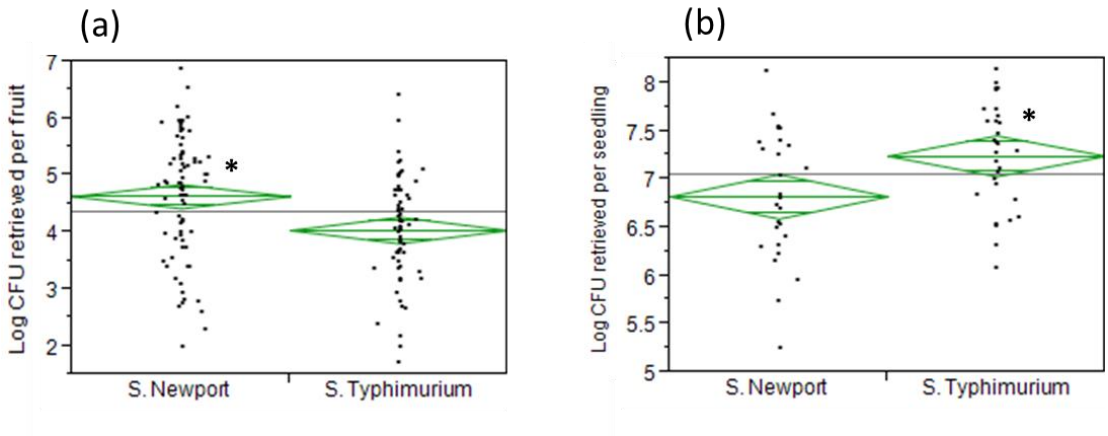


FIGURE 4. *S.* Newport and *S.* Typhimurium colonization of tomato (a) fruit in log CFU/fruit and (b) seedling leaves in log CFU/seedling, for all cultivars. Asterisks denote the significance by one-way ANOVA at $p<0.05$.

3.4. Potential role of plant innate immunity to *S*. Newport colonization

To analyze the effects of plant innate phytopathogen resistance on colonization by *Salmonella*, the population levels of *S*. Newport obtained from 'Movione' and 'Mobox' fruit and 'Movione' seedling leaves were compared against 'Moneymaker'. Both 'Movione' and 'Mobox' are a near isogenic lines (NIL) bred from their parent cultivar 'Moneymaker', selected for resistance to *Pseudomonas syringae* pv. tomato or *Fusarium oxysporum* f. sp. *lycopersici* as a result of harboring the *Pto* or the *I-2* gene, respectively. *Salmonella* counts obtained from leaves of 'Movione' were significantly lower for *S*. Newport (p=0.0124) than the counts from 'Moneymaker' (Fig. 2a). By contrast, there were no differences in the population levels of *S*. Newport on tomato fruit between 'Movione' and 'Moneymaker' (p=0.8131) (Fig. 1a). Similarly, no significant difference in *Salmonella* cell counts on tomato fruit between 'Mobox' and 'Moneymaker' was observed for *S*. Newport (p=0.6450). The same patterns were observed with the laboratory strain *S*. Typhimurium LT2.

4. Discussion

While interest in understanding biological factors involved in *Salmonella*-fresh produce crop plant interactions is growing (Klerks et al., 2007; Berger et al., 2009; Noel et al., 2010), the role of plant genotypes or enteric pathogen serotype remains less investigated. Adaptability of *Salmonella* strains isolated from tomato outbreaks has also not been well addressed. In this study, associations between thirteen tomato genotypes and two *Salmonella* serotypes yielded differential levels of *Salmonella* populations colonizing tomato fruit and seedling leaves. Fruit and leaves of the same cultivar differed in their ability to suppress/support *Salmonella* growth. The tomato

outbreak strain of *S.* Newport was a better colonizer of fruit than *S.* Typhimurium. Susceptibilities for fruit and leaves for individual cultivars did not always follow the same trend. The NIL of 'Moneymaker', cultivar 'Movione', was less susceptible to *S.* Newport leaf colonization, compared to the background genotype, although no expression data to support this observation was obtained.

A few studies have been conducted to evaluate cultivar effects on the colonization of tomato plants with *Salmonella*, testing tomato leaves to investigate cultivar effects, although differences in *Salmonella* population levels on tomato seedling leaves were more obvious between tomato (*Solanum lycopersicum*) and its closely related species (*S. pimpinellifolium*) than between different tomato cultivars (Barak et al., 2011). Recently, Gu et al. (2013) also reported cultivar effects on the internalization and survival of *S.* Typhimurium in tomato leaves. Barak et al. (2008) reported that *Salmonella* contamination incidence rates of soil-germinated tomato seedlings varied depending on the cultivar they screened, with the cultivars 'Nyagous' and 'Yellow Pear' being less frequently contaminated. In the present study, seedling leaves of 'Nyagous' were also the least colonized by *S.* Typhimurium among the 7 cultivars, but by contrast 'Nyagous' fruit supported the largest *S.* Newport populations and among the largest *S.* Typhimurium populations. This suggests that the cultivar-dependent susceptibility to *Salmonella* colonization observed on tomato seedlings is not necessarily correlated with that of tomato fruit, a significant finding, since only fruit are consumed. This discrepancy between leaves and fruit is best observed with the cultivars 'Florida 91 VFF' and 'Heinz-1706'. Young leaves of 'Florida 91 VFF' were among the least colonized by either *S.* Newport or *S.* Typhimurium compared to

the other cultivars, whereas fruit of 'Florida 91 VFF' were among the most favored by both *Salmonella* serotypes. The opposite pattern, with higher population levels on young leaves but lower on fruit, was recorded for 'Heinz-1706'. Interestingly, increases in *Salmonella* population levels were higher in leaves compared to fruit, revealing complex and tissue-specific interactions and responses between this pathogen-crop pair.

Although tomato leaves are not edible, data on susceptibility to leaf colonization are relevant since *Salmonella* residing on leaves can be transmitted to fruit (Barak et al., 2011; Gu et al., 2011). However, since only fruit is eaten, data on *Salmonella* colonization on/in tomato fruit of various cultivars also needs to be considered for establishment of food safety recommendations. Beuchat and Mann (2008) concluded that survival and growth of *Salmonella* was unaffected by tomato variety when *Salmonella* grew in stem scar and pulp tissues using store-bought tomatoes sorted by shape and size (round, Roma, and grape). In contrast, Xia et al. (2012) reported that tomato fruit of the cultivar 'Mountain Spring' were less susceptible to *S.* Thompson internalization than the cultivars 'Applause' and 'BHN961'. Knowledge about the susceptibility of field-grown fruit of various cultivars to surface attachment and colonization by *Salmonella* could be an important criterion in cultivar selection by growers, particularly in geographical areas where *Salmonella* appears to be endemic. Such is the case on the Delmarva peninsula, east of the Chesapeake Bay in Virginia, Maryland, and Delaware, an area supporting intensive tomato cultivation. Multiple serotypes, including *S.* Newport, *S.* Javiana and *S.* Thompson, have been isolated

from tomato farms in this area (Micallef et al., 2012), and even linked to outbreaks (Greene et al., 2008).

To our knowledge, this is the largest screen of field-grown tomato fruit of different cultivars assessing susceptibility to *Salmonella* fruit colonization, including ones that farmers in the mid-Atlantic region can select for cultivation. Under consistent field conditions, several cultivar-specific differences were observed. For instance, compared to the dark pigmented cultivar 'Nyagous', fruit of cultivar 'Heinz-1706' supported significantly lower concentrations of both *S.* Newport (p=0.0002) and *S.* Typhimurium (p=0.0582). Recognizing that *Salmonella* responses to green and mature fruit differ (Noel et al., 2010), only mature fruit were used across all the cultivars examined throughout the experimental protocols. The cultivar-dependent differences in levels of *Salmonella* population on tomato fruit, therefore, can be attributed to genetic variation among cultivars, since all cultivars were grown simultaneously and harvested at equivalent ripeness stage. Recently, tomato maturity and genotype were also found to be factors for *Salmonella* proliferation for cultivars 'Florida-47', 'Solar Fire', and 'Bonny Best' (Marvasi et al., 2013). Yet differences in fruit surface morphology or chemistry between cultivars, on which phyllospheric microbes rely for their food and protection from abiotic stresses, have not been examined. Further research in this area is needed to begin to unravel the mechanisms regulating these differences.

Studies that have used cocktail inocula consisting of multiple *S. enterica* serovars preclude the distinction of serovar-specific responses to various tomato cultivars (Barak et al., 2008; Beuchat and Mann, 2008; Barak et al., 2011). *S.* Newport

exhibited a higher survival rate on tomato cultivar 'Micro-Tom' leaves than *S.* Typhimurium following a *Salmonella* cocktail inoculation (Zheng et al., 2013b). Shi et al. (2007) inoculated red tomato fruit of cultivar 'Abigail VFET' with different *Salmonella* serovars individually and found that *S.* Enteritidis, *S.* Typhimurium, and *S.* Dublin were less adapted to grow on/in tomato fruit than *S.* Hadar, *S.* Montevideo, and *S.* Newport. In this study, serotype-specific, cultivar-dependent, and plant part-specific *Salmonella* colonization for the outbreak strain of *S.* Newport was revealed, providing support to the idea of selecting cultivars on the basis of their resistance to enteric pathogen colonization and the endemic pathogens of a given geographical area of cultivation, although this has not been validated in the field. Leaves provided a more favorable niche for *S.* Typhimurium, while *S.* Newport grew best on tomato fruit, suggesting that the tomato outbreak strain is better equipped to colonize and persist on tomato fruit. This could be one explanation for the frequency of *S.* Newport infections associated with tomato consumption in the mid-Atlantic, compared to other serotypes, in spite of a diversity of serotypes being prevalent in that region (Micallef et al., 2012). A comparison of mid-Atlantic serotypes could validate this.

Tomato cultivar 'Movione' contains the Pto bacterial resistance locus in cultivar 'Moneymaker' background. The *Pto* gene encodes a kinase that confers resistance in tomato to *Pseudomonas syringae* pv. tomato expressing the avirulence gene *avrPto* by directly interacting with type III secretion system effector proteins, AvrPto and AvrPtoB from *P. syringae* pv. tomato (Ronald et al., 1992). Although no expression data for *Pto* was obtained in this study, when comparing the cultivar 'Moneymaker' with 'Movione', 'Movione' was significantly less susceptible to colonization of

seedling leaves by *S.* Newport. Further studies could investigate whether the *Pto* gene in tomato leaves responds to type III secretion system effector proteins in *Salmonella,* and whether a different response is elicited in fruit, as suggested in this study. Cultivar 'Mobox' is another NIL bred from 'Moneymaker' harboring the *I-2* gene conferring resistance to *Fusarium oxysporum* f. sp. *lycopersici*, a wilt-inducing fungus in tomato, probably through recognition of effector proteins (van Ooijen et al., 2007). No difference in fruit colonization was observed between these two cultivars. In a repeated experiment with *S.* Typhimurium colonizing tomato seedlings, it is interesting to note that equivalent *S.* Typhimurium populations – 7 to 8 log CFU per seedlings – were recovered in 3 days, irrespective of initial levels loaded per seedling – 3.5 vs. 7 log CFU. Additionally, leaves supported higher *Salmonella* population densities compared to fruit. These findings suggest that there are spatial and/or nutritional limiting factors impacting *Salmonella* growth on the tomato phyllosphere that differ with different plant parts. Assessing the role that plants play in influencing their associating microbiota is of interest from a food safety standpoint, and requires further research.

In conclusion, these findings reveal that tomato plant genetics play a crucial role in determining the success of *Salmonella* establishment, colonization and persistence on various plant parts. The highly variable predisposition of tomato fruit to *Salmonella* colonization offers the opportunity to use this heterogeneity to a food safety advantage. More research is required to better elucidate what other factors might interplay with this plant-enteric pathogen interaction to determine pathogen

colonization success. Ultimately, a cultivar's inherent susceptibility to *Salmonella* colonization could be one important criterion for cultivar selection for cultivation.

Chapter 4: Potential role of plant exudates on the fate of *Salmonella enterica* in the phyllosphere and root system of tomato

1. Introduction

Salmonella growth on plants appears to be influenced by plant factors, as indicated by differential growth of this pathogen on leaves and fruit of various tomato cultivars (Han and Micallef, 2014), yet the mechanisms regulating this differential cultivar susceptibility remain unexplained. The plant surface, especially the above-ground parts, has long been regarded as a hostile environment for bacterial colonists due to the rapid fluctuation in abiotic conditions such as temperature and relative humidity. Additionally, the availability of nutrients on plants is a major determinant of successful epiphytic colonization (Mercier and Lindow, 2000). Some evidence has been provided to show that plant-derived nutrients or exudates are actively metabolized by enteric pathogens and, therefore, could enhance their persistence in this niche. *Salmonella* movement toward lettuce root exudates and sugar-like compounds in root exudates is driven by chemotaxis (Klerks et al., 2007). Population sizes of *Salmonella* on lettuce leaf surfaces are correlated with the availability of leaf exudates, especially total N content (Brandl and Amundson, 2008). *Salmonella* in the tomato phyllosphere preferentially colonizes type 1 trichomes which are thought to release more exudates than other microsites on the leaf surface (Barak et al., 2011). *Salmonella* levels on cilantro and lettuce leaves increased when co-inoculated with *Dickeya dadantii*, a phytopathogen that can liberate nutrients from plant cells (Goudeau et al., 2013). Another enteric pathogen, *Escherichia coli* O157:H7, causing

foodborne illness frequently associated with consumption of leafy greens, was found to maintain higher population levels on mechanically or biologically damaged lettuce leaves (Aruscavage et al., 2008), enhancing persistence of the enteric pathogen attributed to increased sugar availability on the leaves (Aruscavage et al., 2010). Plant exudation is a regulated process that is integral to plant growth and development. Plants passively and actively exude an enormous range of potentially valuable compounds, ranging from ions, amino acids and simple sugars, to complex sugars and secondary metabolites including fatty acids, phenolics, flavonoids and terpenoids. Exudation via roots alters the chemical and physical properties of soil in their immediate vicinity to form the rhizosphere, influencing resistance to pests and recruitment of beneficial symbionts (Bais et al., 2006). The phyllosphere, although covered with a hydrophobic waxy cuticle that reduces evaporation of water and leaching of plant metabolites, still harbors some nutrients leaked through leaf surface appendages such as trichomes as well as secondary metabolites and antimicrobial compounds produced by plants for defense. These plant factors are involved in shaping microbial communities in the phyllosphere (Vorholt, 2012; Ottesen et al., 2013). Ongoing work is showing that composition of phyllosphere-associated microbial communities is not influenced by foliar application of a bacterial biocontrol agent *Paenibacillis alvei* TS-15 (personal communication, Sarah Allard), active against *Salmonella* on tomato (Allard et al., 2014). A similar robustness in the composition of the tomato fruit microbiome was observed during a season long experiment invovling pesticide application with water sources of varying microbial quality (Telias et al., 2011). The composition of plant exudates is considered both

species and cultivar specific. Micallef et al. (2009a) showed that different variants of the genetic plant model *Arabidopsis* released unique root exudate cocktails into the rhizosphere soil. In turn, these plant variants supported unique rhizobacterial assemblages in the root-soil interface in response to differences in exudate composition, in an age-dependent manner (Micallef et al., 2009b). Overall there was a strong plant-driven regulation of phytobiomes, mediated by plant exudates. Assessment of *S.* Newport and *S.* Typhimurium epiphytic colonization of tomato seedling leaves and fruit of different cultivars revealed noticeable variation in detectable population sizes (Han and Micallef, 2014). In this study, we hypothesized that this differential epiphytic colonization could be explained by differences in tomato exudation among cultivars. To test, tomato root, leaf, stem and fruit exudates were evaluated for their potential to support the growth of *S.* Typhimurium. Fruit at two different ripeness stages (red ripe and pre-breaker, green) and their exudates were tested against a foodborne illness outbreak strain of *S.* Newport (Greene et al., 2008). Root, leaf and fruit exudates were chemically characterized to evaluate the role of exuded phytochemicals on epiphytic *Salmonella* growth on tomato.

2. Materials and Methods

2.1. Tomato cultivars and growth conditions

Tomato cultivars used in this study were prepared as described in Han and Micallef (2014) to collect exudates from shoots and roots of seedlings. Briefly, seeds of the thirteen tomato (*Solanum lycopersicum*) cultivars selected based on distinct fruit phenotypes (Table 1) were surface-sterilized in half-strength household bleach for 30 min, followed by six to seven rinses with sterile water, as recommended by the

Tomato Genetics Resources Center (UC Davis, Sacramento, CA) and transferred aseptically onto Murashige and Skoog medium (MP Biomedicals LLC, Solon, OH) supplemented with 2% sucrose and 1.2% agar medium in culture plates. Germinated seedlings were grown sterilely in the plates at a 16L:8D photoperiod and at 26°C during the day and 18°C at night for 3 weeks. To collect exudates from mature plants and fruit, the tomato cultivars were started in a potting mix soil (Sunshine LC1, Sungro Horticulture, Canada) at the Research Greenhouse Complex, University of Maryland. They were grown under controlled light and temperature conditions (16L:8D photoperiod and 26°C/18°C day/night temperature) following the irrigation and nutrition management regimes preset at the facility. Leaf and root exudates were collected 6 weeks after seeds were sown in the potting soil, when flowers were about to emerge. Stem exudates were collected at 15 weeks. For the collection of fruit exudates, plants were grown under the same greenhouse conditions to fruit maturity, avoiding pesticide application.

Table 1. Tomato (*Solanum lycopersicum*) cultivars used in this study

Cultivar	Source	Note*
cv. 'CA Red Cherry'	Tomato Genetics Resource Center	Cherry variety
cv. 'Heinz-1706'		Genome sequenced by International Sequencing Project
cv. 'Moneymaker'		Suitable for Maryland
cv. 'Nyagous'		Black variety; Suitable for Maryland
cv. 'LA4013'		*hp-2* (High pigment-2) mutant in Moneymaker background
cv. 'Mobox'		Near isogenic line in Moneymaker background with R gene immunity to *Fusarium oxysporum* f. sp. *lycopersici*
cv. 'Movione'		Near isogenic line in Moneymaker background with R gene immunity to *Pseudomonas syringae* pv. tomato
cv. 'Micro-Tom'		Miniaturized cultivar
cv. 'Florida 91 VFF'	Tomato Growers Supply Co.	VFF resistance; Recommended for Mid-Atlantic[1]
cv. 'Rutgers Select'		Recommended for Mid-Atlantic[2]
cv. 'Rutgers VFA'		VFA resistance; Recommended for Mid-Atlantic[2]
cv. 'Virginia Sweets'		Heirloom; Bi-color variety
cv. 'Plum Dandy VF'	Territorial Seed Co.	Recommended for Mid-Atlantic[1]

* V = Resistance to *Verticillium* wilt; F = Resistance to *Fusarium* wilt; A = *Alternaria* resistance; Double letters mean resistance to two or more strains of the disease.

[1] http://www.mdipm.umd.edu/state_resources/MD%20VEG%20REC%202009.pdf

[2] http://www.hgic.umd.edu/content/documents/HG70RecommendedVegetableCultivarsrevised2_2010.pdf.

2.2. Exudates collection

Exudates from shoots and roots were collected from 3-week old seedlings grown sterilely and from plants grown in soil. Seedlings were carefully and aseptically pulled off the MS medium when two true leaves had fully emerged, and placed in a 6-well sterile tissue culture dish having shoots and roots in separate wells filled with 5

ml of sterile phosphate-buffered saline (PBS). The culture dish was shaken for 24 h at room temperature and 100 rpm on an orbital shaker to collect water-soluble exudates from each part of the seedling. For collection of 6-week exudates, mature plants grown in soil were uprooted, washed with tap water and then rinsed thoroughly with deionized water to remove soil particles off the plants. Rinsed shoots and roots were separately put into sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) containing 200 ml of sterile PBS, and shaken for 3 h to collect exudates, following the same shaking procedure described above. Stem exudates were collected from 15-week old plants by applying a sterile cotton swab gently onto stems until the swab is wetted. Ten strokes per each swab were performed in a standardized manner across samples. Each wetted swab was immerged into 10 ml of sterile PBS in a 15-ml conical tube and the tube was vortexed thoroughly to release stem exudates into the solution. Ripe fruit from each cultivar was aseptically harvested and placed in an open petri-dish containing 30 ml of sterile deionized water inside a biosafety cabinet with the blower turned on (Labconco, Kansas City, MO) for 3 h. Number of fruit in each petri dish depended on the fruit surface area (SA) submerged in solution, to approximate SA contact among cultivars. For small fruited cultivars, i.e. cv. 'California Red Cherry' and cv. 'Micro-Tom', more fruit were added into the dish. Exudate solutions were filter-sterilized using 0.2 μm syringe filters (VWR, Radnor, PA) and stored at -20^{o}C until used in a bacterial growth assay.

2.3. Preparation of *Salmonella* inoculum

Salmonella enterica Typhimurium LT2 (ATCC 700720) was used throughout most of this study. The other *Salmonella* strain used was *S. enterica* Newport, an isolate

recovered form a salmonellosis outbreak associated with tomato consumption (Greene et al., 2008), adapted for rifampicin resistance. *S.* Newport was only used in experiments evaluating bacterial growth on tomato fruit and in their exudates at two different ripeness stages, red ripe tomatoes ready for consumption, and tomatoes of mature-size but at a pre-breaker green stage. The *Salmonella* strain was maintained at -80°C in Brucella broth (BD, Sparks, MD) containing 15% glycerol, and plated on Trypticase soy agar (TSA; BD) plates incubated at 35°C overnight, prior to experiments. For growth of rifampicin resistant *S.* Newport, archiving and culture media were supplemented with 50 μg/ml rifampicin (Tokyo Chemical Industry Co. LTD., Japan). Overnight cultures of *S. enterica* grown on TSA at 35°C were suspended in sterile PBS at an OD_{600} of 0.5, which yields approximately 10^9 CFU/ml. Further dilutions were made in sterile PBS to inoculate the tomato exudate solutions. Actual cell concentrations of *Salmonella* suspension were enumerated on TSA plates.

2.4. *Salmonella* growth evaluation in tomato plant exudates and on tomato fruit

Salmonella cell suspension, prepared beforehand as an inoculum, was diluted to 10^6 CFU/ml. Twenty μl of the cell suspension were added to 2 ml of each shoot, root, stem or fruit exudate solution in a sterile culture tube at an initial concentration of 10^4 CFU/ml, or sterile PBS, followed by incubation at 35°C and 200 rpm in a shaking incubator. Multiplication of *Salmonella* cells was monitored at 0, 2, 4, 6 and 24 h following *Salmonella* inoculation. At each time point, serial dilutions were prepared from the cultures and direct plated on TSA for CFU enumeration. Measurement at a time point prior to *Salmonella* inoculation was included to ensure sterility of the

exudate solutions. Negative controls were carried out along with the experiments by inoculating 2 ml of sterile PBS with 20 µl of 10^6 CFU/ml *Salmonella* cell suspension. For fruit inoculation with *S*. Newport and cell recovery, the protocols described in Han and Micallef (2014) were followed. Briefly, 50 µl of 4 log CFU/ml *S*. Newport were aseptically loaded onto the intact fruit surface of each surface-sterilized fruit forming five droplets of 10 µl, spotted as tightly within a minimum diameter as possible. The inoculated fruits were incubated in closed sterile bags at room temperature for 24 h. The fruit skin where the *Salmonella* inocula had been mounted was cut off asceptically and transferred into sterile 1.5-ml microcentrifuge tubes containing 1 ml of PBS to prepare serial dilutions that were plated on TSA with 50 µg/ml rifampicin for CFU quantification.

2.5. Gas Chromatography Time-of-Flight Mass Spectrometry (GC-TOF-MS) analysis of exudates

Frozen exudate samples were shipped to the Genome Center Core Services at the University of California, Davis for GC-MS analysis. Briefly, all samples were spiked with a mixture of fatty acid methyl esters of C8, C9, C10, C12, C14, C16, C18, C20, C22, C24, C26, C28 and C30 linear chain length which served as an internal retention index (Fiehn et al., 2008; Sana et al., 2010). An Agilent 6890 gas chromatograph (Santa Clara, CA) containing a 30 m long, 0.25 mm i.d. rtx5Sil-MS column with an additional 10 m integrated guard column was used to run the samples. The Agilent 6890 was controlled by the Leco ChromaTOF software version 2.32 (St. Joseph, MI). Resulting text files were exported to a data server with absolute spectra intensities and further processed by a filtering algorithm implemented in the metabolomics

BinBase database (Fiehn et al., 2005). Metabolites were unambiguously assigned by the BinBase identifier numbers using retention index and mass spectrum as the two most important identification criteria. Additional confidence criteria were used by giving mass spectral metadata, using the combination of unique ions, apex ions, peak purity and signal/noise ratios. All database entries in BinBase were matched against the Fiehn mass spectral library (http://fiehnlab.ucdavis.edu/Metabolite-Library), which includes sugars, sugar alcohols, amino acids, organic acids, fatty acids, phenolics, etc. Metabolites lacking full structural identification ("unidentified") were unambiguously described by BinBase numbers and full mass spectra, quantifier ions and retention indices. Data normalization was performed as described in Fiehn et al. (2008), using total metabolite content. The data were given as peak heights for the quantification ion at the specific retention index.

2.6. Hierarchical Cluster analysis of metabolites in exudates

Hierarchical Cluster Analysis (HCA) was performed as described in Micallef et al. (2009a). Briefly, reported data for both the identified and unidentified metabolites were log transformed to down weight highly abundant compounds and outliers and ensure a more Gaussian-type frequency distribution, and imported into PRIMER 6 (Plymouth Routines in Multivariate Ecological Research - version 6.1.15) from PRIMER-E Ltd., Plymouth, UK, a statistical software package for the analysis of biological, multivariate data. Similarity matrices for the metabolites profiles were constructed by calculating similarities between each pair of samples using the Bray–Curtis coefficient. To visualize the relationship among samples, the similarity matrices were analyzed by Hierarchical Cluster Analysis (HCA), a classification

method that aims to group samples into discrete clusters based on similarity. HCA was performed by the group-average linkage agglomerative method and dendrograms were constructed from the ranked similarities. For significance testing of sample data, the non-parametric permutation procedure ANOSIM (Analysis of Similarity), available in PRIMER 6, was employed. This test applies ranks to similarity matrices used for HCA and combines this ranking similarity with Monte Carlo randomization to generate significance levels (*p* values). ANOSIM tests the null hypothesis, for which a test statistic R will have a value of 0, that all samples are the same. As R approaches 1, the null hypothesis is rejected and this describes a case where replicates from one group are more similar to each other than to replicates from other groups.

2.7. Statistical Analysis

Each treatment (i.e. tomato cultivar) was tested in replicate. The experiments were repeated and data were pooled from separate experiments for statistical analysis. Enumeration data in CFU per ml were log transformed to satisfy the assumptions on normality of residuals and homogeneity of variances. Differences in log CFU per ml detected between treatments were tested for significance using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. Student's *t* test was performed when comparing only two levels of treatment. Pearson or Spearman correlation analysis was performed when measuring a statistical dependence between two parametric or nonparametric variables with a small sample size, respectively. Statistical analyses were carried out using JMP Pro 11 (SAS Institute Inc., Cary, NC). For all analyses, $p<0.05$ was considered significant.

Bacterial growth data were fitted to a growth model using IPMP 2013, a predictive microbiology tool, available at http://www.ars.usda.gov/Main/docs.htm?docid=23355 (Huang, 2014). Of the models available in the program, the three-phase linear model was selected as proposed by Buchanan et al. (1997) for its consideration of known bacterial physiological behavior as individual cells and as populations. This model can be described by:

Lag phase: $N_t = N_0$, if $t \leq t_{Lag}$,

Exponential growth phase: $N_t = N_0 + k(t - t_{Lag})$, if $t_{Lag} < t < t_{Max}$,

and Stationary phase: $N_t = N_{max}$, if $t \geq t_{Max}$

where N_t = log of the population density at time t (log CFU/ml); N_0 = log of the initial population density (log CFU/ml); N_{max} = log of the maximum population density supported by the environment (log CFU/ml); t = elapsed time (h); t_{Lag} = time when the lag phase ends (h); t_{Max} = time when the maximum population density is reached (h); k= specific growth rate [(log CFU/ml)/h].

Growth kinetic parameters (N_0, Lag, k, and N_{max}) generated by the IPMP 2013 program were compared for significance using 95% confidence intervals associated with the parameters.

3. Results

3.1. Tomato fruit exudates

3.1.1. Tomato fruit exudates can support *S*. Typhimurium growth in a cultivar-dependent manner

Fruit exudates collected from different cultivars were inoculated with *S.* Typhimurium at a level of 4.3–5.1 log CFU/ml. Increases in *Salmonella* populations were observed on all fruit exudates examined in this study, and showed a cultivar-dependent pattern (Fig.1). At 6 h post inoculation, following an inoculation concentration of 4.6 log CFU/ml, 0.6 to 1.7 log CFU increases in population levels were observed. Fruit exudates of tomato cultivars cv. 'Heinz-1706' and cv. 'Plum Dandy VF' were significantly less supportive of bacterial growth than those of cv. 'Florida 91 VFF', cv. 'LA4013', cv. 'Micro-Tom', and cv. 'Rutgers VFA' ($p<0.05$) (Fig. 1a). When bacterial growth reached a plateau, a stationary phase, as measured at the 24 h time point, populations in fruit exudates of cv. 'Plum Dandy VF' ($p=0.0361$) and 'Heinz-1706' ($p=0.0572$) remained smaller than those in exudates of cv. 'Rutgers VFA' (Fig. 1b). Overall, population levels at the 24 h measurement were 1.5 to 2.4 log CFU higher relative to the initial inoculation level.

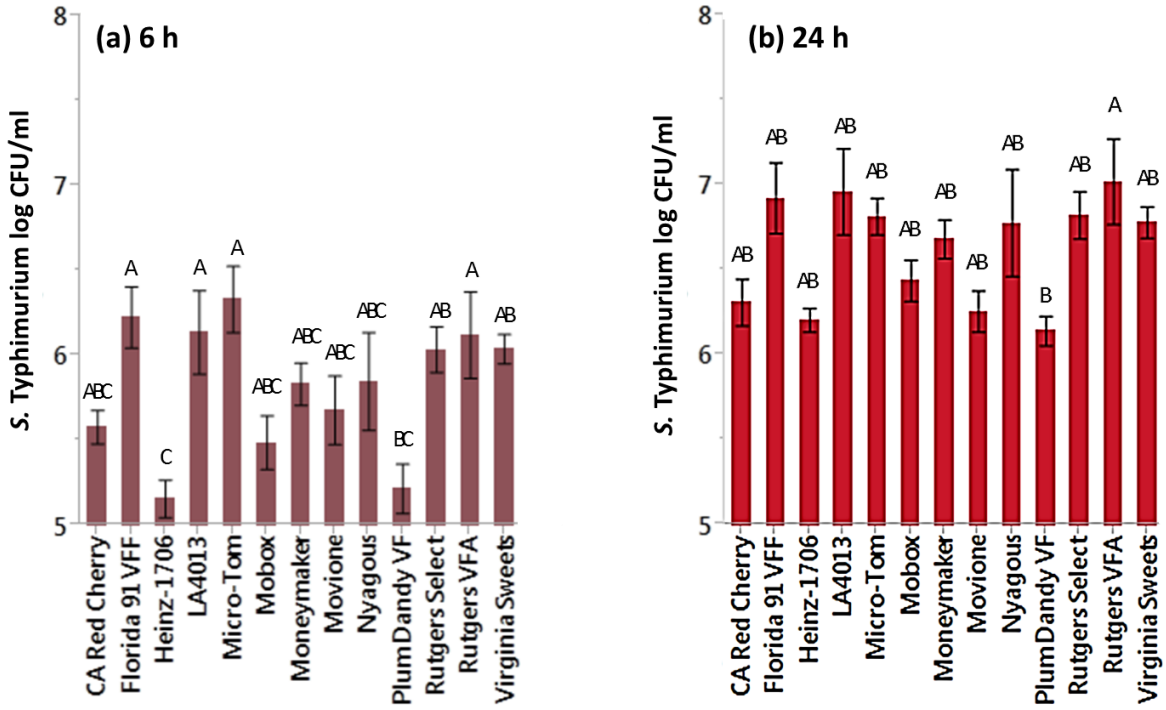


Fig. 1. Growth of *S.* Typhimurium in fruit exudates; population densities measured at 6 hours (a) and 24 hours (b) post inoculation. Error bars indicate standard error of the mean; bars labeled with the same letter are not significantly different within the same time point measurement by Tukey's HSD test (p<0.05).

The observed bacterial growth in fruit exudates was fitted to Buchanan's three-phase linear model using IPMP 2013 (Huang, 2014). Fig. 2 illustrates the curves fitted for *S.* Typhimurium growth in fruit exudates of different tomato cultivars, depicting three growth phases - lag, exponential growth and stationary phases. The growth phase-specific parameters describing the curves are presented in Table 2. Statistically significant differences in k, Lag, and N_{max} were detected among the different cultivars (Table 2). The observations shown in Fig. 1b are well supported by the maximum

population density parameters denoted as N_{max}. The N_{max} of *S.* Typhimurium grown in cv. 'Rutgers VFA' fruit exudates is the highest, whereas the lowest was obtained from cv. 'Plum Dandy VF', followed by that of 'Heinz-1706' ($p<0.05$). The lower N_{max} in the fruit exudates of cv. 'Plum Dandy VF' could be due to the significantly longer lag phase observed for this cultivar. Other cultivars exhibiting higher $N_{max,}$ such as cv. 'Moneymaker', cv. 'Rutgers Select', and cv. 'Virginia Sweets' showed significantly shorter lag phase durations ($p<0.05$). Growth rate, *k,* also could affect N_{max}. *S.* Typhimurium grown in the fruit exudates of cv. 'Micro-Tom' showed the highest growth rate and reached a higher N_{max} although the lowest growth rate observed in cv. 'Mobox' is not necessarily followed by a lower N_{max}. Pairwise correlation of N_0, *k,* Lag, and N_{max} showed that there is a negative correlation identified only between Lag and N_{max} ($p = 0.002$), indicating that a longer lag phase duration will likely lead to a lower maximum population density.

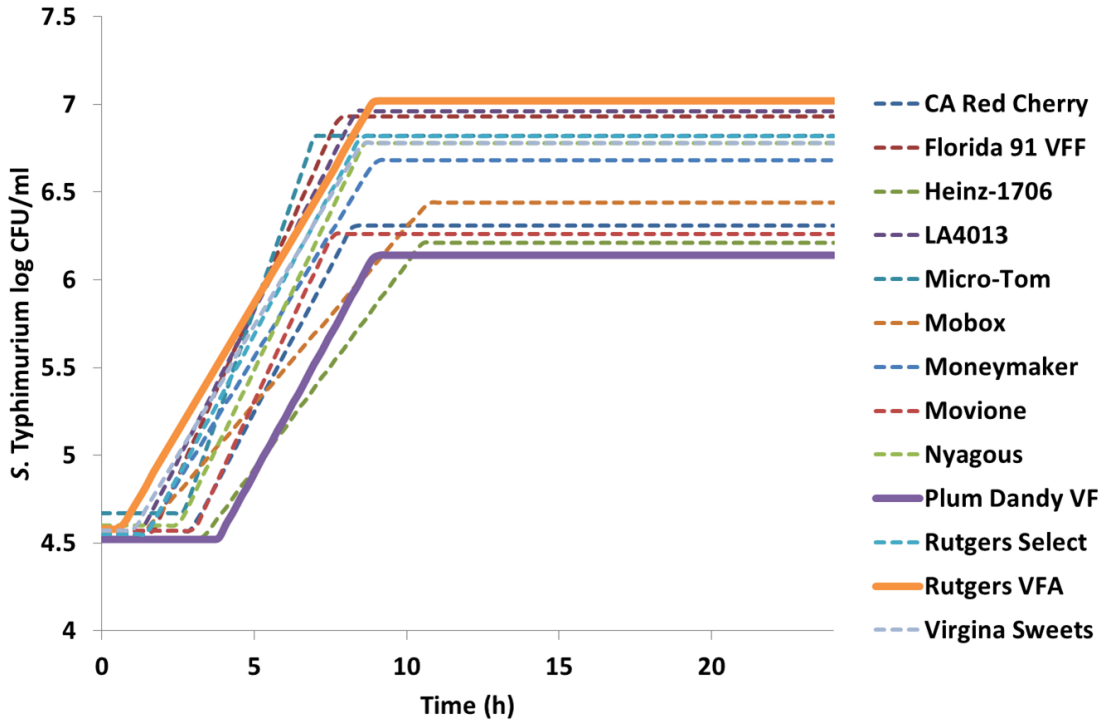


Fig. 2. Growth curves of *S.* Typhimurium in fruit exudates of thirteen tomato cultivars predicted by Buchanan's three phase linear model, based on CFU measurements made at five time points over 24 h (using IPMP 2013).

Table 2. Three Phase Model parameters for growth of *S.* Typhimurium in fruit exudates from various tomato cultivars (see Fig. 2); Measurements labeled with the same letter are not significantly different within the same column at $p<0.05$ by Tukey's HSD test.

Cultivar	N_0 ± C.I. (log CFU/ml)	k ± C.I.	Lag ± C.I. (h)	N_{max} ± C.I. (log CFU/ml)
CA Red Cherry	4.57 ± 0.15 a	0.33 ± 0.15 ab	2.96 ± 1.28 ab	6.31 ± 0.18 cd
Florida 91 VFF	4.57 ± 0.32 a	0.39 ± 0.14 ab	1.71 ± 1.42 ab	6.93 ± 0.32 a
Heinz-1706	4.52 ± 0.12 a	0.23 ± 0.13 ab	3.30 ± 1.31 ab	6.21 ± 0.15 cd
LA4013	4.55 ± 0.40 a	0.34 ± 0.17 ab	1.28 ± 2.18 ab	6.96 ± 0.41 a
Micro-Tom	4.67 ± 0.19 a	0.50 ± 0.19 a	2.70 ± 1.13 ab	6.82 ± 0.24 a
Mobox	4.54 ± 0.22 a	0.20 ± 0.09 b	1.29 ± 2.01 ab	6.44 ± 0.22 abcd
Moneymaker	4.54 ± 0.19 a	0.28 ± 0.08 ab	1.41 ± 1.18 b	6.68 ± 0.19 ab
Movione	4.57 ± 0.20 a	0.37 ± 0.21 ab	3.03 ± 1.50 ab	6.26 ± 0.26 bcd
Nyagous	4.60 ± 0.38 a	0.36 ± 0.39 ab	2.54 ± 3.38 ab	6.78 ± 0.45 abc
Plum Dandy VF	4.52 ± 0.14 a	0.32 ± 0.15 ab	3.80 ± 0.95 a	6.14 ± 0.18 d
Rutgers Select	4.55 ± 0.21 a	0.32 ± 0.09 ab	1.48 ± 1.17 b	6.82 ± 0.21 a
Rutgers VFA	4.58 ± 0.40 a	0.29 ± 0.17 ab	0.61 ± 2.81 ab	7.02 ± 0.40 a
Virginia Sweets	4.57 ± 0.15 a	0.30 ± 0.06 ab	1.06 ± 0.97 b	6.78 ± 0.15 a

Although care was taken to standardize the surface area of fruit immersed in water during exudates collection, there still existed a large variation in fruit shape depending on cultivar type. To best analyze the influence of fruit shape effect, immersed fruit surface area for each cultivar was estimated using an equation to calculate partial surface area of a sphere and then a pairwise correlation was carried out with the growth curve parameters in Table 2. No correlation was observed for

any of the pairs, indicating that fruit shape was not a major factor contributing to the cultivar-specific differential growth curves of *S.* Typhimurium (data not presented). The growth of *S.* Typhimurium in fruit exudates was correlated with the epiphytic growth of the same bacterial strain on tomato fruit. The N_{max} obtained in this study for each cultivar was plotted against the epiphytic population level on tomato fruit (available in Fig. 1 of Han and Micallef (2014), Chapter 3 in this document) (Fig. 3). A positive correlation (Spearman's ρ=0.7143; p=0.0061) was detected. Differential growth of *S.* Typhimurium on fruit of different cultivars appears to be, at least in part, explained by variation in fruit exudates among cultivars.

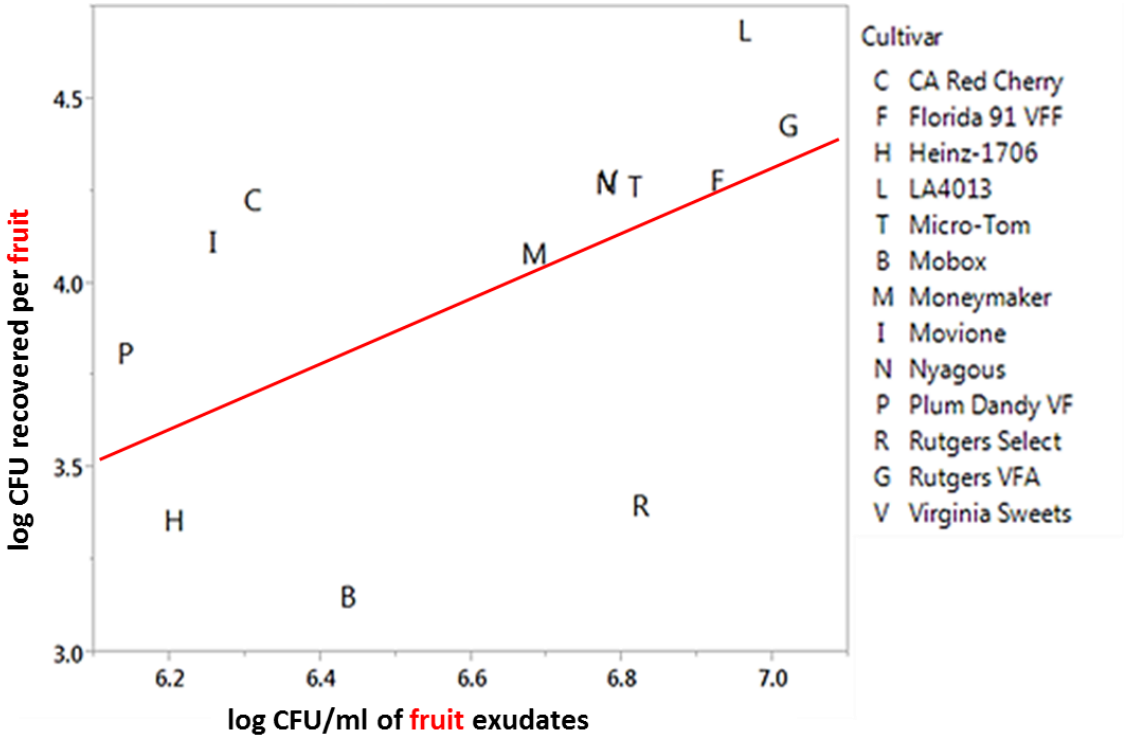


Fig. 3. Scatter plot displaying the relationship between the growth of *S.* Typhimurium in fruit exudates and the epiphytic growth of the pathogen on fruit of corresponding cultivars.

3.1.2. Lower population levels of *S*. Newport were recovered from unripened green fruit and their exudates, than from ripe ones

The surfaces of green mature or ripe red cv. 'Nyagous' fruit were spot inoculated with a tomato outreak strain of *S*. Newport. After 24 h incubation with the fruits, there was about 2.5-3.5 log CFU increase in population level in comparison to the initial level at 2.5 log CFU/fruit for both conditions, but higher population levels of *S*. Newport were recovered from the surface of red ripe fruit than from green fruit (p=0.0394) (Fig. 4a). In addition, the exudates collected from red fruit were more supportive of *S*. Newport growth than those from green tomatoes (p=0.0290) (Fig. 4b).

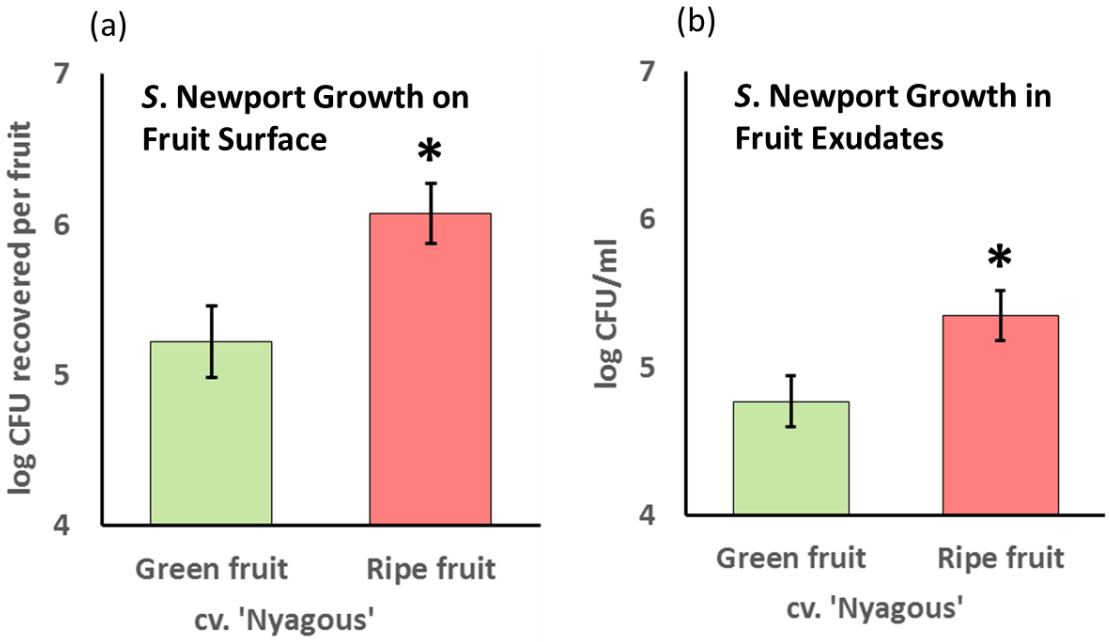


Fig. 4. Growth of *S*. Newport on fruit surface (a) and in fruit exudates (b) from cv. 'Nyagous' fruit of different ripeness 24 hours post inoculation (n=8); Error bars indicate standard error of the mean; Asterisks denote the significance at p<0.05 by student's *t*-test.

3.2. Cultivar-dependent growth of *S*. Typhimurium was also observed in shoot and root exudates, but the patterns differed from fruit

3.2.1. Shoot and root exudates from 3-week old plants

Leaf and root exudates of various tomato cultivars were able to support *S.* Typhimurium growth. Exudates inoculated with about 4.6 log CFU/ml *S.* Typhimurium showed a 3.8-5.3 log CFU increase after 24 h. The growth of *S.* Typhimurium in shoot and root exudates from 3-week-old seedlings grown sterilely is depicted in Fig. 5. For shoot exudates, at 6 hours post *S.* Typhimurium inoculation, *Salmonella* levels had increased to about 7.4 log CFU/ml, but no significant differences among cultivars were detectable (Fig. 5a). On the other hand, at 24 hours post inoculation, the population levels of *S.* Typhimurium in shoot exudates of cv. 'Plum Dandy VF' were significantly higher than in exudates from cv. 'California Red Cherry', cv. 'Florida 91 VFF', cv. 'Heinz-1706', cv. 'Mobox', cv. 'Moneymaker', cv. 'Movione', cv. 'Nyagous', and cv. 'Virginia Sweets' ($p<0.05$) (Fig. 5b). In contrast, the exudates collected from the fruit of cv. 'Plum Dandy VF' were the least supportive of growth of *S.* Typhimurium (Fig. 1).

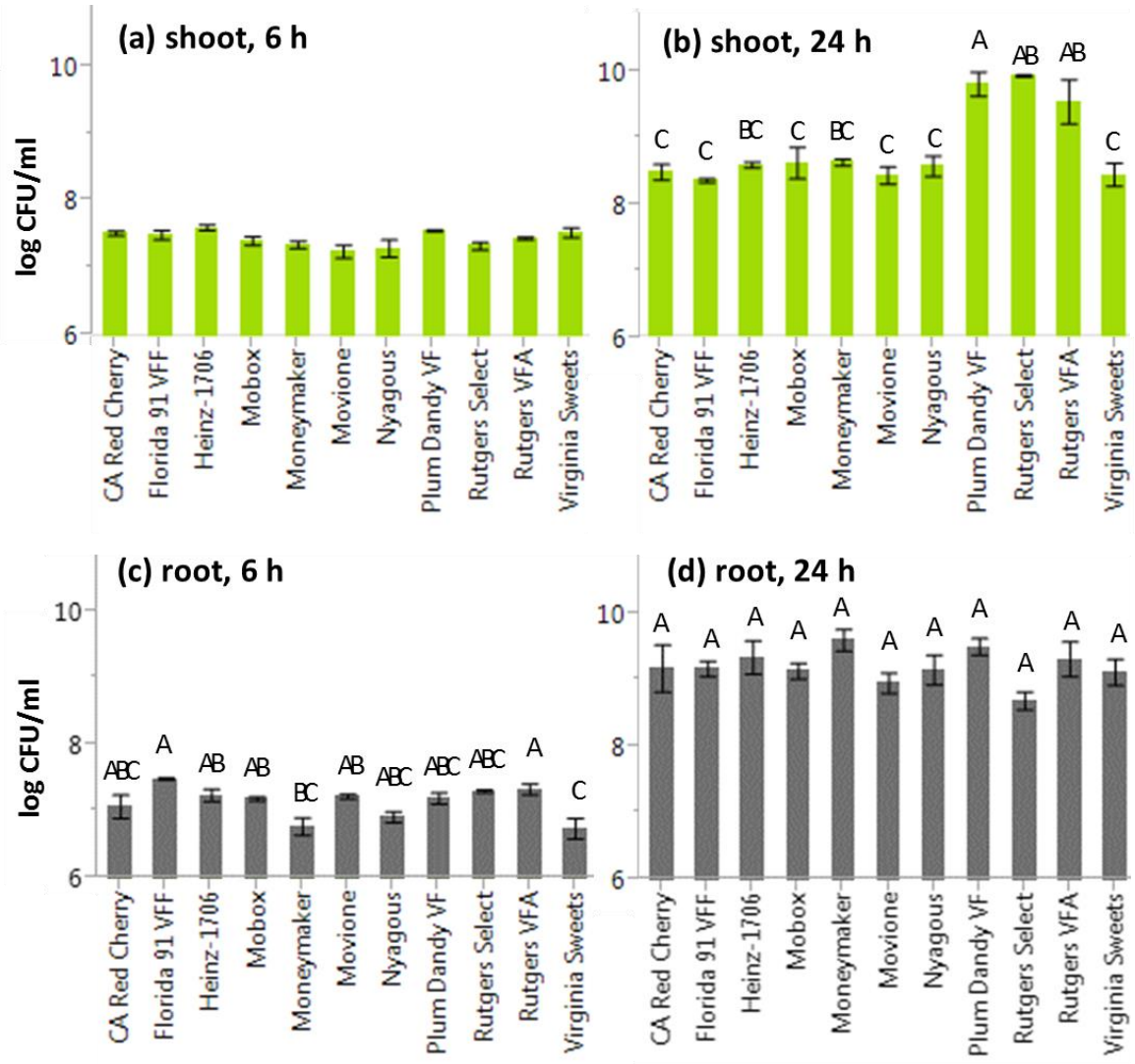


Fig. 5. Growth of *S.* Typhimurium in 3-week old seedling exudates; *S.* Typhimurium cells recovered from the shoot (top a and b) and from the roots (bottom c and d); population densities measured at 6 hours (a and c) and 24 hours (b and d) post inoculation. Error bars indicate standard error of the mean; bars labeled with the same letter are not significantly different within the same time point measurement by Tukey's HSD test ($p<0.05$).

The fitted growth curves using the Buchanan three-phase model showed that shoot exudates from cv. 'Plum Dandy VF', cv. 'Rutgers Select', and cv. 'Rutgers VFA' allowed *S.* Typhimurium to reach higher N_{max}, although none of the other growth parameters, N_0, *k*, and Lag, explain why the higher N_{max} values were obtained for these three cultivars (Fig. 6a and Table. 3).

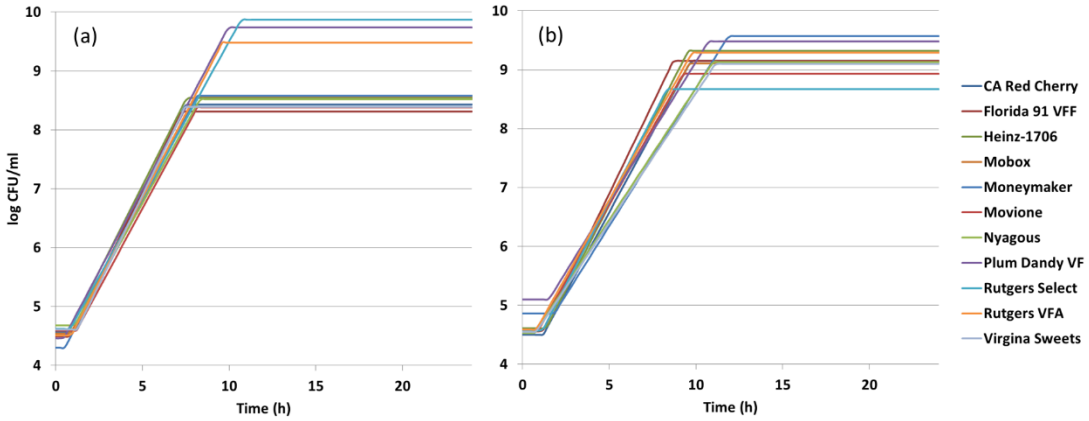


Fig. 6. Growth curves of *S.* Typhimurium in 3-week old seedling leaf (a) and root (b) exudates of eleven tomato cultivars predicted by Buchanan's three phase linear model, based on CFU measurements made at five time points over 24 h (using IPMP 2013).

Table 3. Three Phase Model parameters for growth of *S.* Typhimurium in 3-week old seedling leaf exudates from various tomato cultivars (see Fig. 6a); Measurements labeled with the same letter are not significantly different within the same column at $p<0.05$ by Tukey's HSD test.

Cultivar	N_0 ± C.I. (log CFU/ml)	k ± C.I.	Lag ± C.I. (h)	N_{max} ± C.I. (log CFU/ml)
CA Red Cherry	4.57 ± 0.17 a	0.60 ± 0.05 a	1.04 ± 0.39 a	8.43 ± 0.13 b
Florida 91 VFF	4.49 ± 0.16 a	0.60 ± 0.06 a	0.86 ± 0.44 a	8.31 ± 0.16 b
Heinz-1706	4.52 ± 0.14 a	0.59 ± 0.03 a	0.66 ± 0.33 a	8.54 ± 0.11 b
Mobox	4.54 ± 0.43 a	0.56 ± 0.09 a	0.84 ± 0.98 a	8.57 ± 0.25 b
Moneymaker	4.30 ± 0.26 a	0.56 ± 0.04 a	0.51 ± 0.54 a	8.58 ± 0.11 b
Movione	4.59 ± 0.35 a	0.54 ± 0.08 a	1.17 ± 0.80 a	8.38 ± 0.22 b
Nyagous	4.68 ± 0.46 a	0.53 ± 0.08 a	1.09 ± 1.01 a	8.52 ± 0.23 b
Plum Dandy VF	4.46 ± 0.48 a	0.56 ± 0.07 a	0.45 ± 1.00 a	9.74 ± 0.20 a
Rutgers Select	4.62 ± 0.11 a	0.53 ± 0.04 a	0.82 ± 0.34 a	9.87 ± 0.14 a
Rutgers VFA	4.51 ± 0.60 a	0.57 ± 0.13 a	0.89 ± 1.30 a	9.48 ± 0.35 a
Virginia Sweets	4.61 ± 0.40 a	0.61 ± 0.08 a	1.24 ± 0.79 a	8.39 ± 0.23 b

Root exudates of cv. 'Moneymaker' and cv. 'Virginia Sweets' were less supportive of bacterial growth at 6 hour post inoculation, compared to cv. 'Florida 91 VFF' and cv. 'Rutgers VFA' (Fig. 5c). Growth in root exudates at 24 hours post *S.* Typhimurium inoculation, however, revealed no significant cultivar-dependent differences (Fig. 5d). Any obvious cultivar-dependent signals were also not discernable in the fitted growth curves although subtle differences in log CFU/ml of N_{max} existed (Fig. 6b and Table 4). This indicates that plant genotype may not be a major determinant for *Salmonella* growth in root systems at this early plant growth stage.

Table 4. Three Phase Model parameters for growth of *S.* Typhimurium in 3-week old seedling root exudates from various tomato cultivars (see Fig. 6b); Measurements labeled with the same letter are not significantly different within the same column at $p<0.05$ by Tukey's HSD test.

Cultivar	N_0 ± C.I. (log CFU/ml)	k ± C.I.	Lag ± C.I. (h)	N_{max} ± C.I. (log CFU/ml)
CA Red Cherry	4.50 ± 0.60 a	0.55 ± 0.15 ab	1.18 ± 1.42 a	9.15 ± 0.43 abc
Florida 91 VFF	4.56 ± 0.14 a	0.61 ± 0.05 a	1.15 ± 0.34 a	9.15 ± 0.14 b
Heinz-1706	4.61 ± 0.38 a	0.57 ± 0.10 ab	1.35 ± 0.84 a	9.32 ± 0.27 ab
Mobox	4.57 ± 0.26 a	0.52 ± 0.05 ab	0.86 ± 0.61 a	9.11 ± 0.15 b
Moneymaker	4.86 ± 0.61 a	0.47 ± 0.09 b	1.83 ± 1.39 a	9.58 ± 0.25 a
Movione	4.55 ± 0.26 a	0.52 ± 0.06 ab	0.85 ± 0.64 a	8.93 ± 0.16 bc
Nyagous	4.53 ± 0.48 a	0.45 ± 0.08 b	0.70 ± 1.28 a	9.13 ± 0.24 ab
Plum Dandy VF	5.10 ± 0.42 a	0.48 ± 0.06 ab	1.51 ± 0.95 a	9.48 ± 0.17 a
Rutgers Select	4.57 ± 0.16 a	0.57 ± 0.06 ab	1.13 ± 0.43 a	8.67 ± 0.16 c
Rutgers VFA	4.59 ± 0.49 a	0.52 ± 0.10 ab	0.78 ± 1.16 a	9.29 ± 0.28 ab
Virginia Sweets	4.55 ± 0.46 a	0.44 ± 0.09 b	0.78 ± 1.29 a	9.10 ± 0.26 ab

In order to test whether the cultivar-dependent epiphytic growth of *S.* Typhimurium observed on leaves of 3-week-old tomato seedlings was also due to a factor existing in leaf exudates, the maximum population density of the bacteria grown in the exudates from shoots of 3-week-old tomato seedlings for each cultivar was plotted against the epiphytic population levels on leaves of the tomato seedlings at the same growth stage which are available in the Fig. 2 of Han and Micallef (2014) (Chapter 3 in this document). A significant correlation was detected only at $p<0.1$ (Spearman's $\rho=0.7143$; $p=0.0713$). Although this suggests that at this early developmental stage, shoot exudates appear to be a factor driving the differential cultivar susceptibility to

epiphytic colonization with the pathogen, the influence of small sample size (n=7) on correlation analysis should be considered.

3.2.2. Shoot and root exudates from 6-week old plants

The differential growth of *S.* Typhimurium was also observed in shoot and root exudates collected from 6-week-old plants grown under greenhouse conditions (Fig. 7), although population levels reached were lower compared to those in 3-week exudates presented in Fig. 5. Throughout the course of incubation, cv. 'Plum Dandy VF' shoot exudates were the least supportive of *Salmonella* growth (2.2 log CFU/ml increase from inoculation level) (Fig. 7a and 7b). Interestingly, the lowest population levels of *S.* Typhimurium recovered from fruit exudates were from cv. 'Plum Dandy VF' (Fig. 1), which suggests that at later plant growth stages, cv. 'Plum Dandy VF' may exude chemical compounds via both leaves and fruit that are less utilizable by the enteric pathogen. The exudates that were most supportive of bacterial growth were from cv. 'California Red Cherry' and cv. 'Virginia Sweets' at 6 hours and 24 hours post *S.* Typhimurium inoculation, respectively.

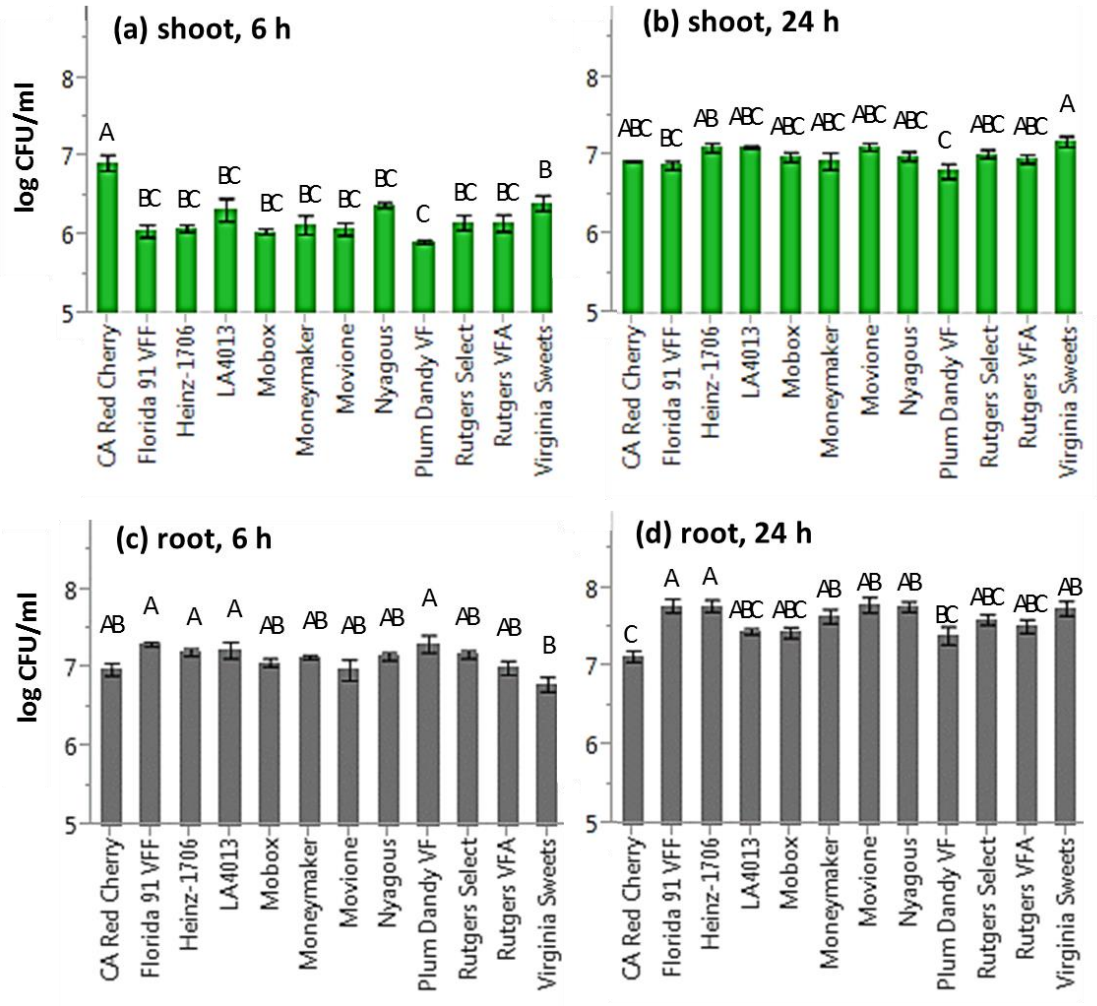


Fig. 7. Growth of *S.* Typhimurium in 6-week old plant exudates (shoots and roots); *S.* Typhimurium cells recovered from the shoot (top a and b) and from the roots (bottom c and d); population densities measured at 6 hours (a and c) and 24 hours (b and d) post inoculation. Error bars indicate standard error of the mean; bars labeled with the same letter are not significantly different within the same time point measurement by Tukey's HSD test ($p<0.05$).

Assessment of *Salmonella* growth kinetics using the Buchanan three-phase model, presented in Fig. 8a and Table 5, revealed that *S.* Typhimurium grown in leaf

exudates of 6-week-old plants of cv. ‘California Red Cherry’ had the highest growth rate, denoted as *k*, compared to other cultivars ($p<0.05$). Despite this higher growth rate, the maximum population levels were achieved in leaf exudates of cv. ‘Heinz-1706’, cv. ‘Movione’, and cv. ‘Virginia Sweets’. The smallest N_{max} values were obtained for cv. ‘California Red Cherry’, cv. ‘Florida 91 VFF’, and cv. ‘Plum Dandy’.

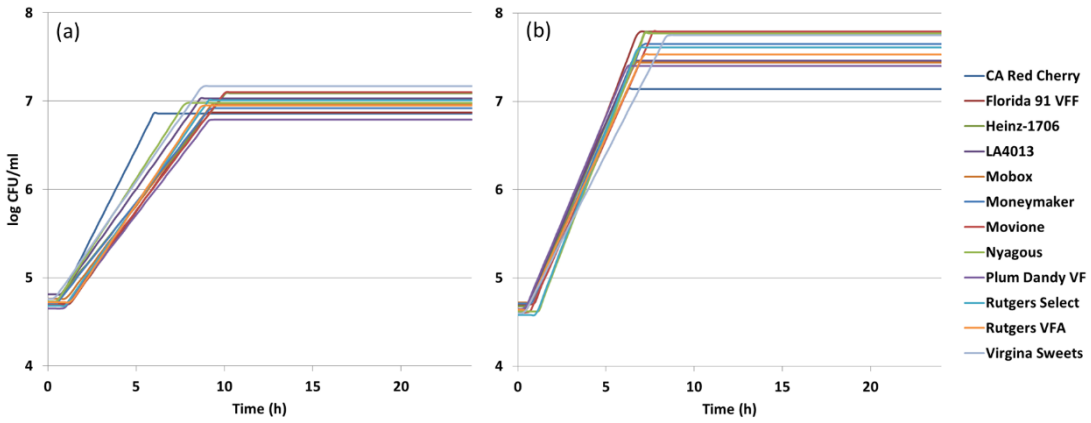


Fig. 8. Growth curves of *S.* Typhimurium in 6-week old plant leaf (a) and root (b) exudates of twelve tomato cultivars predicted by Buchanan’s three phase linear model, based on CFU measurements made at five time points over 24 h (using IPMP 2013).

Table 5. Three Phase Model parameters for growth of *S.* Typhimurium in 6-week old plant leaf exudates from various tomato cultivars (see Fig. 8a); Measurements labeled with the same letter are not significantly different within the same column at $p<0.05$ by Tukey's HSD test.

Cultivar	N_0 ± C.I. (log CFU/ml)	k ± C.I.	Lag ± C.I. (h)	N_{max} ± C.I. (log CFU/ml)
CA Red Cherry	4.73 ± 0.12 a	0.40 ± 0.05 a	0.65 ± 0.60 a	6.86 ± 0.12 b
Florida 91 VFF	4.70 ± 0.09 a	0.28 ± 0.05 b	1.26 ± 0.60 a	6.87 ± 0.09 b
Heinz-1706	4.70 ± 0.08 a	0.25 ± 0.04 b	0.36 ± 0.77 a	7.09 ± 0.08 a
LA4013	4.81 ± 0.18 a	0.28 ± 0.06 b	0.75 ± 1.04 a	7.03 ± 0.18 ab
Mobox	4.76 ± 0.16 a	0.27 ± 0.06 b	1.04 ± 0.93 a	6.97 ± 0.16 ab
Moneymaker	4.70 ± 0.18 a	0.26 ± 0.06 b	0.49 ± 1.18 a	6.92 ± 0.18 ab
Movione	4.69 ± 0.11 a	0.27 ± 0.04 b	1.01 ± 0.67 a	7.10 ± 0.11 a
Nyagous	4.73 ± 0.10 a	0.31 ± 0.05 ab	0.52 ± 0.72 a	6.98 ± 0.10 ab
Plum Dandy VF	4.65 ± 0.12 a	0.26 ± 0.06 b	0.92 ± 0.93 a	6.79 ± 0.12 b
Rutgers Select	4.68 ± 0.12 a	0.29 ± 0.04 b	1.00 ± 0.66 a	7.01 ± 0.12 ab
Rutgers VFA	4.72 ± 0.09 a	0.30 ± 0.05 ab	1.31 ± 0.57 a	6.95 ± 0.09 ab
Virginia Sweets	4.76 ± 0.11 a	0.29 ± 0.05 b	0.39 ± 0.83 a	7.17 ± 0.11 a

In the root exudates of 6-week-old tomato, the higher population levels of *S.* Typhimurium were recovered from cv. 'Florida 91 VFF', cv. 'Heinz-1706', cv. 'LA4013' and cv. 'Plum Dandy VF' at 6 hours post inoculation. Only populations growing in exudates of cv. 'Florida 91 VFF' and cv. 'Heinz-1706', remained significantly higher following 24 hours incubation (Fig. 7c and 7d). The higher population densities of the pathogen in the root exudates from cv. 'Florida 91 VFF' could be attributable to the higher growth rate *k,* although this interpretation does not hold for cv. 'Heinz-1706' (Table 6).

Table 6. Three Phase Model parameters for growth of *S.* Typhimurium in 6-week old plant root exudates from various tomato cultivars (see Fig. 8b); Measurements labeled with the same letter are not significantly different within the same column at $p<0.05$ by Tukey's HSD test.

Cultivar	$N_0 \pm$ C.I. (log CFU/ml)	k ± C.I.	Lag ± C.I. (h)	$N_{max} \pm$ C.I. (log CFU/ml)
CA Red Cherry	4.68 ± 0.12 a	0.44 ± 0.05 ab	0.60 ± 0.51 ab	7.14 ± 0.12 b
Florida 91 VFF	4.70 ± 0.11 a	0.53 ± 0.06 a	0.93 ± 0.42 ab	7.79 ± 0.11 a
Heinz-1706	4.68 ± 0.12 a	0.47 ± 0.06 ab	0.62 ± 0.51 ab	7.78 ± 0.11 a
LA4013	4.62 ± 0.12 a	0.47 ± 0.04 ab	0.39 ± 0.45 b	7.46 ± 0.12 b
Mobox	4.72 ± 0.13 a	0.47 ± 0.05 ab	0.82 ± 0.45 ab	7.44 ± 0.13 b
Moneymaker	4.71 ± 0.11 a	0.47 ± 0.04 ab	0.83 ± 0.39 ab	7.65 ± 0.11 ab
Movione	4.61 ± 0.18 a	0.45 ± 0.06 ab	0.67 ± 0.65 ab	7.79 ± 0.18 a
Nyagous	4.62 ± 0.09 a	0.52 ± 0.04 a	1.17 ± 0.32 a	7.78 ± 0.09 a
Plum Dandy VF	4.65 ± 0.15 a	0.48 ± 0.07 ab	0.43 ± 0.67 ab	7.40 ± 0.14 b
Rutgers Select	4.58 ± 0.14 a	0.53 ± 0.05 a	1.05 ± 0.41 ab	7.61 ± 0.14 ab
Rutgers VFA	4.64 ± 0.12 a	0.44 ± 0.06 ab	0.53 ± 0.61 ab	7.53 ± 0.12 ab
Virginia Sweets	4.60 ± 0.12 a	0.39 ± 0.06 b	0.38 ± 0.70 ab	7.75 ± 0.12 a

3.3. No cultivar-specific differences were observed in growth of *S.* Typhimurium in stem exudates from 15 week old tomato

Unlike shoot, root, or fruit exudates, the cultivar-specific differential growth of *S.* Typhimurium was not found in stem exudates collected from 15-week old plants grown under greenhouse conditions (Appendix 1 Fig. 1.). On average, a 2.4 log

CFU/ml increase in population in stem exudates was observed after the initial inoculation of 4.7 log CFU/ml.

3.4. Tomato plant exudation changes over a developmental course and differs by plant organ

The primary and secondary metabolites present in tomato exudates of four different cultivars at different developmental stages and by different plant organ were analyzed by GC-TOF mass spectrometry for each sample (multiple biological replicates within the same growth condition were pooled for the chemical analysis). Four tomato cultivars, cv. 'Heinz-1706', cv. 'Nyagous', cv. 'Plum Dandy VF', and cv. 'Rutgers VFA', were chosen for this chemical analysis since they had shown a pattern of supporting different levels of *S.* Typhimurium populations in the exudates collected from shoots, roots or fruit. In summary, as measured at the 24 h time point, *S.* Typhimurium populations in fruit exudates of cv. 'Plum Dandy VF' (p=0.0361) and 'Heinz-1706' (p=0.0572) were lower than those in fruit exudates of cv. 'Rutgers VFA' (Fig. 1b). For leaf exudates from 3-week old seedlings, at 24 h post inoculation, cv. 'Heinz-1706' (p=0.0049) and cv 'Nyagous' (p=0.0008) were less supportive to S. Typhimurium growth than cv. 'Plum Dandy VF' (Fig. 5b). After 24 h culture, less *S.* Typhimurium populations were recovered from the shoot and root exudates from 6-week old plants of cv. 'Plum Dandy VF' than from those of cv. 'Heinz-1706', at p=0.0342 and p=0.0441, respectively (Fig. 7b and 7d).

GC-TOF-MS analysis resulted in, after data normalization, a total of 287 compounds detected in tomato exudates. Among these, 145 compounds were identified based on

the mass spectral library database developed by Fiehn laboratory (University of California, Davis), which includes sugars, sugar alcohols, amino acids, organic acids, fatty acids, and phenolics. The resulting metabolite profile data including unidentified metabolites were subjected to Hierarchical Cluster Analysis (HCA) using PRIMER 6 analysis tool package after $\log_{10}$ transformation of the original peak height data. HCA revealed that shoot and root exudate profiles of 3-week-old seedlings clustered together (93% similarity), being distinct from the exudate profiles collected from plants at later developmental stages including tomato fruit (83% similarity) (Fig. 9). Within the 3-week old seedling group, the exudate profiles clustered separately by plant organ, root versus shoot. The exudate profiles of 6-week old shoots and roots formed two distinct clusters, with cv. 'Heinz-1706' and cv. 'Plum Dandy VF' fruit exudate profiles clustering with shoots (Fig. 9).

The 145 identified exudate compounds were broadly categorized into six groups: amino acids, fatty acids, organic acids, phenolics, sugars, and sugar alcohols. ANOSIM on total exudates from 3-week and 6-week leaves and fruit revealed significant differences among all groups (Global R=0.867; $p<0.001$) (Fig 9). By classifying known compounds into metabolite groups, age- and organ-dependent differences could be further examined. The exudate profiles of 3-week old seedlings where significantly different from those of both 6-week old plants and fruits for all metabolic groups ($p<0.05$) (Fig. 10). However, fruits and 6-week old plants were not different with regards to the compositions of phenolics, amino acids, and fatty acids (Fig. 10).

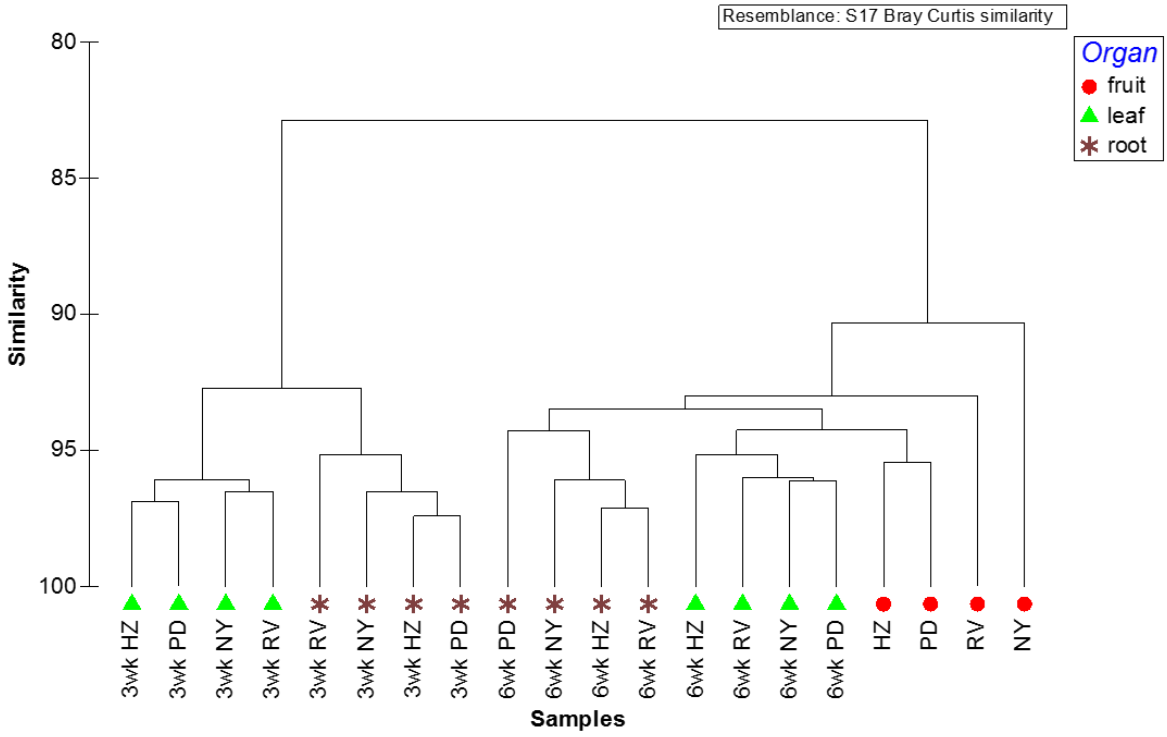
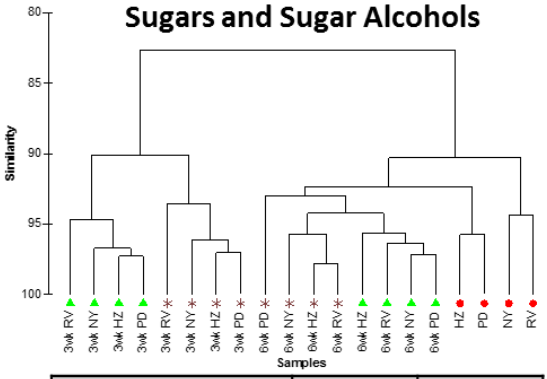


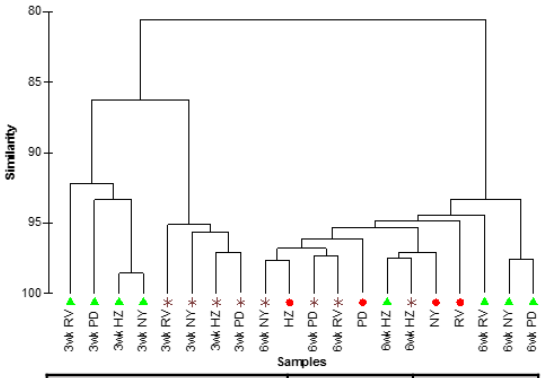
Fig. 9. Hierarchical Cluster Analysis (HCA) of tomato root, shoot and fruit exudate samples, generated from ranked similarities of metabolites data obtained by GC-TOF-MS. Similarity was determined using Bray-Curtis similarity coefficient.

Sugars and Sugar Alcohols



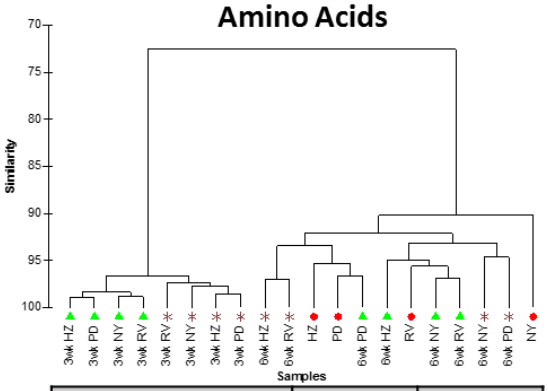
ANOSIM	R value	*p* value
Fruit vs. 6 week	0.717	0.002
Fruit vs. 3 week	1	0.002
6 week vs. 3 week	0.968	0.001

Phenolics



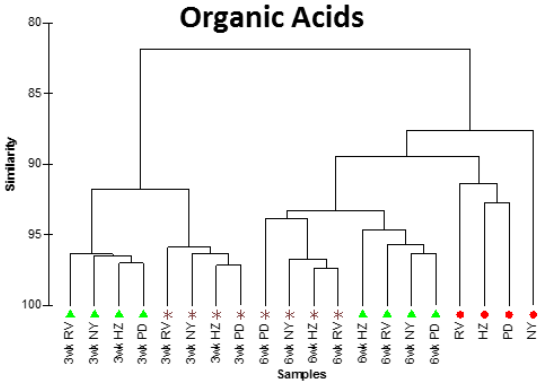
ANOSIM	R value	*p* value
Fruit vs. 6 week	-0.028	0.513
Fruit vs. 3 week	0.950	0.002
6 week vs. 3 week	0.945	0.001

Amino Acids



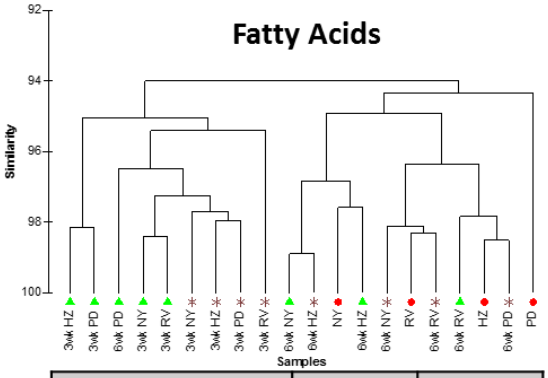
ANOSIM	R value	*p* value
Fruit vs. 6 week	0.294	0.053
Fruit vs. 3 week	1	0.002
6 week vs. 3 week	1	0.001

Organic Acids



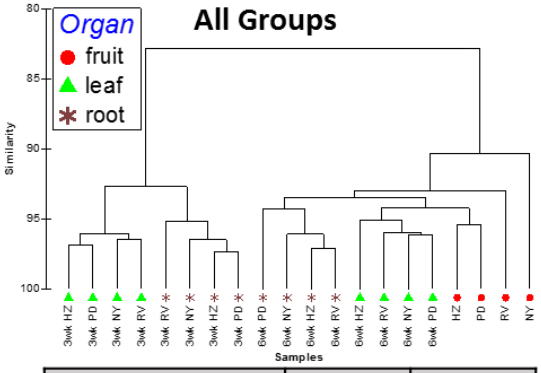
ANOSIM	R value	*p* value
Fruit vs. 6 week	0.912	0.002
Fruit vs. 3 week	1	0.002
6 week vs. 3 week	1	0.001

Fatty Acids



ANOSIM	R value	*p* value
Fruit vs. 6 week	0.055	0.315
Fruit vs. 3 week	0.636	0.002
6 week vs. 3 week	0.617	0.001

All Groups



ANOSIM	R value	*p* value
Fruit vs. 6 week	0.539	0.002
Fruit vs. 3 week	1	0.002
6 week vs. 3 week	1	0.002

Fig. 10. Hierarchical Cluster Analysis (HCA) of tomato root, shoot and fruit exudate samples by classification of identified metabolites, generated from ranked similarities of metabolites data obtained by GC-TOF-MS. Similarity was determined using Bray-Curtis similarity coefficient; Analysis of Similarity (ANOSIM) results are attached to each HCA panel indicating similarity coefficient R for pair-wise comparisons between different plant developmental stages and *p*-value.

3.5. Potential effects of chemical composition of the exudates on the growth of *S*. Typhimurium

Of the 145 identified exudate compounds, 110 were categorized into six metabolite groups: 26 amino acids, 10 fatty acids, 35 organic acids, 5 phenolics, 21 sugars, and 13 sugar alcohols. For each group of compounds, cumulative peak heights were calculated and plotted against the *S.* Typhimurium growth data at 24 hour measurements provided in Fig. 1, 2, and 3 above. These cumulative peak heights somewhat showed a pattern that could explain the bacterial growth pattern among cultivars. For fruit, fatty acids and phenolics were relatively more abundant in exudates from cv. 'Heinz-1706' and cv. 'Plum Dandy VF' compared to the other cultivars while the maximum population levels of *S.* Typhimurium in those exudate solutions were lower (Fig. 11). In contrast, organic acids, and sugars and sugar alcohols were abundant in the fruit exudates from cv. 'Nyagous' and cv. 'Rutgers VFA', and the maximum population levels of the pathogen in those exudate solutions were higher (Fig. 11).

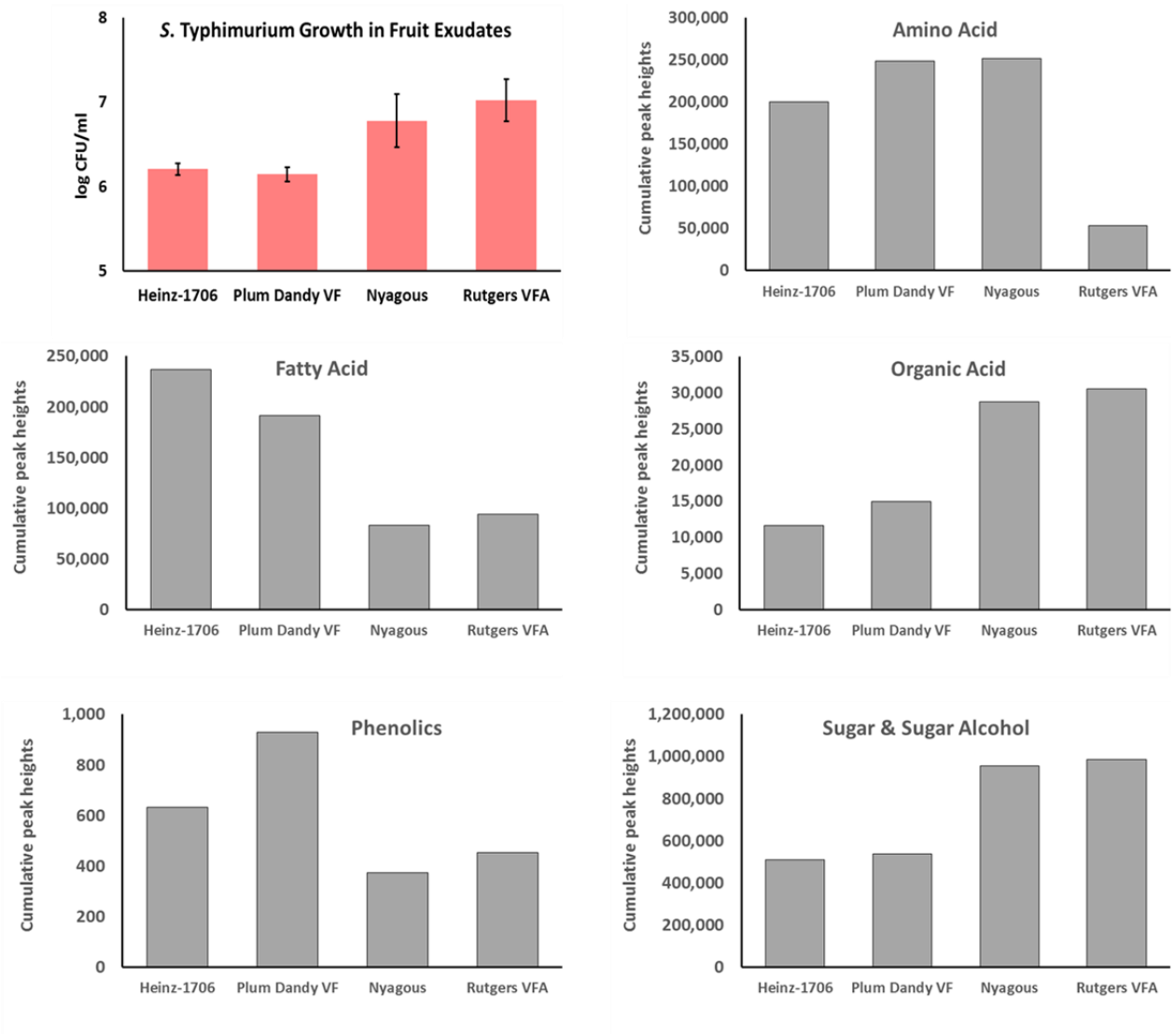


Fig. 11. Total amounts of phytochemicals, grouped into five metabolite categories, found in fruit exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA'. The bar chart in the upper left panel displays growth of *S.* Typhimurium in tomato fruit exudates 24 hours post inoculation. Bar charts in grey display amounts of metabolites found in fruit exudates of each cultivar.

This pattern of higher epiphytic bacterial growth correlating with higher organic acids and sugar compounds, and reduced growth correlating with higher fatty acid and

phenolic concentration, was only weakly observed with 6-week-old plants (Fig. 12 and 13), and not detected in 3-week-old seedlings (Fig. 14and 15). A stoichiometric relationship among metabolites could explain the growth differences observed at a given developmental stage.

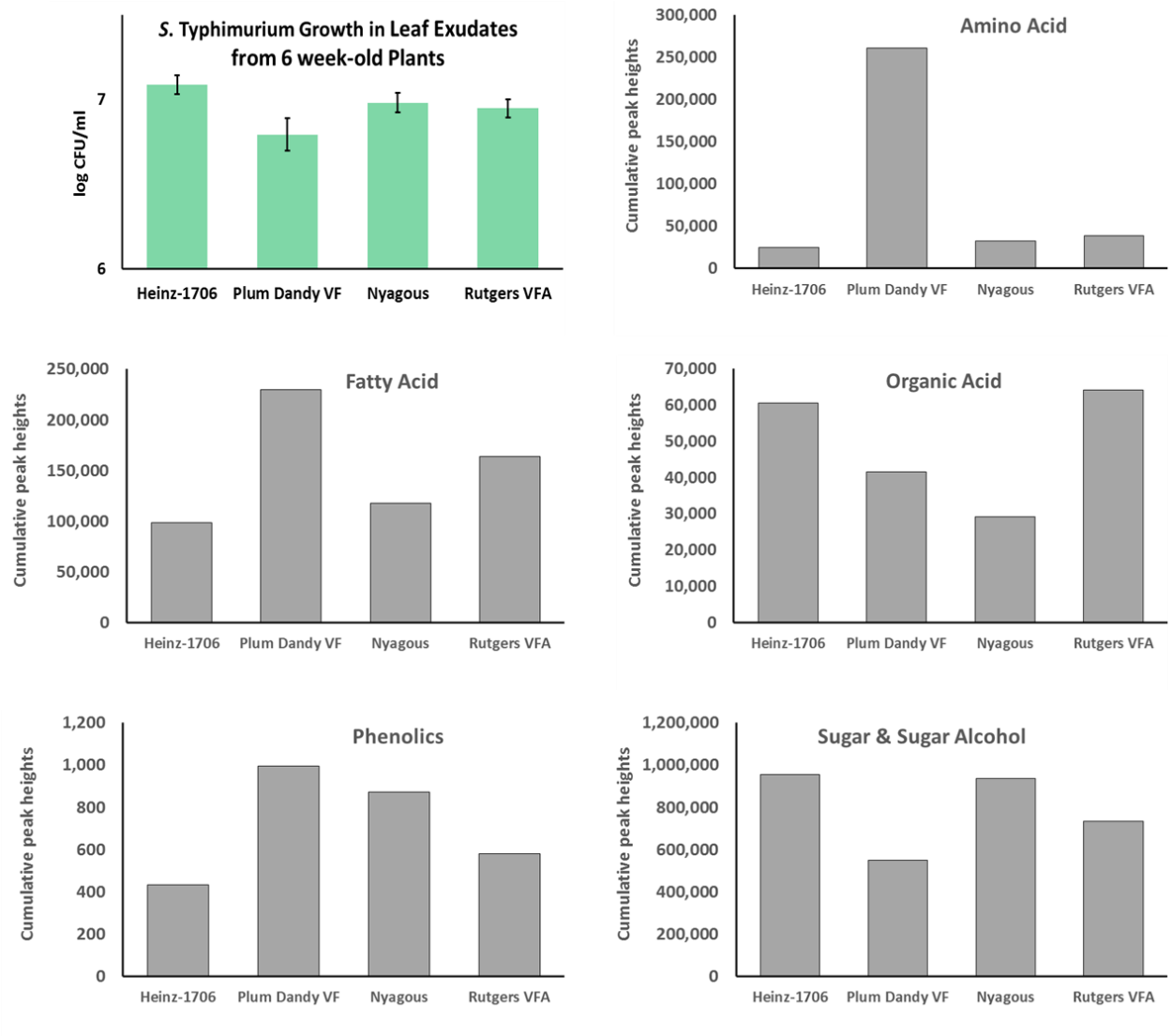


Fig. 12. Total amounts of phytochemicals, grouped into five metabolite categories, found in 6-week old plant shoot exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA'. The bar chart in the upper left panel displays growth of *S.* Typhimurium in 6-week old plant shoot exudates 24 hours post inoculation. Bar charts in grey display amounts of metabolites found in 6-week old plant shoot exudates of each cultivar.

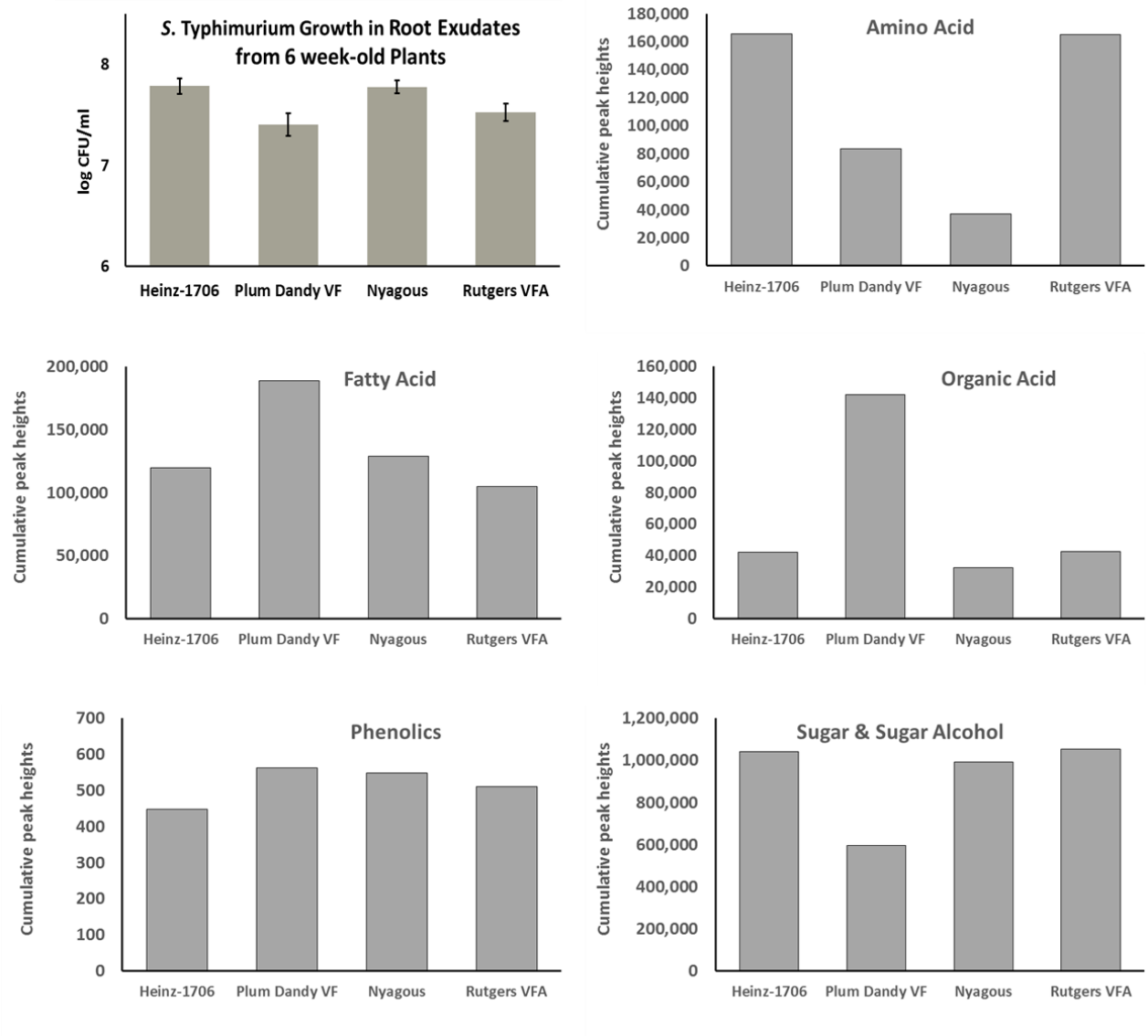


Fig. 13. Total amounts of phytochemicals, grouped into five metabolite categories, found in 6-week old plant root exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA'. The bar chart in the upper left panel displays growth of *S.* Typhimurium in 6-week old plant root exudates 24 hours post inoculation. Bar charts in grey display amounts of metabolites found in 6-week old plant root exudates of each cultivar.

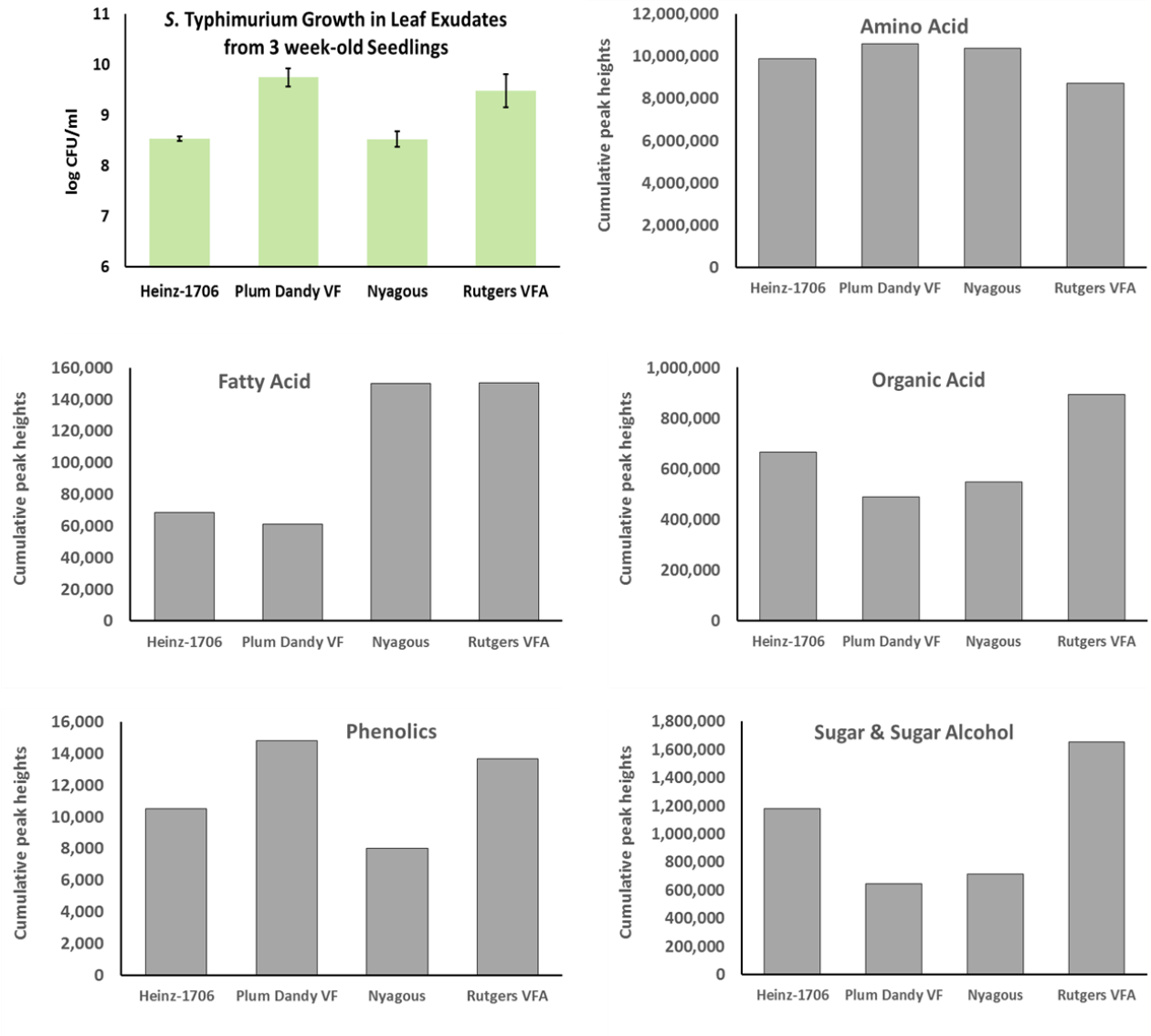


Fig. 14. Total amounts of phytochemicals, grouped into five metabolite categories, found in 3-week old seedling shoot exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA'. The bar chart in the upper left panel displays growth of *S.* Typhimurium in 3-week old seedling shoot exudates 24 hours post inoculation. Bar charts in grey display amounts of metabolites found in 3-week old seedling shoot exudates of each cultivar.

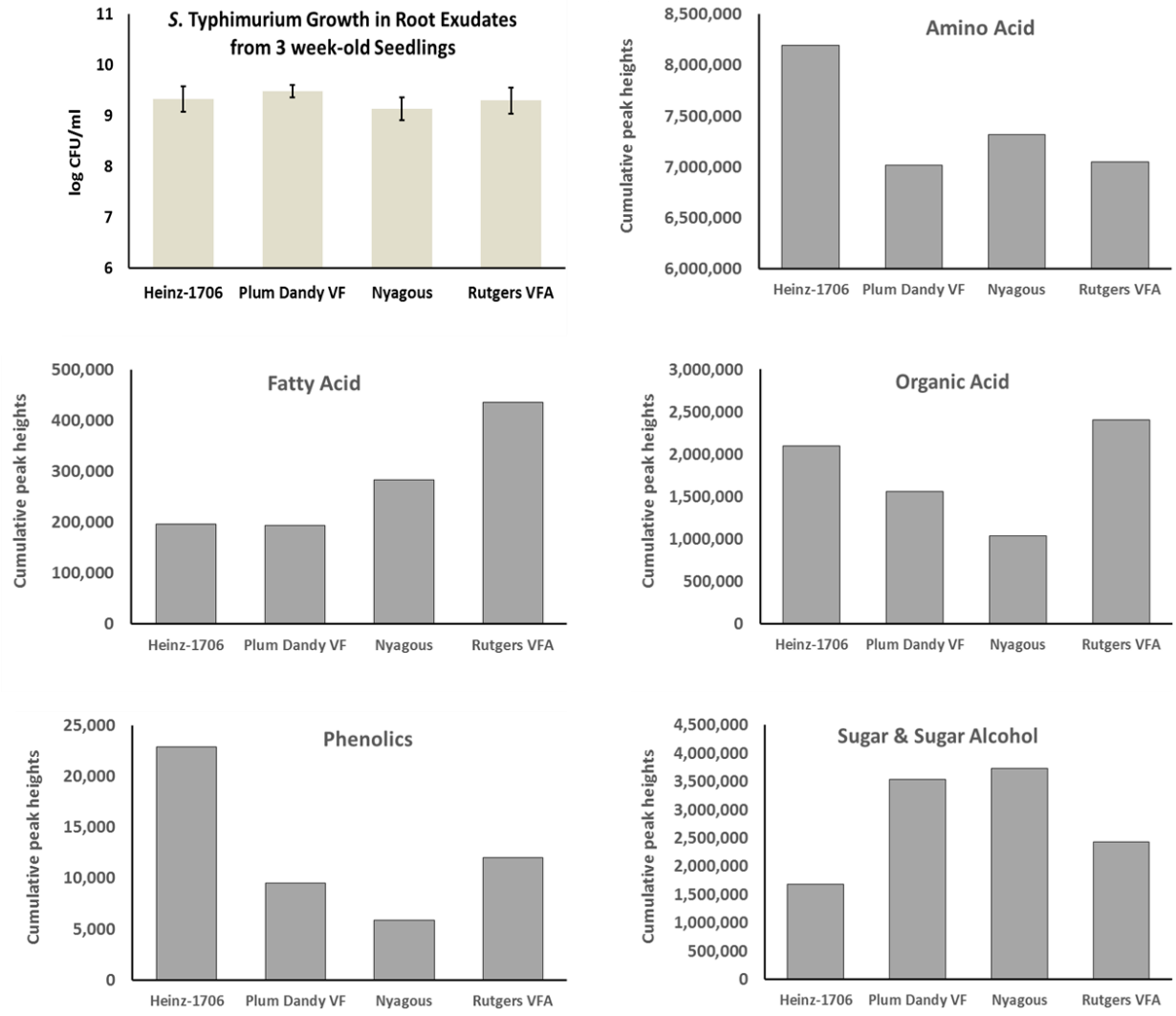


Fig. 15. Total amounts of phytochemicals, grouped into five metabolite categories, found in 3-week old seedling root exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA'. The bar chart in the upper left panel displays growth of *S.* Typhimurium in 3-week old seedling root exudates 24 hours post inoculation. Bar charts in grey display amounts of metabolites found in 3-week old seedling root exudates of each cultivar.

3.6. Differences between green and red fruit is potentially due to secondary metabolites in the exudates of green tomatoes

The GC-TOF-MS analysis revealed that fruit exudates collected from green tomatoes were lower in sugar and sugar alcohol (Fig. 16a) but higher in fatty acids and phenolics (Fig. 16b and 16c), compared to red. There was no difference in pH between ripe and green tomato exudates which were 5.5-5.6 (Appendix 1 Fig. 2), which could therefore not explain differences in growth between ripe and immature tomatoes. Growth trends between green and red fruit in relation to exudates are in agreement with *S*. Typhimurium growth data in fruit exudates of various tomato cultivars, along with the metabolites data. One exception was organic acids, depicted in Fig. 11. This suggests that certain secondary metabolites such as fatty acids and phenolics could have an inhibitory effect on *Salmonella* growth.

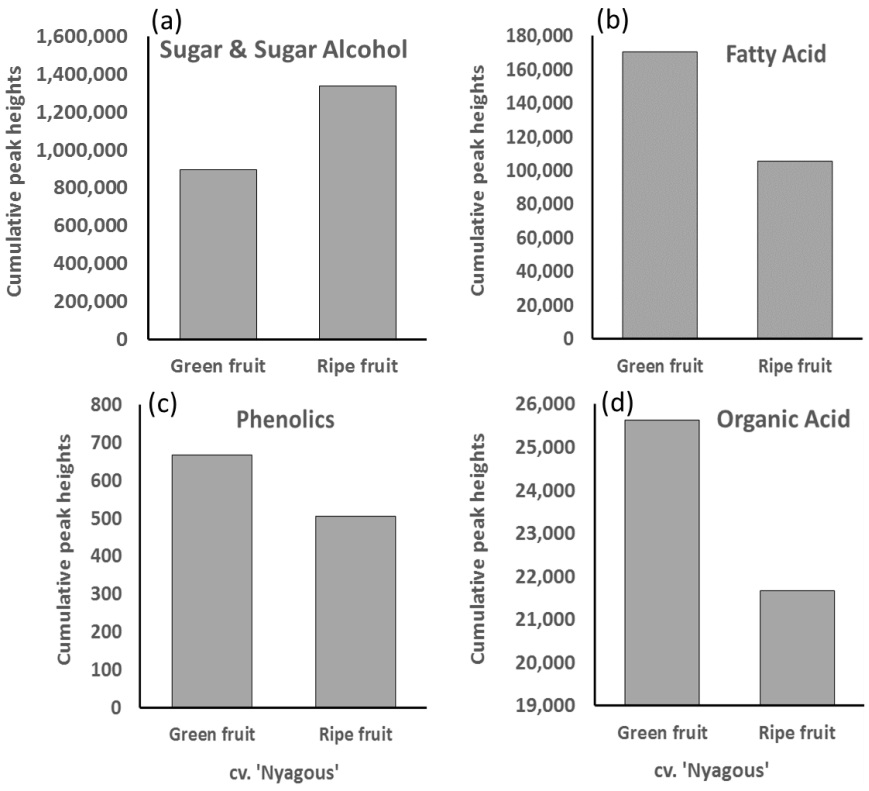


Fig. 16. Total amounts of phytochemicals, grouped into four metabolite categories, found in fruit exudates at different ripeness of cv. ‘Nyagous’.

3.7. Compositional transition in exudates over developmental stages

Assessing the proportion of metabolite groups within given exudate profiles revealed a quantitative transition in leaf metabolites as the plant developed, and by plant organ (Fig. 17). Amino acids were in proportion more predominant in the exudates of 3-week old seedlings compared to 6-week old shoot and root exudates and fruit. On the other hand, sugars and sugar alcohols comprised at least 50% of 6-week old plant and fruit exudates, compared to 5-30% in 3-week old plant exudates. Relative quantity of fatty acids, a group of plant secondary metabolites, increased with plant age (av. 14.6 % in fruit, av. 14.2% in 6 week shoots, and av. 11.5 % in 6 week roots versus av. 0.9

% in 3 week shoots, and av. 2.2 % in 3 week roots). The other important group of plant secondary metabolites, phenolics, comprised 0.06%, 0.07%, 0.04%, 0.10%, and 0.10% of the total identified metabolites in the exudates from tomato fruit, 6-week shoots, 6-week roots, 3-week shoots, and 3-week roots, respectively.

The proportional amounts of metabolite groups in ripe and green tomato fruit exudates are presented in Fig. 17. Sugars and sugar alcohols made up a higher proportion in red fruit, compared to while fatty acids were proportionally higher in green fruit.

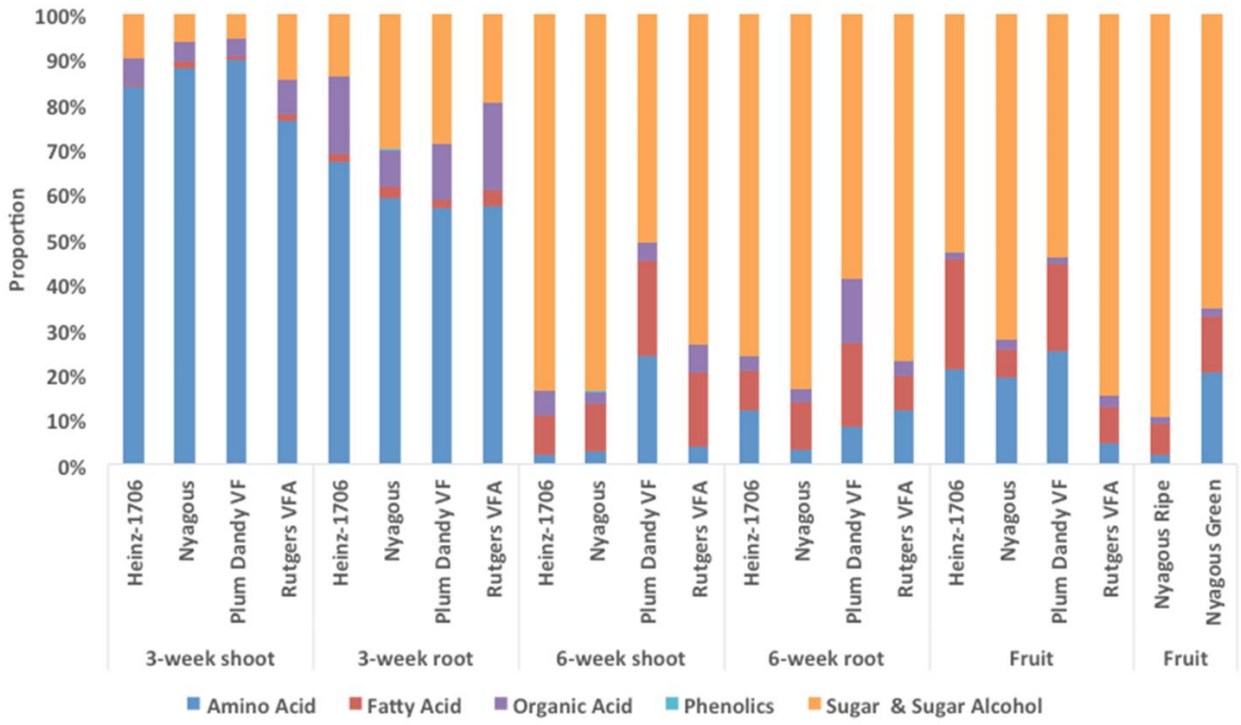


Fig. 17. Proportions of primary and secondary metabolites in exudates of four different tomato cultivars, 'Heinz-1706', 'Plum Dandy VF', 'Nyagous', and Rutgers VFA' at different developmental stages and by organ. Metabolites found in each sample were grouped into five metabolite categories. Relative abundance of each category is displayed in relation to the total amounts of metabolites found per sample.

4. Discussion

This is the first study to undergo an exhaustive investigation of plant exudates as a potential factor determining the fate of human pathogens on plants. Quantitative and qualitative analysis of tomato exudates presented in this study provide a possible explanation for the differential susceptibility to pathogen colonization among tomato cultivars, previously documented in the literature and in our own work (Barak et al., 2011; Han and Micallef, 2014). These findings strongly support the hypothesis that

plant-regulated mechanisms influence enteric pathogen colonization. Here I present evidence that tomato exudates alone are capable of supporting *Salmonella* growth, and that the growth kinetics of *S.* Typhimurium in tomato exudates differ by cultivar ("plant genotype effects"). This cultivar-dependent pattern of *Salmonella* growth responds to exudate changes due to plant developmental stage and plant organ. In addition, the differential epiphytic colonization of tomato fruit by *Salmonella* which is reported in Han and Micallef (2014) could be in part explained by the differential growth kinetics of *Salmonella* in fruit exudates. Characterization of the chemical composition of primary and secondary metabolites in tomato exudates point to potential causes for the differential growth of *S.* Typhimurium observed in the exudates of various tomato cultivars.

Plants secrete an enormous range of potentially valuable compounds (Bais et al., 2006). Sugars and sugar alcohols serve as readily metabolizable sources of energy for microbial growth. Simple sugars such as glucose, fructose, and sucrose are the dominant carbon sources on the plants that have been examined and are thought to simply leach from the interior of the plant (Mercier and Lindow, 2000). Tomato leaves were found to have on average 1.55 µg/g of sugar compounds available (Mercier and Lindow, 2000). Lindow and Brandl (2003) discuss that "oases" on a leaf, where abundant carbon-containing nutrients are available due to localized leakage such as from glandular trichomes or sites of injury, are mostly where large bacterial aggregates form. Secreted organic acids act as metal chelators in the rhizosphere and are thought to increase phosphorous availability for plants by forming complexes with phosphates (Dakora and Phillips, 2002). Several plant

species are known to increase organic acid secretion into the soil substantially in response to phosphorous deficiencies (Lipton et al., 1987; Hoffland et al., 1992; Johnson et al., 1994). The roles of organic acids in the rhizosphere were well reviewed by Jones that they are implicated in mobilization and uptake of nutrients by plants as well as microorganisms (Jones, 1998). Shi et al. (2011) demonstrated that organic acids in the rhizosphere increase the richness of soil bacterial communities, indicating a significant role of organic acids in root exudates in shaping soil bacterial communities. Organic acids are among major chemo-attractants for some biocontrol agents. For instance, *Pseudomonas fluorescens* WCS365, known to present biocontrol properties and be the best competitive root-tip colonizer, showed flagella-driven chemotaxis towards organic acids such as malic acid and citric acid contained in tomato root exudates (de Weert et al., 2002). Recently, natural organic acids such as galacturonic, glucuronic, citric, and alginic acid have been found to diminish the toxic effects of metal ions on soil bacterial cells and thus significantly increase microbial cell growth rate (Dogan et al., 2014).

This study strengthens an insight that phytochemical components play a critical role in determining the fate of the enteric pathogen not only nourishing with sugary compounds but also secreting a potential antibacterial compounds during the interaction. The chemical composition of exudates is regulated genetically by plants so that this trait could be one important criterion for cultivar selection for enhanced microbiological safety of fresh produce or for breeding program.

Plant secondary metabolites including fatty acids and phenolics are known to play important roles in disease resistance (Nicholson and Hammerschmidt, 1992; Bennett

and Wallsgrove, 2006; Li et al., 2009). Their release is known to increase at later developmental stages in the plant life cycle (De-la-Pena et al., 2010). Chaparro et al. (2013) reported that as *Arabidopsis* develops, the quantity of sugars and sugar alcohols decrease in root exudates while those of phenolics and amino acids increase, inferring that this programmed transition is necessary to adopt a more defensive strategy against various plant pathogens and abiotic stresses. In this study, however, among the identified metabolites, amino acids were found to be the most abundant in proportion compared to other groups of metabolites at young seedling stages (Fig. 17). The portion of amino acids were substituted by sugars and sugar alcohols at later stages. The chemical data presented in this study were not measured in absolute quantity (per unit of biomass) so that it is not inferable whether there was a decrease in absolute amount of sugar and sugar alcohol in exudates over the course of plant development, which has been observed to occur according to the previous reports (Aulakh et al., 2001; Chaparro et al., 2013). However, the amount and chemistry of root exudates can vary considerably with plant species, cultivar, age, and stress factors (Bertin et al., 2003).

For other groups of metabolites, it was observed that as tomato plants develop the proportion of organic acids decreases whereas that of fatty acids increases (Fig. 17), and at later stages the relative quantities of fatty acids seemed to negatively coincide with the levels of *Salmonella* populations in the exudates (Fig. 11, 12, and 13). It is probable to hypothesize that certain fatty acids may suppress bacterial populations when their concentrations exceed a certain threshold in plant exudates.

However, it is apparent that the relationship between exudates' chemical composition and bacterial growth is more complex and goes beyond concentrations of a few chemical components. For example, even though relative amounts of fatty acids, organic acids, and sugars and sugar alcohols were high in shoot exudates of 3-week cv. 'Rutgers VFA' in comparison to those of cv. 'Plum Dandy VF', similar growth kinetics of S. Typhimurium were obtained from the two different exudates (Fig. 14 and Table 3).

In this study, the fruit exudates collected from tomato cultivars 'Heinz-1706' and 'Plum Dandy VF' were found to be less supportive than other tested cultivars of *S.* Typhimurium growth (Fig. 1, Fig. 2, and Table 2). This observation could be attributed to lower levels of sugars, sugar alcohols and organic acids detected in their exudates (Fig. 11), in combination with the secondary metabolite contents in the exudates: fatty acids and phenolics were highest in this group and one or more of these compounds could potentially have a suppressive effect on bacterial growth whereas organic acids and sugars sugar alcohols could have posed an opposing impact on the bacterial growth (Fig. 11). This fruit finding is the most significant from the consumer point of view since only fruit are edible. However, from a food safety perspective, leaf or root exudate analyses are relevant at all plant developmental stages, since pre-harvest contamination can occur throughout the plant life cycle. Exudates on leaves or roots can allow for prolonged persistence of the enteric pathogen on the plant, and could increase the risk of fruit contamination (Gu et al., 2011) Phenolics identified from the tomato plant exudates in this study are chlorogenic acid, ferulic acid, salicylaldehyde, tyrosol, and vanillic acid. Phenolics

occur in plant tissues as simple substituted phenols, glycosides and amides, or complex and polymerized molecules. Several well-characterized plant phenolics have been described as playing putative roles in protection against or response to infection by plant pathogens (Cowan, 1999). There are considerable efforts, in the food and pharmaceutical industries, being invested to identify beneficial phenolic compounds from plant resources for their antibacterial effects. For example, phenolic compounds extracted from berries showed inhibitory effects on intestinal bacteria including *Staphylococcus* and *Salmonella* (Puupponen-Pimia et al., 2005). A wide range of rumen bacteria was negatively influenced by plant phenolic acids (Chesson et al., 1982). All the fatty acids identified from the tomato plant exudates in this study are long-chain fatty acids. Along with other natural antimicrobial compounds such as organic acids and phenolics, non-volatile long fatty acids were investigated several decades ago. The inhibitory effects of low concentration of long-chain fatty acids on the growth of certain rumen bacteria were recorded although considerable variation in growth responses was noted among the tested strains (Maczulak et al., 1981). The antibacterial activities of oleic acid and stearic acid were enhanced under anaerobic conditions (Angelidaki and Ahring, 1992). Thus, the phenolic compounds and long chain fatty acids identified in the tomato plant exudates could have exerted suppression on *Salmonella* growth.

In this study, a potential negative correlation between the quantity of secondary metabolites and the growth of *S.* Typhimurium was only seen with the exudates collected from fruits or plants at later flowering stage, not from young seedlings. This phenomenon observed in this study could be due to secondary metabolites being

predominant on plants at later stages, while the sugary compounds are usually exuded in greatest abundance at seedling stages to recruit beneficial microorganisms, so that the impact of the secondary metabolites is diluted or masked. However, within this study whether there was an increase in absolute amount of secondary metabolites exudation at older plants and fruits compared to young seedlings are not known mainly because the chemical analysis was not done with unit of biomass of plant given time. Thus, a comparison between different developmental stages or different plant organs is not doable. Different sampling methods were used to sample exudates from plants at different stages and different plant organs. There could be another caveat during sampling that might have affected the results. Water or a water-based buffer solution was used to remove leachate from the surface of fruit, shoots and roots. It is possible that the water or buffer did not extract non-water-soluble metabolites. Nonetheless, Fig. 9 provides evidence that the exudates from young seedlings are very distinct from those from older plants and fruits in quantitative composition. Therefore, the impacts of young seedlings exudates on the bacterial growth are not the same as fruit or older plant exudates.

Only a few studies have been conducted to evaluate plant genotype effects on the colonization of tomato plants with *Salmonella* (Barak et al., 2008; Barak et al., 2011; Gu et al., 2013). Density of trichomes on leaves was correlated with the population levels of *Salmonella* as nutrients leaked from the trichomes (Barak et al., 2011). To my knowledge, this study is the first attempt to test exudates from different cultivars and different plant organs as a plant genotype factor having crucial impacts on the fate of *Salmonella*. I present here that exudates can be different quantitatively and

qualitatively depending on cultivar type and plant organ so that the impacts on *Salmonella* growth vary. In other words, to conclude, these findings reveal that tomato plant genetics can be a determinant of the fate and persistence of *Salmonella*, suggesting that fatty acids and phenolics in the exudates are potential inhibitors of bacterial growth. More research is required to discover what metabolites are specifically suppressing the bacterial population. Ultimately, this study and future progress can be contributed to a breeding program that is to find a cultivar containing more of those metabolites.

Chapter 5: Genome-wide Transcriptional Profiling of *Salmonella enterica* serovar Typhimurium Epiphytically Attaching and Colonizing Tomato

1. Introduction

Salmonella enterica subsp. *enterica* being implicated in numerous foodborne illness outbreaks associated with the consumption of tomatoes (CDC, 2007b; Gupta et al., 2007; Greene et al., 2008). It is possible that these enteric pathogens have evolved a strategy to survive the transition from one host to another by successfully colonizing and even propagating in plant-associated habitats, thus exploiting plants as vectors to re-enter herbivorous animal hosts.

The phyllosphere is regarded as a harsh habitat for human enteric pathogens such as *Salmonella enterica*, having to contend with restricted nutrient availability, as well as abiotic stresses such as desiccation, temperature fluctuation, and UV irradiation. Moreover, plant innate immunity could be another factor suppressing enteric pathogen populations on plants, through the hypersensitive response or the production of antimicrobials (Melotto et al., 2006). Our previous work demonstrated that populations levels of *S.* Typhimurium and *S.* Newport colonizing tomato shoots and fruit rapidly increased in a cultivar and plant organ dependent manner (Han and Micallef, 2014). This suggests that *Salmonella* was responding to cultivar differences in tomato phytochemicals and or plant innate immune responses. However, our understanding of the genetic responses of *Salmonella* during this interaction with tomato is limited.

Previous studies have begun to describe bacterial genes involved in attachment and colonization of *Salmonella* on food plants. *S*. Enteritidis mutants defective in aggregative fimbria/curli nucleator (*agfB*), stationary phase sigma factor (*rpoD*), bacterial cellulose synthesis (*bcsA*), and *O*-antigen capsule assembly and translocation (*yihO*) exhibit reduced attachment and colonization of alfalfa sprouts (Barak et al., 2005; Barak et al., 2007). It was reported that *S.* Typhimurium genes required for metabolism of unsaturated fatty acids (*fadH*), cysteine acquisition (*cysB*), and another gene predicted to encode a DNA-binding prophase protein (STM2006) were differentially regulated in tomato fruit tissues (Noel et al, 2010). Salazar et al. (2013) demonstrated that in-frame deletions of *ycfR* that is a putative stress regulatory gene, *sirA* involved in biofilm formation, and *yigG* of unknown function in *S.* Typhimurium and *S.* Saintpaul reduced bacterial attachment to spinach and grape tomatoes as well as glass and polystyrene.

In spite of these advances, the full scope of adaptations by *Salmonella* while colonizing plants is not well understood. We can hypothesize that *Salmonella*'s ability to survive a range of environmental stress conditions found in agricultural settings, outside animal hosts, could be governed by highly responsive transcriptional regulators which respond to specific stimuli and switch on a cascade of signaling events to overcome the given stress. With the availability of high throughput molecular tools such as RNA-seq, a genome-wide view of the transcriptomic landscape of *Salmonella* colonization of tomato can be obtained. To better understand how *Salmonella* is capable of thriving on plants, the objective of this study was to identify genes that are differentially expressed when *Salmonella* attaches

and colonizes tomato, compared to growth in a nutrient-rich medium. We assessed the transcriptional profile of *Salmonella* on tomato using a genome-wide RNA-seq approach. An understanding of the molecular mechanisms responsible for the establishment of pathogenic bacteria on plants is critical for devising targeted measures to improve the safety of our food supply.

2. Materials and Methods

2.1. Bacterial strain, tomato cultivar, growth medium and conditions

Salmonella enterica Typhimurium LT2 (ATCC700720), maintained at -80°C in Brucella broth (BD, Sparks, MD, U.S.A.) containing 15% glycerol, was plated on trypticase soy agar (TSA) (BD, Sparks, MD, U.S.A.) and incubated for 18 h at 35°C, prior to experiments. The fresh culture of *S.* Typhimurium was suspended in sterile phosphate buffered saline (PBS) at an OD_{600} of 0.5, which yields ~10^9 CFU/ml. Further dilutions were made in sterile PBS to enumerate actual cell concentrations of the suspension on TSA.

Tomato seeds (*Solanum lycopersicum* cv. Heinz-1706, Tomato Genetics Resource Center (TGRC), U.C. Davis, Davis, CA, U.S.A.) were sterilized by soaking in half-strength household bleach for 30 min, followed by 6-7 rinses in sterile water, as recommended by the TGRC. Seeds germinated in the dark were grown gnotobiotically in an upright position in 245 mm × 245 mm square culture dishes (Corning, Acton, MA, U.S.A.) containing Murashige and Skoog (MS) medium (MP Biomedicals LLC, Solon, OH, U.S.A.) 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.

At 5 weeks post-germination, 10 locations on leaves per plant were spotted with 10 µl of ~10^9 CFU/ml *S.* Typhimurium. The same spot inoculation procedures were followed for roots on separate plants. Square culture dishes holding the inoculated plants were re-sealed with micropore tape (3M, St. Paul, MN, U.S.A.) to maintain high relative humidity and aeration inside the dishes as well as to prevent airborne contamination. The culture dishes were re-incubated for 3 days at 28°C under a 16L:8D photoperiod until *Salmonella* cell retrieval.

Three days after *Salmonella* inoculation, the plants were removed carefully from the culture dishes and cut in half to separate leaves and roots. Then the inoculated part, shoots or roots, was immediately put in a Whirl-Pak bag containing a mix of 30 ml of RNAprotect cell reagent (Qiagen, Valencia, CA, U.S.A.) and 15 ml of sterile PBS to stabilize microbial RNA. The bags were sonicated in Branson Ultrasonic Cleaner (Branson Ultirasonics Corperation, Danbury, CT, U.S.A.) for 2 min and hand-rubbed for another 1 min to dislodge attached *Salmonella* cells from the plant. The RNAprotect cell reagent-PBS washates were collected in sterile 50 ml conical tubes and the tubes were centrifuged for 1 h at 10,000 rpm at 4°C. Cell pellets were kept at -80°C until total RNA isolation. For a control growth condition, the same *Salmonella* strain grown for 18 hours on Luria-Bertani agar (LB) (BD, Sparks, MD, U.S.A.) plates at 28°C was directly resuspended in 1 ml of RNAprotect cell reagent and pelletized following the manufacturer's instructions. Each treatment was carried out in replicates of 3.

The bacterial cell retrieval procedures described above were repeated using PBS in place of RNAprotect cell reagent to ensure that the cells recovered and thus used in

later RNA isolation were no other organisms but *Salmonella*. Briefly, the inoculated plants removed from the culture dishes, cut in half, and put in 45 ml of sterile PBS. After sonication and hand-rubbing, the washates were centrifuged to collect cell pellets, then resuspended in PBS to prepare serial dilutions. These were plated on TSA and xylose lysine tergitol-4 (XLT4) (BD, Sparks, MD, U.S.A.) plates for CFU enumeration. XLT4 is a highly selective medium for *Salmonella* so any discrepancy in CFU between TSA and XLT4 plates served as an indication of contamination.

2.2. Total RNA isolation and rRNA removal

Total RNA from the pre-stabilized pellets was extracted using RNeasy mini kit (Qiagen, Valencia, CA, U.S.A.) following the manufacturer's instructions and then quantitated on NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, U.S.A.) as well as Bioanalyzer (Agilent, Santa Clara, CA, U.S.A.) at the sequencing facility of the Institute of Bioscience and Biotechnology Research (IBBR), University of Maryland (UMD). The total RNA was depleted of prokaryotic ribosomal RNA using Ribo-Zero rRNA removal kit (Epicentre, Madison, WI, U.S.A.) following the manufacturer's instructions.

2.3. cDNA synthesis and RNA-seq libraries

All procedures hereafter followed the protocols of ScriptSeq v2 RNA-seq Library Preparation kit (Epicentre, Madison, WI, U.S.A.). Briefly, the rRNA-depleted RNA was precipitated in a mix solution of 3 M NaOAC, glycogen, and ice-cold 100% EtOH. mRNA pellet was washed with ice-cold 70% EtOH and resuspended in RNase-free water. Then this purified rRNA-depleted mRNA was fragmented in a

reaction with RNA fragmentation solution heated at 85°C for 5 min. The first strand cDNA were synthesized from the cleaved mRNA fragments using Epicentre's StarScript reverse transcriptase and random hexamers with a tagging sequence. The reaction was incubated at 25°C for 5 min, 42°C for 20 min, and paused at 37°C to add finishing solution. The finishing solution was inactivated at 95°C for 3 min, followed by cooling at 25°C. The resultant cDNA fragments were ligated with 3'-terminal-tags (adaptor). The adaptor-ligated cDNA were purified using Agencourt AMPure XP System (Beckman Coulter, Brea, CA, U.S.A.), 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. The PCR underwent denaturation for 1 min at 95°C, 15 cycles of 30 s at 95°C, 30 s at 55°C, and 3 min at 68°C, and incubation for 7 min at 68°C after the final cycle. Each PCR product was purified using Agencourt AMPure XP System. The prepared RNA-seq libraries were checked for quality and quantity on a Bioanalyser, and sequenced on an Illumina Hi-Seq 1000 to obtain 100 bp paired-end reads, at the sequencing facility of the IBBR, UMD.

2.4. Mapping and statistical analysis

Data cleanup and analysis was carried out through the UNIX command-line interface using a high performance computing cluster at the UMD. 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). Briefly, the

reference genome downloaded from the NCBI FTP site (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/*Salmonella_enterica*_serovar_Typhimurium_LT2_uid57799/) for *S.* Typhimurium was indexed to be used for read alignments and mapping, which was done using Bowtie2 (Langmead et al., 2009). Sequence reads for each treatment condition was mapped to the reference genome with Tophat2. The resulting alignment files were provided to Cufflinks to generate a transcriptome assembly for each treatment condition. The assemblies from all conditions were then merged together using the Cuffmerge utility which is included in the Cufflinks package. 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). The original read counts were normalized in order to ensure that samples were comparable by removing systematic differences between samples that were likely due to differences in sample preparation rather than the result of the underlying biology. Significance of differentially transcribed genes was corrected for multiple testing errors by taking into account the Benjamini-Hochberg false discovery rate approach (Benjamini and Hochberg, 1995), which is represented with *q*-values; *q*-values lower than 0.1 were considered as significant in this study.

Functions of the differentially regulated genes found in this study were classified according to the database of the Clusters of Orthologous Groups of proteins (COGs) available at http://eggnog.embl.de/version_4.0.beta/. For each differentially regulated gene, genetic pathways showing molecular interaction and reaction networks were

searched using the KEGG PATHWAY database available at http://www.genome.jp/kegg/pathway.html.

2.5. Quantitative reverse transcription-PCR verification

A number of genes that were significantly differentially transcribed for both leaf and root conditions, compared to LB culture, was selected for qRT-PCR to validate RNA-seq data. 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). The specificity of the primer pairs were tested on DNA samples collected from fresh *S.* Typhimurium culture and tomato plant tissues beforehand qRT-PCR experiment.

Table 1. List of genes and primers for qRT-PCR verification

Gene	NCBI tag	Gene Description	Primer	Sequence (5' → 3')	Primer Design
nmpC	STM1572	outer membrane porin protein OmpD	Forward	GTCCGTCCATCGCTTACCTG	This study
			Reverse	GCTTTGGTGAAGTCGCTGTC	
lamB	STM4231	maltoporin	Forward	GTATTGGCTGGACGGGAAGC	This study
			Reverse	TCGCCCTCTTTCCACACTTC	
malE	STM4229	maltose ABC transporter substrate-binding protein MalE	Forward	ATCGCCGACTTCCCTTTCAC	This study
			Reverse	ACAAAGACCTCGTCCCGAAC	
ydaA	STM1661	universal stress protein E	Forward	GACCACCCTGCTTTCTCCTG	This study
			Reverse	GGACGATTGTGCCAGACCAC	
aphA	STM4249	class B acid phosphatase	Forward	AACGGCTGGGATGAGTTCAG	This study
			Reverse	CGTCTGACTACGACCAGTGAC	
ygbA	STM2860	cytoplasmic protein	Forward	GTGGGCACTGGCTTTCATAC	This study
			Reverse	GCCTGGTAAACGTATCGCTC	
wza	STM2118	polysaccharide export protein	Forward	TACCGACGACGCTAACCTTG	This study
			Reverse	CGATGTGCTGAATGTCACCG	
yoaG	STM1272	cytoplasmic protein	Forward	ATAGCAACGGCGTCTCTGTG	This study
			Reverse	GGTATCGTAGGAACGCACGG	
rpoD	STM3211.S	RNA polymerase sigma factor RpoD	Forward	TGAAATGGGCACTGTTGAACTG	This study
			Reverse	CCAGCAGATAGGTAATGGCTTC	

Total RNA from *S.* Typhimurium LT2 colonizing tomato shoots or roots was extracted in replicates of four, under the same growth conditions as the RNA-seq procedures described above. Verso cDNA synthesis kit (Thermo Scientific, Wilmington, DE, U.S.A.) with random hexamers was used for reverse transcription

according to the manufacturer's instructions. All qPCR reactions were done with PerfeCTa® SYBR Green SuperMix (Quanta Biosciences, MD, U.S.A.) following the manufacturer's instructions. Amplification of gene transcripts of interest was performed on Bio-Rad qPCR/Real-Time PCR System (Bio-Rad, U.S.A.). Briefly, each of real-time PCR reactions was consist 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 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C). Relative gene expression was calculated for each gene of interest relative to a calibrator (*rpoD*, internal control) gene following the comparative C(T) method by Schmittgen and Livak (2008), referred as the ΔΔCt method.

For data normalization, housekeeping gene *rpoD* (Botteldoorn et al., 2006) was used as an internal control. *rpoD* was observed as not being differentially transcribed on both leaves and roots of tomato, compared to LB culture, based on the RNA-seq data presented in this study. All qRT-PCR experiments were done in four independent replicates and additionally three technical replications within each qPCR run. Gene expression intensity in Ct was calculated using the software incorporated within the qPCR machine. For each gene, ΔΔCt values calculated following the manufacturer's data reporting manual were used to determine a differential gene expression between different levels of treatment. Student's t test was performed on ΔΔCt values to compare expression levels of a gene in the treatment condition with those in the control condition.

3. Results

3.1. Recovery of *S.* Typhimurium from tomato

Following inoculation of gnotobiotically-grown tomato plants, levels of *S.* Typhimurium populations were maintained on both shoots and roots 11 days after inoculation (Fig. 1). Cells of *S.* Typhimurium were treated with an RNA stabilizing solution while still attached to plants 3 days post inoculation in order to examine their transcriptional profiles associated with this plant-colonizing state. *Salmonella* cells were then retrieved from the plants and used for bacterial RNA isolation.

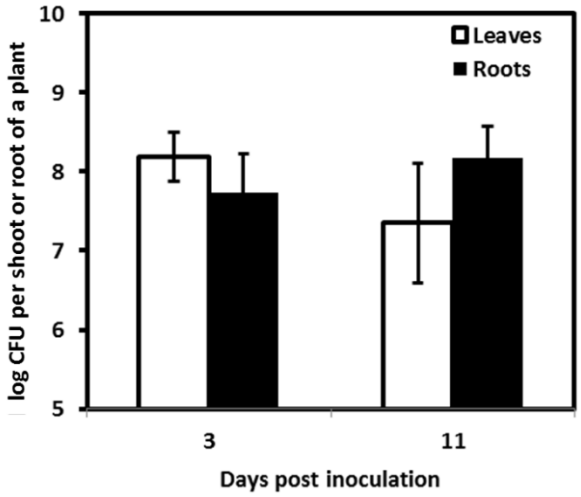


Fig. 1. Log CFU retrieved from each part of a tomato plant 3 and 11 days post inoculation; Initial load was 8.0 log CFU per shoot or roots of a plant; Error bars represent standard deviation; n=6.

3.2. Global gene expression profiling on tomato

Global analysis of the transcriptome of *S.* Typhimurium epiphytically attaching and colonizing tomato resulted in expression signals for 4,454 chromosomal genes and 102 plasmid genes out of the totals of 4,634 and 111 annotated genes, respectively,

indicating >96% coverage of the whole transcriptome of *S.* Typhimurium. Genes with no expression signals mostly belonged to rRNA or tRNA processing which had been depleted during the mRNA isolation step. Of these, 162 and 330 chromosomal genes were differentially expressed on tomato shoots and roots, respectively, relative to growth in LB culture (q<0.1). These represented 3.6% and 7.4% of the expressed genes. Approximately 25% of plasmid genes were found to be differentially expressed on both shoots and roots, relative to LB culture (Table 2).

Table 2. Number of differentially expressed *S.* Typhimurium genes colonizing tomato compared to LB culture [a].

	S. Typhimurium Chromosome		*S.* Typhimurium Plasmid	
Expression type	On shoots	On roots	On shoots	On roots
Up-regulated	51	124	9	4
Down-regulated	111	206	17	20
Total	162	330	26	24

[a] Differentially expressed genes were determined based on q-values (q<0.1) which are adjusted from original p values to correct for multiple testing errors by using the Benjamini-Hochberg false discovery rate (FDR) approach (Benjamini and Hochberg, 1995).

Out of the 51 chromosomal genes up-regulated in *Salmonella* colonizing shoots, 38 genes were also observed as being up-regulated in *Salmonella* colonizing roots, while out of the 111 down-regulated genes on shoots, 99 genes were also down-regulated on roots (Fig. 2, and Tables 2 and 3). On the plasmid, 3 up-regulated genes and 15 down-regulated genes were common to both shoots and roots. This indicates that

there is a core set of *S.* Typhimurium genes needed for adaptation to tomato shoot and root colonization.

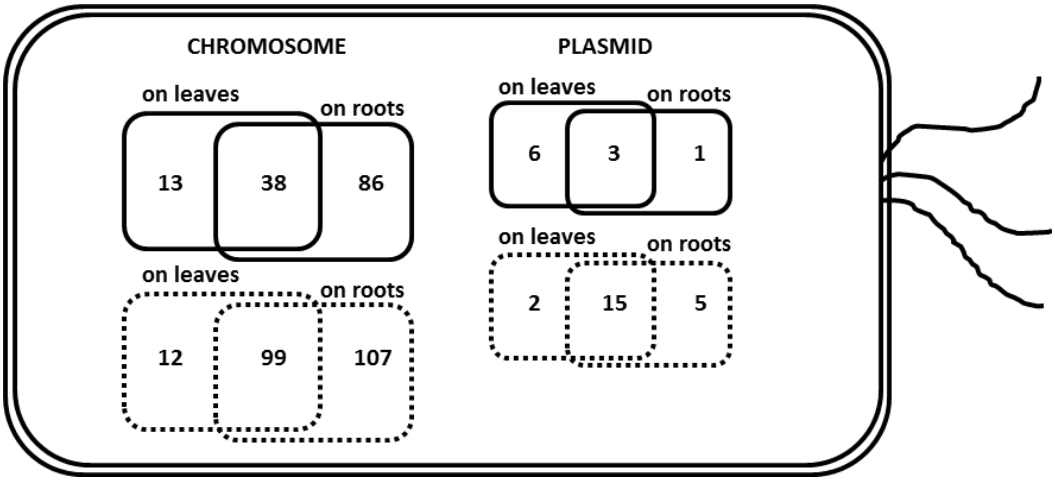


Fig. 2. Number of significantly (q<0.1) differentially expressed genes in *S.* Typhimurium when colonizing shoots or roots of tomato, altered in expression by at least 1.7-fold (q<0.1); Solid lines represent up-regulated genes and dashed lines represent down-regulated genes.

Table 3. Genes in *S.* Typhimurium LT2 up-regulated on tomato (A) shoots and roots, (B) shoots only, and (C) roots only, relative to LB culture.

A

Function and gene	NCBI tag	Annotation	Shoots		Roots	
			log2 (Fold change)	*q* value	log2 (Fold change)	*q* value
CELLULAR PROCESSES AND SIGNALING						
[M] Cell wall/membrane/envelope biogenesis						
wza	STM2118	polysaccharide export protein	3.6	0.023	5.5	0.022
yhdV	STM3392	outer membrane lipoprotein	1.7	0.002	3.7	0.002
[O] Post-translational modification, protein turnover, and chaperones						
hslJ	STM1648	heat-inducible protein HslJ	1.0	0.059	1.1	0.018
STM1251	STM1251	molecular chaperone	1.0	0.076	2.2	0.002
INFORMATION STORAGE AND PROCESSING						
[J] Translation, ribosomal structure and biogenesis						
trpS2	STM4508	tryptophanyl-tRNA synthetase II	1.1	0.022	1.0	0.038
[K] Transcription						
marA	STM1519.S	DNA-binding transcriptional activator MarA	4.8	0.002	2.5	0.002
marR	STM1520	DNA-binding transcriptional repressor MarR	3.6	0.002	1.6	0.002
soxR	STM4266	redox-sensitive transcriptional activator SoxR	3.1	0.002	1.9	0.002
yfhH	STM2572	DNA-binding transcriptional regulator	1.0	0.086	1.2	0.017
yneJ	STM1523	LysR family transcriptional regulator	1.0	0.091	1.2	0.009
[L] Replication, recombination and repair						
deaD	STM3280.S	ATP-dependent RNA helicase DeaD	2.1	0.002	1.0	0.094
METABOLISM						
[C] Energy production and conversion						
hycC	STM2851	hydrogenase 3 membrane subunit	1.3	0.045	1.4	0.023
hycD	STM2850	hydrogenase 3 membrane subunit	1.0	0.080	1.0	0.076
STM1787	STM1787	hydrogenase 1 large subunit	1.1	0.094	1.2	0.033
STM1792	STM1792	cytochrome oxidase subunit I	0.9	0.100	1.2	0.011
yneI	STM1524	succinate semialdehyde dehydrogenase	1.4	0.002	1.6	0.002
[E] Amino acid transport and metabolism						
aroF	STM2670	phospho-2-dehydro-3-deoxyheptonate aldolase	1.0	0.046	1.4	0.002

hisG	STM2071	ATP phosphoribosyltransferase	1.2	0.002	1.0	0.016
mtr	STM3279	HAAAP family tryptophan-specific transport protein	1.9	0.002	1.2	0.008
trpC	STM1725	bifunctional indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase	2.0	0.002	1.0	0.028
trpD	STM1724	bifunctional glutamine amidotransferase/anthranilate phosphoribosyltransferase	3.0	0.002	1.8	0.016
trpE	STM1723	anthranilate synthase component I	3.1	0.002	2.0	0.002
[G] Carbohydrate transport and metabolism						
ydeA	STM1522	sugar efflux transporter	1.1	0.013	0.9	0.036
[P] Inorganic ion transport and metabolism						
cysD	STM2935	sulfate adenylyltransferase subunit 2	3.0	0.002	2.5	0.002
cysN	STM2934	sulfate adenylyltransferase subunit 1	2.7	0.002	2.2	0.002
marB	STM1518	multiple antibiotic resistance protein MarB	3.7	0.002	1.7	0.027
[Q] Secondary metabolites biosynthesis, transport, and catabolism						
hpaB	STM1099	4-hydroxyphenylacetate 3-monooxygenase oxygenase subunit	0.9	0.068	1.2	0.006
FUNCTION UNKNOWN OR POORLY CHARACTERIZED						
iap	STM2936	alkaline phosphatase isozyme conversion aminopeptidase	0.8	0.091	1.3	0.002
yqfA	STM3049	hemolysin	0.9	0.095	1.2	0.006
STM1851	STM1851	cytoplasmic protein	1.3	0.004	1.6	0.002
STM1869	STM1869	phage-tail assembly-like protein	1.4	0.016	1.3	0.020
ygbA	STM2860	cytoplasmic protein	3.5	0.002	3.5	0.002
ygbE	STM2932	inner membrane protein	1.5	0.002	1.7	0.002
yjbE	STM4222.S	outer membrane protein	3.0	0.004	5.9	0.002
yoaG	STM1272	cytoplasmic protein	7.7	0.029	7.1	0.038
STM05615	STM05615	hypothetical protein	2.2	0.002	2.0	0.002
STM1650	STM1650	hypothetical protein	1.4	0.050	1.3	0.051
yhaK	STM3236	cytoplasmic protein	2.1	0.002	1.4	0.002

B

Function and gene	**NCBI tag**	**Annotation**	**log2 (Fold change)**	***q* value**
INFORMATION STORAGE AND PROCESSING				
[J] Translation, ribosomal structure and biogenesis				
rsuA	STM2222	rRNA small subunit pseudouridine synthase A	1.0	0.060

[K] Transcription				
ptsJ	STM2436	transcriptional regulator PtsJ	1.1	0.024
[L] Replication, recombination and repair				
stpA	STM2799	DNA binding protein StpA	0.9	0.083
METABOLISM				
[E] Amino acid transport and metabolism				
metA	STM4182	homoserine O-succinyltransferase	1.4	0.002
trpB	STM1726	tryptophan synthase subunit beta	1.8	0.021
[F] Nucleotide transport and metabolism				
yfeJ	STM2437	glutamine amidotransferase	0.9	0.094
[P] Inorganic ion transport and metabolism				
feoB	STM3506	ferrous iron transport protein B	4.8	0.034
fhuF	STM4550	ferric hydroximate transport ferric iron reductase	1.0	0.036
smvA	STM1574	methyl viologen resistance protein SmvA	1.0	0.029
FUNCTION UNKNOWN OR POORLY CHARACTERIZED				
STM1513	STM1513	cytoplasmic protein	3.3	0.002
STM3362	STM3362	periplasmic protein	10.4	0.034
STM4552	STM4552	inner membrane protein	2.3	0.002
yhcN	STM3361	outer membrane protein	8.4	0.014

C

Function and gene	**NCBI tag**	**Annotation**	**log2 (Fold change)**	***q* value**
CELLULAR PROCESSES AND SIGNALING				
[M] Cell wall/membrane/envelope biogenesis				
dgkA	STM4236	diacylglycerol kinase	1.0	0.034
gmd	STM2109	GDP-D-mannose dehydratase	5.8	0.013
pagP	STM0628	lipid A palmitoyltransferase PagP	0.9	0.052
STM0908	STM0908	hypothetical protein	2.0	0.024
STM1540	STM1540	hydrolase	2.7	0.002
wcaJ	STM2103	UDP-glucose lipid carrier transferase	3.2	0.034
[O] Post-translational modification, protein turnover, and chaperones				
ibpB	STM3808.S	heat shock protein IbpB	5.3	0.077
STM0912	STM0912	ATP-dependent Clp protease proteolytic subunit	2.0	0.002
INFORMATION STORAGE AND PROCESSING				
[J] Translation, ribosomal structure and biogenesis				
ymfC	STM1237	rRNA large subunit pseudouridine synthase E	5.8	0.027
[K] Transcription				
STM0898A	STM0898A	hypothetical protein	2.2	0.002

[L] Replication, recombination and repair				
STM1309	STM1309	excinuclease	0.9	0.053
yejH	STM2223	ATP-dependent helicase	0.9	0.069
METABOLISM				
[C] Energy production and conversion				
asrC	STM2550	anaerobic sulfite reductase subunit C	1.2	0.006
hpaC	STM1098	4-hydroxyphenylacetate 3-monooxygenase reductase subunit	1.0	0.086
hycE	STM2849	hydrogenase 3 large subunit	1.2	0.011
hycG	STM2847	hydrogenase	1.7	0.086
pflF	STM0843	pyruvate formate lyase	1.2	0.004
STM0691	STM0691	tricarballylate dehydrogenase	5.3	0.022
STM1253	STM1253	cytochrome b561	1.4	0.002
STM1793	STM1793	cytochrome oxidase subunit II	1.0	0.042
ttrA	STM1383	tetrathionate reductase subunit A	1.3	0.002
ttrB	STM1385	tetrathionate reductase subunit B	1.2	0.020
yqhD	STM3164	alcohol dehydrogenase	4.3	0.085
[E] Amino acid transport and metabolism				
artJ	STM0887	arginine ABC transporter substrate-binding protein ArtJ	0.9	0.092
lysC	STM4220	aspartokinase	0.8	0.090
serA	STM3062	D-3-phosphoglycerate dehydrogenase	1.2	0.011
tyrA	STM2669	bifunctional chorismate mutase/prephenate dehydrogenase	0.9	0.062
[F] Nucleotide transport and metabolism				
purU	STM1756	formyltetrahydrofolate deformylase	4.3	0.020
[G] Carbohydrate transport and metabolism				
gntK	STM3542	gluconate kinase	4.4	0.022
mdfA	STM0866	multidrug translocase	0.8	0.095
STM2757	STM2757	cytoplasmic protein	1.1	0.081
yicI	STM3749	alpha-xylosidase	1.1	0.024
yicJ	STM3750	GPH family transport protein	0.9	0.081
[H] Coenzyme transport and metabolism				
bioA	STM0793	adenosylmethionine--8-amino-7-oxononanoate aminotransferase	4.9	0.042
nadA	STM0756	quinolinate synthetase	6.1	0.071
[I] Lipid transport and metabolism				
ybjG	STM0865	undecaprenyl pyrophosphate phosphatase	1.3	0.014
ydiF	STM1357.S	acetyl-CoA/acetoacetyl-CoA transferase subunit beta	1.0	0.052
[P] Inorganic ion transport and metabolism				
kdpC	STM0704	potassium-transporting ATPase subunit C	5.4	0.043
ygaP	STM2798	rhodanese-like sulfurtransferase	0.9	0.066

FUNCTION UNKNOWN OR POORLY CHARACTERIZED				
phnX	STM0432	phosphonoacetaldehyde hydrolase	1.5	0.009
STM0906	STM0906	hypothetical protein	1.5	0.002
STM0907	STM0907	prophage chitinase	1.7	0.002
STM0910	STM0910	hypothetical protein	2.0	0.002
STM0911	STM0911	hypothetical protein	1.9	0.087
STM2601	STM2601	minor capsid protein FII	1.1	0.044
STM2603	STM2603	phage head-like protein	0.9	0.060
STM2604	STM2604	phage head-like protein	1.4	0.014
STM2986.Sc	STM2986.Sc	integral membrane protein	5.0	0.021
STM4271	STM4271	inner membrane protein	2.2	0.002
yieM	STM3878.S	protein ViaA	4.5	0.024
ssaE	STM1396	secretion system effector SsaE	1.9	0.002
sseB	STM1398	secreted effector protein SseB	6.2	0.072
sseI	STM1051	secreted effector protein SseI	1.2	0.008
sspH2	STM2241	E3 ubiquitin-protein ligase SspH2	1.4	0.004
STM0909	STM0909	hypothetical protein	2.1	0.033
STM1008.S	STM1008.S	hypothetical protein	1.1	0.074
STM1528	STM1528	outer membrane protein	1.9	0.002
STM1585	STM1585	outer membrane lipoprotein	1.4	0.002
STM2240	STM2240	cytoplasmic protein	1.1	0.051
STM2617	STM2617	antiterminator-like protein	1.2	0.034
STM3030	STM3030	periplasmic protein	5.7	0.100
STM3521	STM3521	ribonucleoprotein related-protein	2.9	0.023
ybjM	STM0871	inner membrane protein	0.9	0.069
ydbH	STM1646	periplasmic protein	1.2	0.004
yeaK	STM1282	cytoplasmic protein	7.1	0.041
yebG	STM1882	DNA damage-inducible protein	1.3	0.023
yfcC	STM2339	integral membrane protein	1.1	0.018
ygaC	STM2801	cytoplasmic protein	1.0	0.058
yhfK	STM3467	inner membrane protein	4.8	0.088
yjbH	STM4225	outer membrane lipoprotein	1.2	0.052
yjcB	STM4263	inner membrane protein	1.1	0.099
STM04875	STM04875	hypothetical protein	1.6	0.021
STM04895	STM04895	hypothetical protein	1.5	0.067
STM0894	STM0894	excisionase	1.7	0.002
STM0895	STM0895	hypothetical protein	1.8	0.002
STM0896	STM0896	hypothetical protein	1.7	0.002
STM0897	STM0897	hypothetical protein	2.0	0.002
STM0899	STM0899	hypothetical protein	1.6	0.002
STM0904	STM0904	hypothetical protein	1.1	0.011
STM1010	STM1010	hypothetical protein	1.0	0.094
STM1010.1n	STM1010.1n	hypothetical protein	1.7	0.009

STM1011	STM1011	hypothetical protein	1.8	0.046
STM1530	STM1530	outer membrane protein	1.6	0.002
STM1869A	STM1869A	hypothetical protein	1.1	0.074
STM1870	STM1870	hypothetical protein	1.2	0.031
STM2237	STM2237	inner membrane protein	1.4	0.017

Table 4. Genes in *S.* Typhimurium LT2 down-regulated on tomato (A) shoots and roots, (B) shoots only, and (C) roots only, relative to LB culture.

A

Function and gene	NCBI tag	Annotation	Shoots		Roots	
			log2 (Fold change)	*q* value	log2 (Fold change)	*q* value
CELLULAR PROCESSES AND SIGNALING						
[D] Cell cycle control, cell division, chromosome partitioning						
minD	STM1815	ATPase MinD	-1.3	0.002	-1.8	0.002
minE	STM1816	cell division topological specificity factor MinE	-1.9	0.002	-2.3	0.002
[M] Cell wall/membrane/envelope biogenesis						
mltD	STM0260	membrane-bound lytic murein transglycosylase D	-2.4	0.002	-2.0	0.002
nlpD	STM2925	Murein hydrolase activator NlpD	-0.8	0.088	-2.0	0.002
nmpC	STM1572	outer membrane porin protein OmpD	-5.4	0.002	-5.4	0.002
ompF	STM0999	outer membrane protein F	-2.8	0.002	-2.9	0.002
ompW	STM1732	outer membrane protein W	-3.4	0.002	-3.0	0.002
pagC	STM1246	virulence membrane protein PagC	-1.8	0.002	-1.7	0.002
yaeT	STM0224	outer membrane protein assembly factor BamA	-1.1	0.071	-1.4	0.002
ycgR	STM1798	Cyclic di-GMP binding protein	-1.7	0.002	-1.5	0.002
yfiO	STM2663	outer membrane protein assembly factor BamD	-0.9	0.095	-1.4	0.002
[N] Cell motility						
cheR	STM1918	chemotaxis protein methyltransferase	-5.4	0.028	-5.4	0.021
[O] Post-translational modification, protein turnover, and chaperones						
groEL	STM4330	chaperonin GroEL	-1.7	0.002	-1.0	0.082
groES	STM4329	co-chaperonin GroES	-1.8	0.002	-1.0	0.039
[T] Signal transduction mechanisms						
STM2314	STM2314	chemotaxis signal transduction protein	-1.2	0.004	-1.1	0.021

ttrS	STM1386	tetrathionate sensor histidine kinase TtrS	-1.3	0.009	-1.2	0.029
ydaA	STM1661	universal stress protein E	-1.3	0.008	-1.8	0.002
yebR	STM1847	free methionine-(R)-sulfoxide reductase	-1.1	0.077	-1.0	0.100
ynaF	STM1652	universal stress protein F	-2.7	0.002	-2.6	0.002
[U] Intracellular trafficking, secretion, and vesicular transport						
secG	STM3293	preprotein translocase IISp family protein	-1.1	0.070	-1.8	0.002
tatA	STM3973	Sec-independent protein translocase protein TatA	-1.7	0.073	-2.1	0.021
tatB	STM3974	Sec-independent protein translocase protein TatB	-1.0	0.067	-1.2	0.008
INFORMATION STORAGE AND PROCESSING						
[J] Translation, ribosomal structure and biogenesis						
efp	STM4334	elongation factor P	-1.5	0.038	-2.1	0.002
pnp	STM3282	polyribonucleotide nucleotidyltransferas	-1.3	0.013	-2.1	0.002
rplS	STM2673	50S ribosomal protein L19	-1.4	0.009	-2.2	0.002
rplY	STM2224	50S ribosomal protein L25	-1.1	0.035	-1.4	0.004
rpsB	STM0216	30S ribosomal protein S2	-1.3	0.013	-1.5	0.002
rpsO	STM3283	30S ribosomal protein S15	-1.0	0.092	-1.7	0.002
rpsP	STM2676	30S ribosomal protein S16	-1.3	0.008	-2.2	0.002
STM1549	STM1549	translation initiation inhibitor	-1.1	0.008	-1.3	0.006
trmD	STM2674	tRNA (guanine-N(1)-)-methyltransferase	-1.2	0.027	-2.2	0.002
tsf	STM0217	elongation factor Ts	-1.1	0.065	-1.5	0.004
yfiA	STM2665	translation inhibitor protein RaiA	-1.8	0.002	-1.6	0.002
[K] Transcription						
cspC	STM1837	cold shock-like protein CspC	-1.3	0.036	-2.4	0.002
cspD	STM0943	stress response protein	-1.7	0.002	-2.7	0.002
hns	STM1751	DNA-binding protein H-NS	-2.0	0.016	-2.8	0.002
osmE	STM1311	DNA-binding transcriptional activator	-1.7	0.004	-2.9	0.002
[L] Replication, recombination and repair						
hupA	STM4170	DNA-binding protein HU-alpha	-2.9	0.004	-2.8	0.002
METABOLISM						
[C] Energy production and conversion						
aceA	STM4184	isocitrate lyase	-2.6	0.002	-3.1	0.002
aceB	STM4183	malate synthase	-1.9	0.002	-2.4	0.002
cyoA	STM0443	cytochrome o ubiquinol oxidase subunit II	-2.4	0.074	-3.6	0.009
frdA	STM4343	fumarate reductase flavoprotein subunit	-1.8	0.002	-1.6	0.002

frdC	STM4341	fumarate reductase subunit C	-2.2	0.002	-2.1	0.002
glpQ	STM2282	glycerophosphodiester phosphodiesterase	-1.4	0.009	-1.8	0.002
nuoC	STM2326	bifunctional NADH-ubiquinone oxidoreductase subunit C/D	-1.0	0.095	-1.5	0.002
nuoG	STM2323.S	NADH-quinone oxidoreductase subunit G	-1.3	0.072	-1.6	0.004
nuoI	STM2321	NADH-quinone oxidoreductase subunit I	-1.7	0.002	-2.0	0.002
nuoL	STM2318	NADH-quinone oxidoreductase subunit L	-1.4	0.066	-1.6	0.028
nuoM	STM2317	NADH-quinone oxidoreductase subunit M	-1.4	0.002	-1.3	0.013
nuoN	STM2316.S	NADH-quinone oxidoreductase subunit N	-1.1	0.024	-1.1	0.032
[E] Amino acid transport and metabolism						
astA	STM1304	arginine succinyltransferase	-2.2	0.081	-3.5	0.002
gcvH	STM3054	glycine cleavage system protein H	-2.9	0.002	-3.7	0.002
gcvP	STM3053	glycine dehydrogenase	-2.0	0.002	-2.7	0.002
gcvT	STM3055	glycine cleavage system aminomethyltransferase T	-2.2	0.002	-3.2	0.002
oppA	STM1746.S	oligopeptide ABC transporter substrate-binding protein OppA	-1.3	0.004	-1.4	0.002
STM1795	STM1795	glutamate dehydrogenase	-2.1	0.002	-4.1	0.002
[F] Nucleotide transport and metabolism						
guaC	STM0141	GMP reductase	-1.1	0.021	-1.3	0.008
pyrH	STM0218	uridylate kinase	-1.0	0.052	-1.1	0.044
[G] Carbohydrate transport and metabolism						
celA	STM1312	PTS system N,N'-diacetylchitobiose-specific transporter subunit IIB	-1.6	0.002	-1.7	0.002
glpT	STM2283	sn-glycerol-3-phosphate transporter	-1.5	0.004	-1.8	0.002
lamB	STM4231	maltoporin	-4.1	0.002	-5.2	0.002
malE	STM4229	maltose ABC transporter substrate-binding protein MalE	-3.7	0.002	-5.0	0.002
malF	STM4228	maltose ABC transporter permease MalF	-2.6	0.002	-3.1	0.002
malG	STM4227	maltose ABC transporter permease MalG	-1.9	0.002	-1.9	0.002
malK	STM4230	maltose ABC transporter ATP-binding protein MalK	-2.6	0.002	-3.3	0.002
malM	STM4232	maltose regulon periplasmic protein	-2.9	0.002	-4.1	0.002
manX	STM1830	PTS system mannose-specific transporter subunit IIAB	-1.5	0.002	-2.5	0.002

manY	STM1831	PTS system mannose-specific transporter subunit IIC	-1.2	0.004	-1.9	0.002
manZ	STM1832	PTS system mannose-specific transporter subunit IID	-1.5	0.002	-2.4	0.002
treA	STM1796	trehalase	-1.3	0.009	-2.2	0.002
[I] Lipid transport and metabolism						
accA	STM0232	acetyl-CoA carboxylase carboxyltransferase subunit alpha	-0.9	0.086	-1.3	0.002
acs	STM4275	acetyl-CoA synthetase	-1.7	0.002	-3.3	0.002
cdsA	STM0222	phosphatidate cytidylyltransferase	-1.4	0.006	-1.4	0.002
uppS	STM0221	Ditrans,polycis-undecaprenyl-diphosphate synthase	-1.3	0.004	-1.2	0.009
[P] Inorganic ion transport and metabolism						
chaA	STM1771	calcium/sodium:proton antiporter	-1.5	0.002	-1.5	0.002
katE	STM1318	Catalase	-1.5	0.002	-2.1	0.002
oppB	STM1745	oligopeptide ABC transporter permease OppB	-1.4	0.006	-1.7	0.002
oppC	STM1744	oligopeptide ABC transporter permeaseOppC	-1.3	0.013	-1.3	0.006
phnA	STM4289	hypothetical protein	-1.0	0.017	-0.9	0.075
zraP	STM4172	zinc resistance protein	-4.3	0.002	-2.8	0.002
FUNCTION UNKNOWN OR POORLY CHARACTERIZED						
actP	STM4273	cation/acetate symporter ActP	-2.3	0.002	-2.6	0.002
aphA	STM4249	class B acid phosphatase	-1.4	0.002	-1.3	0.002
pliC	STM1249	lysozyme inhibitor	-1.8	0.002	-0.9	0.065
ybjP	STM0892	lipoprotein	-1.4	0.002	-1.2	0.008
yieF	STM3850	oxidoreductase	-1.1	0.013	-1.6	0.002
STM1254	STM1254	outer membrane lipoprotein	-1.8	0.002	-1.5	0.002
yaiZ	STM0379	inner membrane protein	-1.0	0.074	-0.9	0.095
yciI	STM1738	cytoplasmic protein	-1.3	0.002	-1.3	0.002
ydiZ	STM1325	cytoplasmic protein	-1.1	0.013	-1.3	0.002
ygaM	STM2802	inner membrane protein	-1.1	0.085	-2.3	0.002
yihD	STM3995	cytoplasmic protein	-1.3	0.002	-1.4	0.002
yjaH	STM4171	inner membrane protein	-1.4	0.002	-1.5	0.002
yjbJ	STM4240	stress-response protein	-1.6	0.002	-3.1	0.002
yjeI	STM4331	outer membrane lipoprotein	-1.8	0.002	-2.1	0.002
ynaJ	STM1662	inner membrane protein	-1.0	0.050	-1.1	0.023
yobF	STM1838	cytoplasmic protein	-1.1	0.090	-2.4	0.002
STM1740	STM1740	dsDNA-mimic protein	-1.0	0.046	-1.0	0.023
STM3745	STM3745	cytoplasmic protein	-1.1	0.011	-0.9	0.052
STM4503	STM4503	inner membrane protein	-1.3	0.004	-1.4	0.002

B

Function and gene	NCBI tag	Annotation	log2 (Fold change)	*q* value
CELLULAR PROCESSES AND SIGNALING				
[D] Cell cycle control, cell division, chromosome partitioning				
yhcP	STM3364	p-hydroxybenzoic acid efflux pump subunit AaeB	-4.2	0.014
[O] Post-translational modification, protein turnover, and chaperones				
htpX	STM1844	protease HtpX	-1.2	0.004
INFORMATION STORAGE AND PROCESSING				
[K] Transcription				
lexA	STM4237	LexA repressor	-1.1	0.033
METABOLISM				
[E] Amino acid transport and metabolism				
gltS	STM3746	GltS family glutamate transport protein	-0.9	0.070
[P] Inorganic ion transport and metabolism				
ftn	STM1935	ferritin	-1.6	0.002
FUNCTION UNKNOWN OR POORLY CHARACTERIZED				
STM3133	STM3133	amidohydrolase	-1.0	0.076
STM0905	STM0905	hypothetical protein	-1.5	0.002
yebE	STM1880	inner membrane protein	-1.1	0.009
nanH	STM0928	sialidase	-2.6	0.081
STM04890	STM04890	hypothetical protein	-2.1	0.071
STM1854	STM1854	inner membrane protein	-1.2	0.014
STM2329	STM2329	cytoplasmic protein	-1.6	0.041

C

Function and gene	NCBI tag	Annotation	log2 (Fold change)	*q* value
CELLULAR PROCESSES AND SIGNALING				
[M] Cell wall/membrane/envelope biogenesis				
hlpA	STM0225	chaperone protein Skp	-1.4	0.002
kdsB	STM0988	3-deoxy-manno-octulosonate cytidylyltransferase	-4.8	0.017
lpxD	STM0226	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	-1.6	0.002
yaeL	STM0223	zinc metallopeptidase RseP	-1.1	0.062
yggB	STM3067	mechanosensitive channel	-0.9	0.052
ytfM	STM4409	outer membrane protein	-4.8	0.072
[O] Post-translational modification, protein turnover, and chaperones				
cyoE	STM0439	protoheme IX farnesyltransferase	-3.5	0.018

hflB	STM3296	ATP-dependent metalloprotease	-1.1	0.027
yeaZ	STM1820	tRNA threonylcarbamoyladenosine biosynthesis protein TsaB	-1.5	0.002
[T] Signal transduction mechanisms				
proQ	STM1846	ProP effector	-1.5	0.002
rseC	STM2637	SoxR reducing system protein RseC	-5.8	0.094
[U] Intracellular trafficking, secretion, and vesicular transport				
csgF	STM1140	curli production assembly/transport protein CsgF	-1.6	0.002
tatC	STM3975	Sec-independent protein translocase protein TatC	-1.4	0.028
yidC	STM3842	membrane protein insertase YidC	-1.6	0.002
[V] Defense mechanisms				
oppD	STM1743	oligopeptide ABC transporter ATP-binding protein OppD	-1.5	0.076
INFORMATION STORAGE AND PROCESSING				
[J] Translation, ribosomal structure and biogenesis				
asnC	STM1000	sparagine--tRNA ligase	-1.1	0.046
def	STM3406	peptide deformylase	-1.0	0.018
frr	STM0219	ribosome recycling factor	-1.4	0.004
infB	STM3286	translation initiation factor IF-2	-1.1	0.049
rimM	STM2675	ribosome maturation factor RimM	-2.0	0.002
rluD	STM2662	rRNA large subunit pseudouridine synthase D	-1.2	0.087
rpsU	STM3209	30S ribosomal protein S21	-2.3	0.002
spoU	STM3743	tRNA guanosine-2'-O-methyltransferase	-1.0	0.036
truB	STM3284	tRNA pseudouridine synthase B	-1.2	0.044
ychF	STM1784	ribosome-binding ATPase	-1.0	0.024
yoaB	STM1822	translation initiation inhibitor	-1.1	0.031
[K] Transcription				
fnr	STM1660.S	fumarate/nitrate reduction transcriptional regulator	-1.3	0.013
kdgR	STM1842	IclR family transcriptional repressor	-1.1	0.028
rpoS	STM2924	RNA polymerase sigma factor RpoS	-1.9	0.002
rpoZ	STM3741	DNA-directed RNA polymerase subunit omega	-1.4	0.002
[L] Replication, recombination and repair				
dbpA	STM1655	ATP-dependent RNA helicase DbpA	-1.0	0.023
dnaN	STM3837	DNA polymerase III subunit beta	-1.3	0.006
holE	STM1876	DNA polymerase III subunit theta	-1.6	0.002
ssb	STM4256	single-stranded DNA-binding protein	-1.4	0.008
METABOLISM				
[C] Energy production and conversion				
adhE	STM1749	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	-1.5	0.002

aldB	STM3680	aldehyde dehydrogenase B	-3.7	0.061
gldA	STM4108	glycerol dehydrogenase	-5.7	0.024
nuoA	STM2328	NADH-quinone oxidoreductase subunit A	-1.3	0.008
nuoB	STM2327	NADH-quinone oxidoreductase subunit B	-1.5	0.002
nuoF	STM2324	NADH-quinone oxidoreductase subunit F	-1.4	0.078
pckA	STM3500	phosphoenolpyruvate carboxykinase	-4.3	0.004
qor	STM4245	quinone oxidoreductase	-1.1	0.017
yjgB	STM4486	alcohol dehydrogenase	-1.2	0.002
[E] Amino acid transport and metabolism				
argD	STM1303	bifunctional succinylornithine transaminase/acetylornithine transaminase	-3.8	0.028
artI	STM0890	arginine ABC transporter substrate-binding protein ArtI	-1.3	0.004
artP	STM0891	arginine ABC transporter ATP-binding protein ArtP	-1.2	0.006
astB	STM1306	succinylarginine dihydrolase	-3.0	0.021
astD	STM1305	N-succinylglutamate 5-semialdehyde dehydrogenase	-3.5	0.058
potE	STM0700	APC family putrescine/ornithine antiporter	-3.1	0.049
potF	STM0877	putrescine ABC transporter substrate-binding protein PotF	-1.1	0.039
sdaA	STM1826	L-serine deaminase I/L-threonine deaminase I	-1.6	0.002
yehX	STM2163	proline/glycine betaine ABC transporter ATP-binding protein	-5.3	0.009
[F] Nucleotide transport and metabolism				
gmk	STM3740	guanylate kinase	-1.5	0.002
gpt	STM0317	xanthine phosphoribosyltransferase	-1.4	0.066
prsA	STM1780	ribose-phosphate pyrophosphokinase	-2.3	0.058
udk	STM2122	uridine kinase	-4.5	0.028
udp	STM3968	uridine phosphorylase	-1.0	0.021
upp	STM2498	uracil phosphoribosyltransferase	-0.9	0.073
[G] Carbohydrate transport and metabolism				
fba	STM3068	fructose-bisphosphate aldolase	-1.5	0.002
pfkB	STM1326	6-phosphofructokinase	-1.1	0.009
pgk	STM3069	phosphoglycerate kinase	-1.3	0.002
prpB	STM0368	2-methylisocitrate lyase	-3.0	0.071
rpiA	STM3063	ribose-5-phosphate isomerase A	-1.9	0.002
STM1324	STM1324	cytoplasmic protein	-1.3	0.002
[H] Coenzyme transport and metabolism				
fre	STM3979	NAD(P)H-flavin reductase	-1.7	0.072
menF	STM2310	isochorismate synthase	-0.9	0.073
nadE	STM1310	NH(3)-dependent NAD synthetase	-1.1	0.009

[I] Lipid transport and metabolism				
fabZ	STM0227	(3R)-hydroxymyristoyl-ACP dehydratase	-1.3	0.002
fadA	STM3982	3-ketoacyl-CoA thiolase	-1.7	0.002
fadB	STM3983	multifunctional fatty acid oxidation complex subunit alpha	-1.9	0.002
fadD	STM1818	long-chain-fatty-acid--CoA ligase	-1.4	0.004
idi	STM3039	isopentenyl-diphosphate delta-isomerase	-1.0	0.022
pssA	STM2652	phosphatidylserine synthase	-1.3	0.009
[P] Inorganic ion transport and metabolism				
cyaY	STM3943	frataxin-like protein	-1.4	0.014
STM1731	STM1731	catalase	-3.1	0.002
STM1741	STM1741	voltage-gated potassium channel	-1.0	0.026
STM1874	STM1874	inner membrane protein	-0.9	0.059
yobA	STM1875	hypothetical protein	-1.2	0.011
[Q] Secondary metabolites biosynthesis, transport, and catabolism				
dlhH	STM3967	carboxymethylenebutenolidase	-1.8	0.002
STM0950	STM0950	SlsA protein	-1.0	0.042
FUNCTION UNKNOWN OR POORLY CHARACTERIZED				
chaB	STM1770	cation transport regulator	-2.5	0.002
STM2346	STM2346	Nudix hydrolase	-0.9	0.090
ydfG	STM1511	L-serine/L-allo-threonine dehydrogenase	-1.1	0.022
yhbO	STM3269	intracellular proteinase	-1.8	0.002
elaB	STM2311	inner membrane protein	-1.7	0.002
smg	STM3404	hypothetical protein	-1.5	0.006
spoT	STM3742	bifunctional (p)ppGpp synthetase II/ guanosine-3',5'-bis pyrophosphate 3'-pyrophosphohydrolase	-1.1	0.036
STM1586	STM1586	hypothetical protein	-1.8	0.002
STM2901	STM2901	cytoplasmic protein	-1.2	0.035
STM3841	STM3841	membrane protein insertion efficiency factor	-2.1	0.020
yajQ	STM0435	phage host factor	-1.5	0.002
ybaY	STM0465	outer membrane lipoprotein	-1.9	0.002
yccJ	STM1118	cytoplasmic protein	-1.1	0.026
yciE	STM1730	cytoplasmic protein	-3.1	0.002
yciF	STM1729	cytoplasmic protein	-3.2	0.002
yciG	STM1728	cytoplasmic protein	-2.6	0.002
ydeI	STM1515	periplasmic protein	-1.7	0.002
yeaC	STM1292	cytoplasmic protein	-2.2	0.002
yebF	STM1881	hypothetical protein	-1.7	0.002
yfbU	STM2335	hypothetical protein	-0.9	0.053
yhbC	STM3288	ribosome maturation factor RimP	-1.2	0.004
yjbQ	STM4250	hypothetical protein	-0.9	0.053

ylaC	STM0471	inner membrane protein	-2.8	0.086
ymgE	STM1797	transglycosylase-associated protein	-1.6	0.002
yniB	STM1323	regulatory protein	-1.3	0.006
sseA	STM1397	secretion system chaperone SseA	-1.5	0.002
STM4002	STM4002	cytoplasmic protein	-2.5	0.013

The differentially expressed genes were clustered according to their Clusters of Orthologous Groups of proteins (COGs) and are shown in Figs. 3 and 4. Protein functions known or predicted based on their orthologs were given to each of the differentially regulated gene using the eggNOG 4.0 database. Many *S.* Typhimurium genes that were up-regulated on tomato shoots are involved in the transport and metabolism of amino acids and inorganic ions, and in transcription, although the majority (about 30%) of up-regulated genes remain uncharacterized or unclassified, encoding hypothetical proteins or proteins with unknown function (Fig. 3). On tomato roots, a greater portion (47%) of up-regulated genes were categorized as being unknown for function or poorly characterized and thus the COGs pattern shown for shoots is hardly observed for roots. Interestingly, *ygbA* and *yoaG*, belonging to the nitrosative stress resistance regulon (NsrR) (Karlinsey et al., 2012), showed marked up-regulation on both shoot and root conditions, although they are classified into a COG class of unknown function (Table 3A). This suggests that *S.* Typhimurium is responding to nitric oxide on the surface of tomato plants.

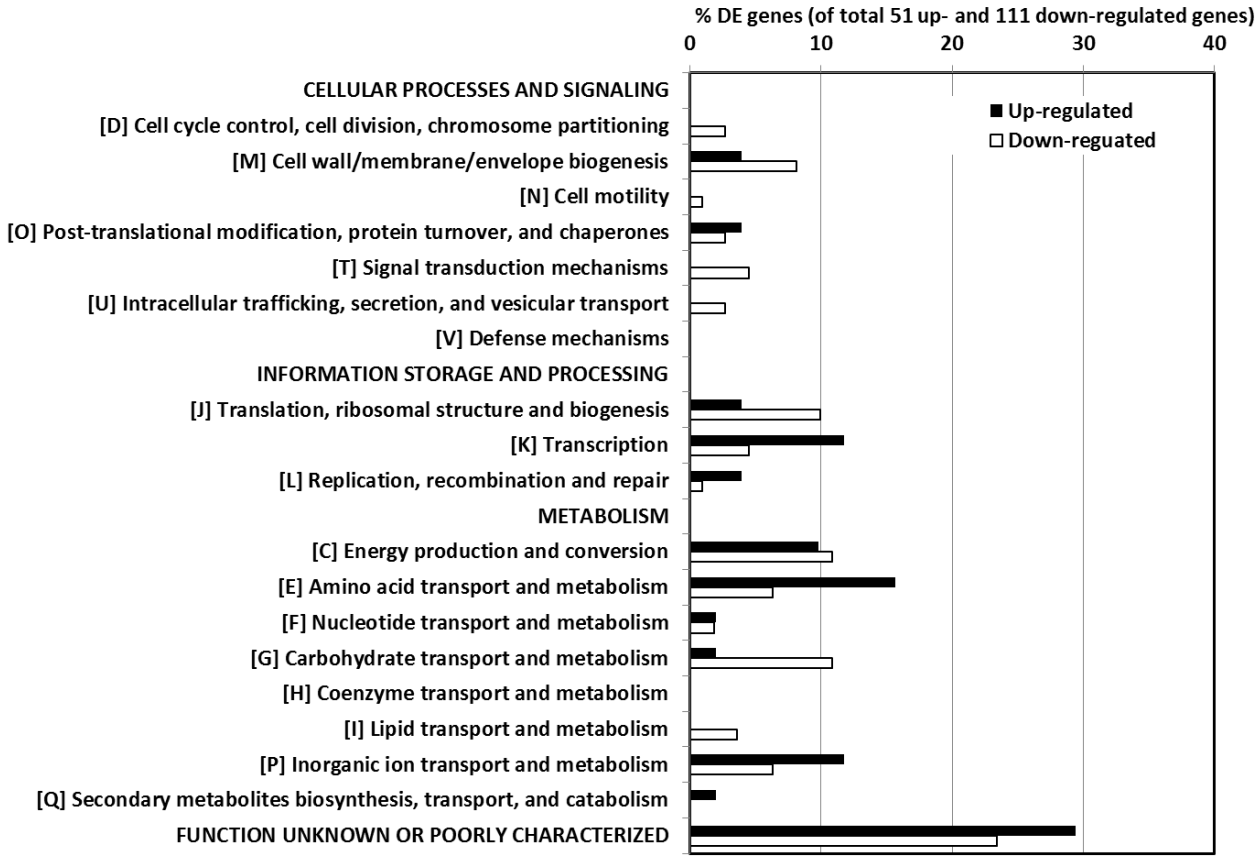


Fig. 3. Relative percentage of genes differentially expressed, altered in expression by at least 1.7-fold, during tomato shoot colonization compared to LB medium; Functions of genes of interest were classified according to the Clusters of Orthologous Groups of proteins (COGs) (http://www.genome.jp/kegg/pathway.html).

The genes that were significantly down-regulated on both tomato shoots and roots are involved in carbohydrate transport and metabolism (Fig. 4 and Table 4A), especially the gene cluster functioning in maltose transport (*lamB* and *malEFGKM*). Genes involved in translation, ribosomal structure and biogenesis were also observed as being down-regulated on tomato plants. Moreover, all the differentially expressed genes that are involved in cellular processes and signaling such as cell cycle, signal

transduction, and intracellular trafficking were down-regulated on both shoots and roots.

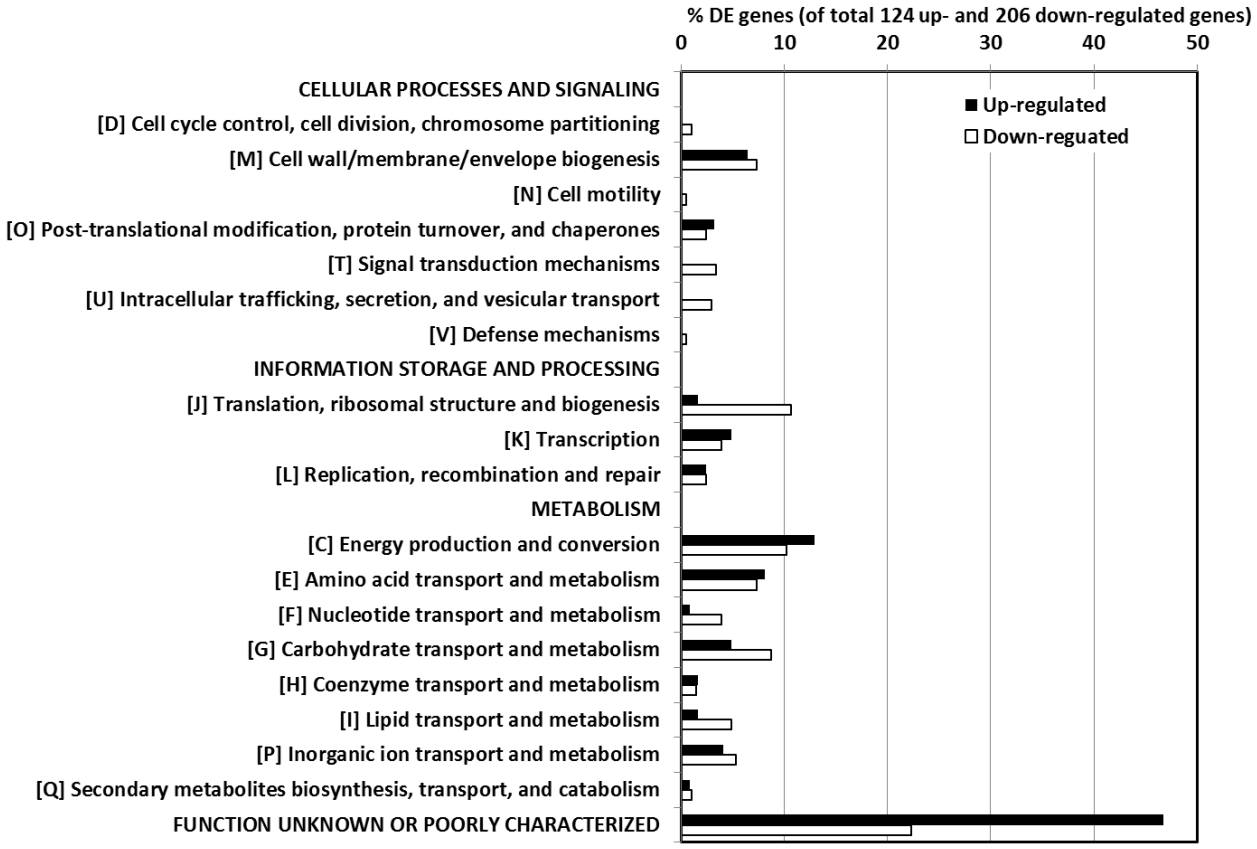


Fig. 4. Relative percentage of genes differentially expressed, altered in expression by at least 1.7-fold, during tomato root colonization compared to LB medium; Functions of genes of interest were classified according to the Clusters of Orthologous Groups of proteins (COGs) (http://www.genome.jp/kegg/pathway.html).

3.3. Amino acid metabolism on tomato plants

Expression of genes involved in metabolic pathways was explored to glean clues on the nutritional environment and metabolic activity of *S.* Typhimurium colonizing tomato. According to the KEGG pathway database for *S.* Typhimurium LT2, which provides microbial pathway maps, along with associated genes drawn from

experimental evidence of molecular interaction and reaction networks within a cell, 29 genes out of all the differentially expressed genes found in this study are known to be involved in various amino acid metabolism pathways (Fig. 5). Among them, the genes *aroF*, *trpB*, *trpC*, *trpD*, and *trpE*, that encode part of phenylalanine, tyrosine, and tryptophan biosynthesis pathways, were induced on shoots, relative to LB culture. On roots, *aroF*, *trpC*, *trpD*, *trpE*, and *tyrA* were up-regulated for these biosynthesis pathways. A role for tryptophan biosynthesis in biofilm formation has been identified (Hamitlon et al., 2009). A group of genes involved in arginine and proline metabolism (*astA* and STM1795 on shoots and roots; *argD*, *astB*, and *astD* on roots) and glycine, serine, and threonine metabolism (*gcvH*, *gcvP*, and *gcvT* on shoots and roots; *sdaA* and *pssA* on roots) were down-regulated on tomato in comparison to LB culture. A few of the glycine, serine, and threonine metabolism genes were found up-regulated on shoots (*trpB*) and roots (*lysC* and *serA*). Of the arginine and proline metabolism genes, STM1795 is also known to be involved in alanine, aspartate and glutamate metabolism, as well as D-glutamine and D-glutamate metabolism.

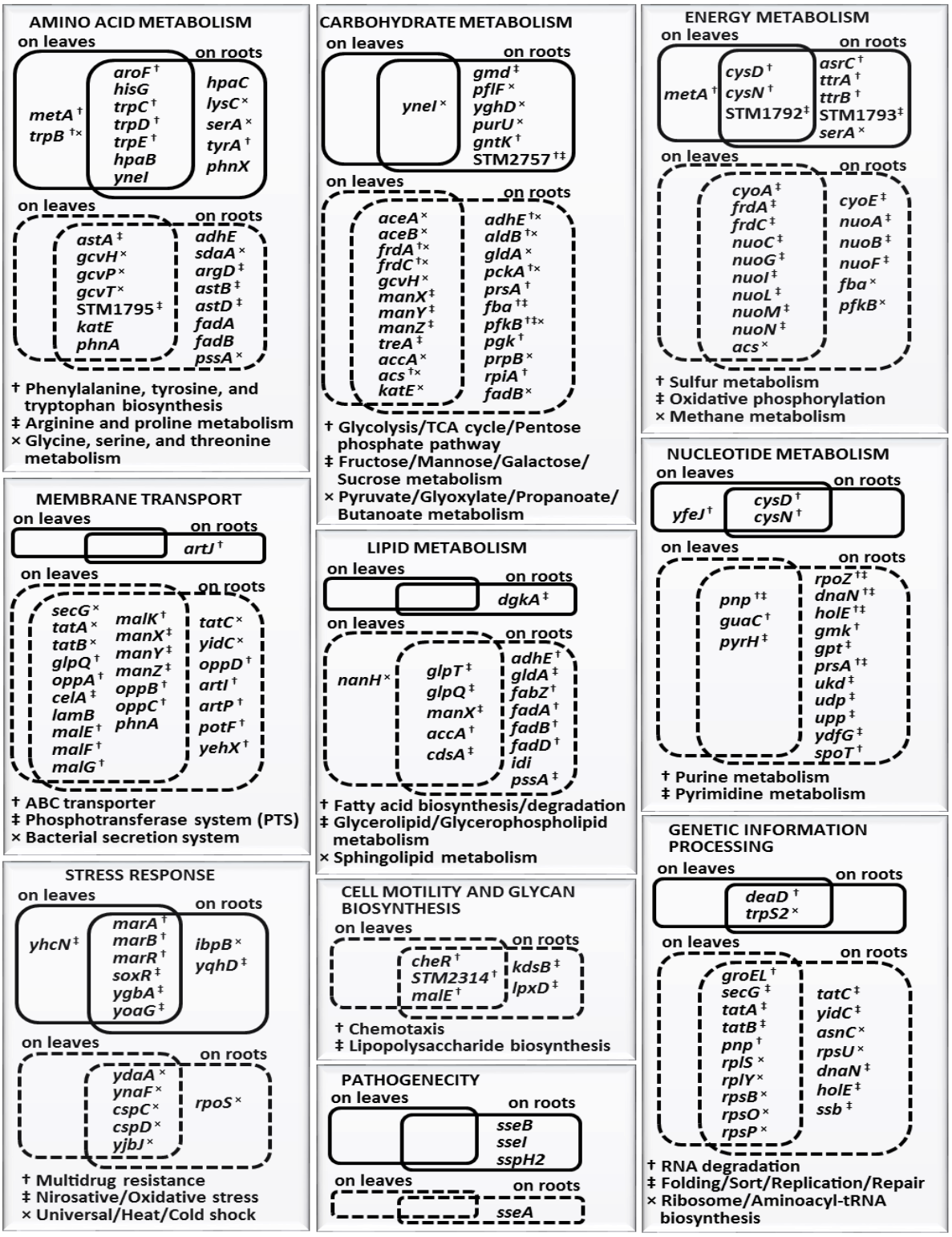


Fig. 5. Lists of differentially regulated genes in *S.* Typhimurium by metabolic pathway when colonizing leaves or roots of tomato plants; Solid line represents up-

regulated genes and dashed line represents down-regulated genes; Metabolic pathways in which genes of interest are associated are classified according to the KEGG pathway database for *S.* Typhimurium LT2.

3.4. Carbohydrate metabolism

Many genes involved in carbohydrate metabolism were less expressed on tomato relative to growth in LB (Fig. 5). The majority of them (*acs, adhE, aldB, fba, frdA, frdC, pckA, pfkB, pgk, prsA,* and *rpiA*) are known to function in the central carbohydrate pathways that produce important precursor metabolites such as glycolysis, the tricarboxylic acid cycle (TCA cycle), and the pentose phosphate pathway (PPP). Root colonization induced a greater magnitude of gene expression changes in *S.* Typhimurium than shoot colonization, with a larger number of genes found differentially expressed on roots.

Another restricted function in carbohydrate metabolism was related to sugar compound metabolism involving *fba, manX, manY, manZ, pfkB,* and *treA*. The *manXYZ* operon encodes three proteins forming the mannose phosphotransferase system (PTS) and has been characterized as the main transporter for mannose as well as glucose, fructose, and many other sugars (Stock et al., 1982). The *treA* gene, which encodes periplasmic trehalase and enables the cell to split periplasmic trehalose into glucose molecules that can subsequently be taken up by the PTS system, was also down-regulated on both shoots and roots.

In alignment with the down-regulation of the central carbohydrate pathways (glycolysis, TCA cycle, and PPP), genes involved in pathways for downstream

precursor metabolites were also repressed, including pyruvate, glyoxylate, propanoate, and butanoate metabolism.

The gene encoding succinate semialdehyde dehydrogenase, *yneI*, was found up-regulated on both shoots and roots. Aldehyde dehydrogenases are known to play an important role in not only the metabolic conversion of carbohydrates but also the detoxification of endogenous and exogenous aldehydes (Zheng et al., 2013a). It is possible that *S.* Typhimurium is responding to natural plant volatile aldehydes.

3.5. Energy and lipid metabolism

Many genes involved in energy generation, associated with the process of oxidative phosphorylation, were down-regulated relative to LB culture (Fig. 5). The majority of these involved genes belonging to the *nuo* locus, which encodes the subunits of the type I NADH dehydrogenase, a key component of the respiratory chain. This type I enzyme translocates protons across the membrane to generate a proton motive force. In addition, the genes encoding parts of the type II succinate dehydrogenase (*frdA* and *frdC*), and the type IV cytochrome c oxidase (*cyoA* and *cyoE*) encoding enzymes for oxidative phosphorylation, were repressed. On the other hand, STM1792 and STM1793, encoding cytochrome oxidase subunit I and II, respectively, were up-regulated on tomato, suggesting that they may serve as alternatives to the well-characterized type I, II, and IV oxidative phosphorylation units.

Genes involved in sulfur metabolism were up-regulated (*cysD*, *cysN*, and *metA* on shoots; *cysD*, *cysN*, *asrC*, *ttrA*, and *ttrB* on roots). Sulfate adenylyltransferase encoded by *cysD* and *cysN* is known to participate in 3 metabolic pathways: purine metabolism and selenoamino acid metabolism as well as sulfur metabolism.

Salmonella is one of the genera in Enterobacteriaceae capable of utilizing tetrathionate as a terminal respiratory electron acceptor and the *ttrRSBCA* locus is required for tetrathionate respiration in *S.* Typhimurium (Barrett and Clark, 1987; Hensel et al., 1999). *Salmonella* gains a competitive advantage in the gut by utilizing tetrathionate produced by oxidation of thiosulphate as a result of inflammation that triggers the release of oxygen radicals (Winter et al., 2010). It is possible that *Salmonella* employs this same strategy during colonization of plant tissue.

It is observed that *metA* was more transcribed, compared to the control LB culture, when *S.* Typhimurium was grown on tomato shoots. In *S.* Typhimurium, MetA protein (homoserine O-succinyltransferase) is known to convert homoserine to O-succinylhomoserine as the first step in the biosynthesis of methionine (Saint-Girons et al., 1988). Price-Carter et al. (2005) showed that organic acid impaired methionine biosynthesis in *S.* Typhimurium, and this led to derepression of MetA and possibly inhibited the bacterial growth by causing toxic accumulation of denatured protein. The authors suggested that the sensitivity of MetA to multiple stress conditions, acidity as well as heat, could be an indication of unfavorable growth conditions.

In cells, lipids often function as an energy reserve. Lipid metabolism genes were repressed on tomato relative to LB culture (Fig. 5). The genes involved in the *glp* regulon-dependent glycerol-3-phosphate transport system, *glpT* and *glpQ* (Hengge et al., 1983), were down-regulated on both shoots and roots. Glycerol-3-phosphate is an organophosphate derived from glycerol and fed to glycolysis. Another group of genes encoding proteins that utilize fatty acids as an energy source were also found down-regulated on roots (*fadA, fadB*, and *fadD*). The repression of energy and lipid

metabolism could be an indication of limited availability of nutrients on tomato, in comparison to LB culture.

3.6. Membrane transport

S. Typhimurium may need to adapt its strategy for translocating metabolites across the cell membrane in response to interaction with the phyllosphere and root system of tomato. Many genes involved in membrane transport were shown to be down-regulated. The genes comprising the oligopeptide permease single operon *OppABCDF* (Hiles et al., 1987), a typical member of the ABC (ATP-binding cassette) superfamily of transporters, were repressed. Oligopeptide permease (Opp) in *S.* Typhimurium is a well-characterized binding protein-dependent system. Opp provides the main pathway for peptide uptake by enteric bacteria and can transport peptides of up to six amino acid residues in length (Payne and Gilvarg, 1968). The genes encoding the maltose ABC transporter *MalE-FGK2*, another member of the ABC superfamily translocating maltose and maltodextrins through protein-dependent and high affinity transport systems, were also down-regulated with marked fold-changes. The gene *lamB*, which encodes maltoporin that facilitates passage of maltose and maltodextrins across the outer membrane was repressed. The maltose transporter is composed of the periplasmic maltose binding protein (MalE), the membrane-spanning subunits MalF and MalG, and two copies of the ATP-hydrolyzing subunit (MalK) (Higgins, 2001). In addition to the genes involved in the ABC transporter system, other ones involved in the phosphotransferase system (PTS) transporting sugar compounds (*manX, manY,* and *manZ*) and in the bacterial secretion

system such as the bacterial twin-arginine translocation (Tat) pathway (*tatA*, *tatB*, and *tatC*) were down-regulated.

3.7. Nucleotide metabolism and genetic information processing

Cellular pathway analysis using the KEGG pathway database identified 17 genes involved in nucleotide metabolism. Purine and pyrimidine metabolisms were found differentially regulated on tomato compared to LB culture. Three genes (*pnp*, *guaC*, and *pyrH*) were found down-regulated on both shoots and roots, while several genes were found repressed specifically when associating with roots. The induction of two genes, *cysD* and *cysN*, reflects the involvement of sulfur metabolism for energy generation.

In addition to genes involved in nucleotide metabolism, genetic information processing genes, such as RNA degradation, protein folding and sorting, DNA replication and repair, and ribosome and aminoacyl-tRNA biosynthesis, were also observed to be down-regulated on tomato. Exceptions included *deaD*, encoding ATP-dependent RNA helicase DeaD, and *trpS2*, encoding tryptophanyl-tRNA synthetase II, both exhibiting up-regulation.

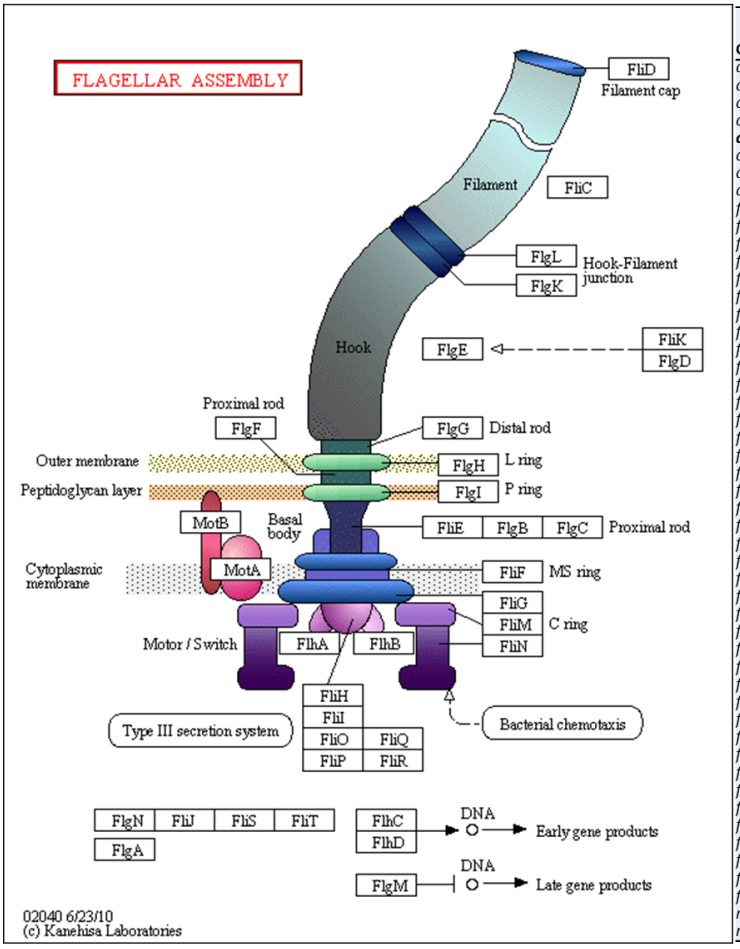
3.8. Stress response

In contrast to the majority of metabolic pathway genes that were mostly down-regulated, a group of genes known to respond to environmental stresses were strongly induced in *Salmonella* colonizing tomato, compared to growth in LB. The genes encoding the multiple antibiotics resistance operon MarRAB (*marR*, *marA* and *marB*), genes in the nitrosative stress regulator operon NsrR (*ygbA* and *yoaG*), and a

gene in the oxidative stress regulator operon SoxRS (*soxR*) were observed to be up-regulated on tomato shoots and roots (Table 3). Similarly, *yhcN* and *yqhD*, found to be associated with oxidative and acid stress responses in *E. coli* (Perez et al., 2008; Lee et al., 2010), were strongly up-regulated in *Salmonella* on tomato shoots and roots, respectively. Genes involved in universal stress, heat, or cold shock responses were found to be down-regulated.

3.9. Cell motility and pathogenicity

During the interaction with tomato, genes encoding proteins involved in bacterial chemotaxis in *S.* Typhimurium were down-regulated, in relation to LB culture. The gene encoding CheR, S-adenosylmethionine-dependent protein methyltransferase that methylates chemotaxis receptor protein and thus initiates signal transduction processes (Djordjevic and Stock, 1998), was repressed with a marked fold change (-5.4). Chemotaxis-related genes STM2314 and *malE*, encoding chemotaxis signal transduction protein and maltose ABC transporter substrate-binding protein which functions as a maltose chemoreceptor, respectively, were also down-regulated. Most genes involved in bacterial chemotaxsis (*aer* and *cheABMRWYZ*) showed a distinct downward trend in expression on tomato shoots and roots in comparison to LB culture, but not sufficiently to result in a significant change at $q<0.1$, except for *cheR* on both shoots and roots (Fig. 6). Similarly, *flgABCDEFGHIJKLMN*, *flhABCD*, *fliABCDEFGHIJKLMNOPQRSTYZ*, and *motAB*, involved in flagella assembly, exhibited a non-statistically significant down-regulation trend.



	on shoots			on roots		
Gene	log2(fold change)	*p* value	*q* value	log2(fold change)	*p* value	*q* value
aer	-5.40	0.22	1.00	-3.37	0.15	0.94
cheA	-2.84	0.11	0.83	-2.74	0.12	0.85
cheB	-5.07	0.03	0.36	-2.83	0.28	1.00
cheM	-2.20	0.33	1.00	-1.98	0.38	1.00
cheR	**-5.41**	**0.00**	**0.03**	**-5.41**	**0.00**	**0.02**
cheW	-3.80	0.11	0.82	-4.12	0.14	0.90
cheY	-2.72	0.10	0.79	-2.37	0.15	0.94
cheZ	-2.44	0.15	0.94	-2.73	0.12	0.86
flgA	-3.57	0.06	0.56	-3.38	0.47	1.00
flgB	-1.67	0.31	1.00	-1.44	0.38	1.00
flgC	-4.20	0.11	0.82	-5.23	0.02	0.28
flgD	-2.91	0.21	1.00	-2.29	0.37	1.00
flgE	-4.28	0.20	1.00	-4.34	0.21	1.00
flgF	-5.01	0.16	0.96	-4.68	0.25	1.00
flgG	-3.83	0.29	1.00	-3.43	0.32	1.00
flgH	-1.62	0.33	1.00	-1.76	0.29	1.00
flgI	-2.76	0.12	0.87	-4.03	0.07	0.63
flgJ	-1.59	0.41	1.00	-1.53	0.41	1.00
flgK	-2.66	0.28	1.00	-2.80	0.26	1.00
flgL	-2.46	0.46	1.00	-2.82	0.40	1.00
flgM	-3.24	0.19	1.00	-5.05	0.24	1.00
flgN	-1.18	0.29	1.00	-1.40	0.22	1.00
flhA	-0.69	0.70	1.00	-0.41	0.82	1.00
flhB	-1.11	0.47	1.00	-0.14	0.92	1.00
flhC	-1.03	0.45	1.00	-1.45	0.30	1.00
flhD	-1.58	0.28	1.00	-2.40	0.10	0.79
fliA	-4.47	0.04	0.45	-5.30	0.03	0.38
fliB	-2.01	0.22	1.00	-1.85	0.27	1.00
fliC	-3.79	0.02	0.30	-5.30	0.17	0.99
fliD	-2.28	0.25	1.00	-2.10	0.25	1.00
fliE	-2.21	0.17	1.00	-2.23	0.17	0.99
fliF	-1.17	0.13	0.89	-1.03	0.16	0.95
fliG	-4.34	0.17	0.99	-4.99	0.26	1.00
fliH	-5.68	0.35	1.00	-2.96	0.35	1.00
fliI	-2.95	0.14	0.92	-2.95	0.14	0.92
fliJ	-1.76	0.34	1.00	-1.28	0.50	1.00
fliK	-3.44	0.28	1.00	-2.95	0.31	1.00
fliL	-3.44	0.15	0.93	-1.34	0.38	1.00
fliM	-2.06	0.39	1.00	-1.56	0.55	1.00
fliN	-5.10	0.07	0.62	-2.93	0.30	1.00
fliO	-3.22	0.06	0.60	-3.90	0.05	0.49
fliP	-0.33	0.84	1.00	0.15	0.93	1.00
fliQ	-0.30	0.89	1.00	-0.30	0.87	1.00
fliR	-0.16	0.92	1.00	-0.57	0.71	1.00
fliS	-4.08	0.02	0.26	-4.77	0.01	0.18
fliT	-0.41	0.78	1.00	-0.77	0.59	1.00
fliY	-0.01	0.99	1.00	-0.21	0.88	1.00
fliZ	-5.97	0.07	0.63	-5.37	0.07	0.62
motA	-2.38	0.26	1.00	-2.72	0.22	1.00
motB	-3.02	0.21	1.00	-3.15	0.20	1.00

Fig. 6. List of genes involved in bacterial flagella assembly and their associated expression levels on shoots and roots compared to LB medium.

3.10. Comparison of *S.* Typhimurium gene expression on shoots versus roots

Bacteria residing on the leaf surface encounter multiple stresses that differ greatly from conditions found below ground. To determine whether *S.* Typhimurium adopts different survival strategies in the phyllosphere and rhizosphere, we compared the transcriptome of the enteric pathogen on tomato leaves with that on tomato roots. A total of 98 genes were found differently regulated at $q<0.1$, of which 54 genes were

up-regulated on leaves while 44 were up-regulated on roots (Table 5). Among the 54 genes that were up-regulated on tomato shoots compared to roots, many encode proteins that are known to be involved in cellular stress responses such as multiple antibiotic resistance loci MarRAB (Sulavik et al., 1997), oxidative stress response SoxR (Farr and Kogoma, 1991), cold shock response CspC and CspD (Shah et al., 2013), and stress response YjbJ. RNA polymerase sigma factor *rpoS* and *rpoS*-dependent *yciGFE* known to respond to general stresses (Beraud et al., 2010) were also induced on shoots although *S*. Typhimurium LT2 is altered in *rpoS* to attenuate the virulence of the strain (Wilmes-Riesenberg et al., 1997). By contrast, only a few of the above mentioned genes were induced on roots, *zraP* and *spy* encoding zinc resistance protein and general stress response protein, respectively. This finding supports the idea that microbes in the phyllosphere have to content with multiple abiotic and biotic stresses, while roots usually provide a less hostile niche to recruit beneficial microbes to the rhizosphere (Lindow and Brandl, 2003; Bais et al., 2006).

Table 5. Genes in S. Typhimurium LT2 up-regulated on tomato (A) shoots relative to roots, and (B) roots relative shoots

A

Gene	NCBI tag	Annotation	log2 (Fold change)	*q* value
acs	STM4275	acetyl-CoA synthetase	1.6	0.00
celC	STM1314	PTS system N,N'-diacetylchitobiose-specific transporter subunit IIA	0.9	0.07
chaB	STM1770	cation transport regulator	2.2	0.00
cspC	STM1837	cold shock-like protein CspC	1.1	0.08
cspD	STM0943	stress response protein	1.0	0.08
dbpA	STM1655	ATP-dependent RNA helicase DbpA	1.1	0.02
deaD	STM3280.S	ATP-dependent RNA helicaseDeaD	1.1	0.07
def	STM3406	peptide deformylase	1.0	0.04
dlhH	STM3967	carboxymethylenebutenolidase	1.0	0.05
elaB	STM2311	inner membrane protein	1.0	0.09
fadA	STM3982	3-ketoacyl-CoA thiolase	1.7	0.00
fadB	STM3983	multifunctional fatty acid oxidation complex subunit alpha	1.8	0.00
fhuF	STM4550	ferric hydroximate transport ferric iron reductase	1.5	0.00
gcvT	STM3055	glycine cleavage system aminomethyltransferase T	1.0	0.04
kdsB	STM0988	3-deoxy-manno-octulosonate cytidylyltransferase	4.2	0.02
malM	STM4232	maltose regulon periplasmic protein	1.2	0.05
manX	STM1830	PTS system mannose-specific transporter subunit IIAB	1.0	0.07
manZ	STM1832	PTS system mannose-specific transporter subunit IID	0.9	0.07
marA	STM1519.S	DNA-binding transcriptional activator MarA	2.3	0.00
marB	STM1518	multiple antibiotic resistance protein MarB	2.1	0.00
marR	STM1520	DNA-binding transcriptional repressor MarR	2.0	0.00
narU	STM1576	nitrate/nitrite transporter NarU	1.2	0.00
nlpD	STM2925	Murein hydrolase activator NlpD	1.2	0.00
osmE	STM1311	DNA-binding transcriptional activator	1.1	0.07
phnB	STM4288	cytoplasmic protein	1.1	0.02
rpiA	STM3063	ribose-5-phosphate isomerase A	1.2	0.04
rpoS	STM2924	RNA polymerase sigma factor RpoS	1.3	0.01
rpsU	STM3209	30S ribosomal protein S21	1.5	0.00
soxR	STM4266	redox-sensitive transcriptional activator SoxR	1.2	0.04

STM05520	STM05520	hypothetical protein	1.2	0.01
STM1324	STM1324	cytoplasmic protein	0.9	0.09
STM1513	STM1513	cytoplasmic protein	3.4	0.00
STM1586	STM1586	hypothetical protein	1.1	0.03
STM1731	STM1731	catalase	3.0	0.00
STM1795	STM1795	glutamate dehydrogenase	2.0	0.00
STM4552	STM4552	inner membrane protein	1.9	0.00
stpA	STM2799	DNA binding protein StpA	0.9	0.08
tonB	STM1737	transport protein TonB	1.5	0.00
udp	STM3968	uridine phosphorylase	1.5	0.00
yabF	STM0085	glutathione-regulated potassium-efflux system ancillary protein KefF	4.1	0.02
yajQ	STM0435	phage host factor	0.9	0.09
ybaY	STM0465	outer membrane lipoprotein	1.5	0.00
yciE	STM1730	cytoplasmic protein	2.9	0.00
yciF	STM1729	cytoplasmic protein	2.9	0.00
yciG	STM1728	cytoplasmic protein	2.7	0.00
ydeI	STM1515	periplasmic protein	1.7	0.00
yeaC	STM1292	cytoplasmic protein	1.5	0.00
yebF	STM1881	hypothetical protein	1.1	0.02
ygaM	STM2802	inner membrane protein	1.2	0.05
yhbO	STM3269	intracellular proteinase	2.1	0.00
yjbJ	STM4240	stress-response protein	1.5	0.00
yjgB	STM4486	alcohol dehydrogenase	1.4	0.01
ymgE	STM1797	transglycosylase-associated protein	1.6	0.00
yobF	STM1838	cytoplasmic protein	1.3	0.04

B

Gene	**NCBI tag**	**Annotation**	**log2 (Fold change)**	***q* value**
dgkA	STM4236	diacylglycerol kinase	1.2	0.00
ftn	STM1935	ferritin	1.1	0.01
ilvL	STM3900	ilvG operon leader peptide	3.1	0.06
pagP	STM0628	lipid A palmitoyltransferase PagP	1.2	0.01
spy	STM1308	stress response protein	1.1	0.08
ssaE	STM1396	secretion system effector SsaE	1.4	0.00
sseI	STM1051	secreted effector protein SseI	1.1	0.04
STM04875	STM04875	hypothetical protein	1.6	0.02
STM04890	STM04890	hypothetical protein	2.1	0.05
STM04895	STM04895	hypothetical protein	1.3	0.08
STM0894	STM0894	excisionase	1.5	0.00

STM0895	STM0895	hypothetical protein	1.6	0.00
STM0895.1n	STM0895.1n	hypothetical protein	1.7	0.07
STM0896	STM0896	hypothetical protein	1.7	0.00
STM0897	STM0897	hypothetical protein	1.5	0.00
STM0898	STM0898	prophage transcriptional regulator	1.3	0.01
STM0898A	STM0898A	hypothetical protein	2.7	0.00
STM0899	STM0899	hypothetical protein	1.8	0.00
STM0904	STM0904	hypothetical protein	1.2	0.01
STM0905	STM0905	hypothetical protein	1.5	0.00
STM0906	STM0906	hypothetical protein	1.7	0.00
STM0907	STM0907	prophage chitinase	1.4	0.01
STM0908	STM0908	hypothetical protein	1.7	0.05
STM0909	STM0909	hypothetical protein	2.0	0.03
STM0910	STM0910	hypothetical protein	1.8	0.00
STM0911	STM0911	hypothetical protein	1.9	0.07
STM0912	STM0912	ATP-dependent Clp protease proteolytic subunit	2.0	0.00
STM1010	STM1010	hypothetical protein	1.1	0.07
STM1010.1n	STM1010.1n	hypothetical protein	1.4	0.08
STM1251	STM1251	molecular chaperone	1.1	0.06
STM1253	STM1253	cytochrome b561	1.7	0.00
STM1528	STM1528	outer membrane protein	1.5	0.00
STM1530	STM1530	outer membrane protein	1.3	0.01
STM1540	STM1540	hydrolase	2.1	0.00
STM1585	STM1585	outer membrane lipoprotein	1.1	0.02
STM1854	STM1854	inner membrane protein	1.0	0.10
STM2629	STM2629	hypothetical protein	1.5	0.08
tatE	STM0632	Sec-independent protein translocase protein TatE	0.9	0.07
ybjG	STM0865	undecaprenyl pyrophosphate phosphatase	0.9	0.10
yebE	STM1880	inner membrane protein	1.6	0.00
yhdV	STM3392	outer membrane lipoprotein	2.0	0.00
yjbE	STM4222.S	outer membrane protein	2.9	0.00
yqhD	STM3164	alcohol dehydrogenase	3.1	0.09
zraP	STM4172	zinc resistance protein	1.5	0.00

3.11. Plasmid

Certain *Salmonella* serovars belonging to subspecies I (*enterica*) and frequently associated with infections of humans and animals, such as Enteritidis, Typhimurium,

Dublin, Choleraesuis, Gallinarum, Pullorum, and Abortus-ovis, carry a large, low-copy-number plasmid that contains virulence genes (Rotger and Casadesus, 1999). Virulence plasmids are required to trigger systemic disease. *Salmonella* virulence plasmids vary in size (50-100 kb), but all share a 7.8 kb region, *spv*, required for virulence (Rotger and Casadesus, 1999). Depending on the serovar, these plasmids code for additional virulence-associated traits. For instance, fimbrial operon (*pef*), conjugal transfer (*tra*), resistance to complement killing (*rck*), and plasmid maintenance and replication (*par*, *rep,* and *rsd*) (Rychlik et al., 2006). In this study, plasmid genes involved in the virulence factors *spvA*, *spvB*, *spvC*, and *spvD* were up-regulated on tomato, especially on shoots, compared to LB culture (Fig. 7). In contrast, the other groups of genes functioning in conjugal transfer and plasmid maintenance and replication were down-regulated on tomato.

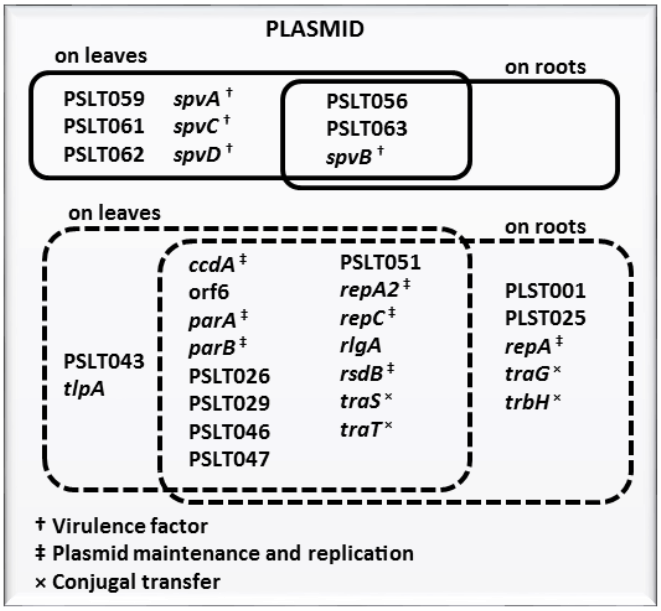


Fig. 7. Differentially regulated genes in *S.* Typhimurium plasmid during colonization of shoots or roots of tomato; Solid line represents up-regulated genes and dashed line represents down-regulated genes; Functions in which genes of interest are associated are classified according to the KEGG pathway database for *S.* Typhimurium LT2 and previous studies (Rotger and Casadesus, 1999; Rychlik et al., 2006).

3.12. Differential gene expression verification

For reproducibility of the genetic response of *S.* Typhimurium on tomato, and validation of RNA-seq analysis results, eight genes were selected for analysis of their transcription by quantitative reverse transcription-PCR (qRT-PCR) on a repeated experiment (Fig. 8). Of the selected target genes, *lamB, malE, nmpC, ydaA,* and *aphA* were shown down-regulated in RNA-seq analysis of *S.* Typhimurium colonizing tomato shoots and roots in comparison to LB culture (Table 4). The other three genes, *yoaG*, *wza*, and *ygbA* were induced in RNA-seq analysis (Table 3). Results between qRT-PCR and RNA-seq were similar with differences in the

magnitude and significance of expression ratios. As in RNA-seq, *lamB* and *malE* were significantly repressed on shoots and roots relative to LB. The genes *yoaG, wza,* and *ygbA* were significantly induced on shoots and upward-trending on roots, agreeing with RNA-seq data. As in RNA-seq, on-root expression of *nmpC* was significantly repressed, but differed on shoots, where no expression change was detected.

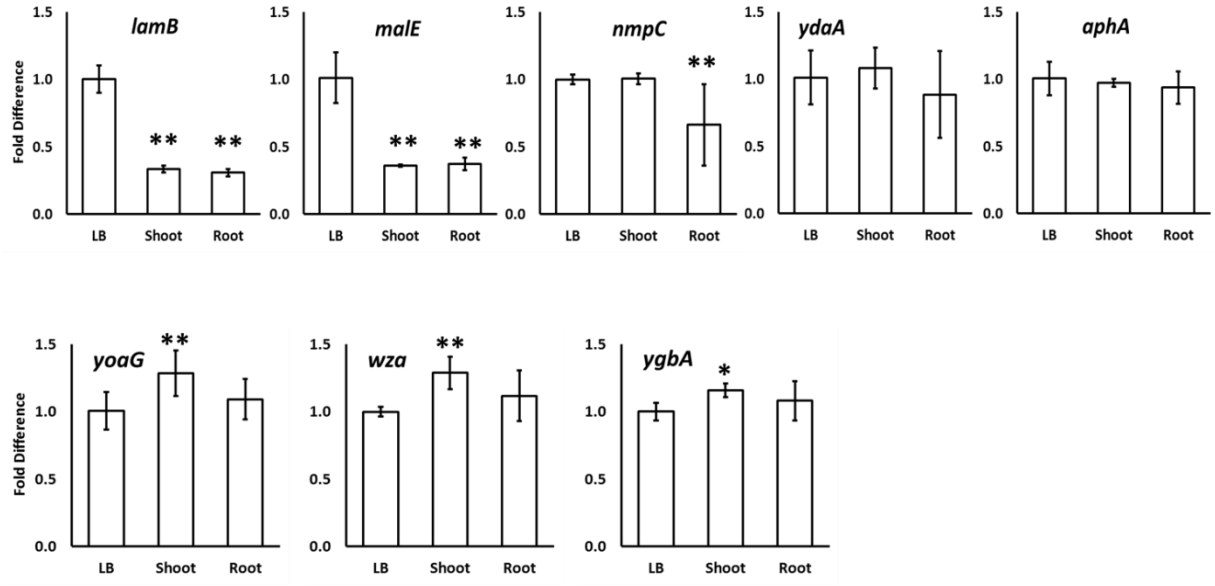


Fig. 8. Transcription ratios of target genes, selected for confirmation of RNA-seq analysis data obtained in this study. Transcription of mRNA was determined by quantitative reverse transcription-PCR. Top five panels represent genes that were differentially down-regulated on shoots and roots compared to LB control by RNA-seq while bottom three panels were for genes up-regulated. Fold difference was determined using the ΔΔCt method which calculates relative changes in gene expression. Error bars indicate standard deviation; n=4 except for LB control with n=3. Asterisks denote significance at $p<0.05$ (**) and $p<0.1$ (*) in relation to LB control.

4. Discussion

The interaction of enteric pathogens on plants is confounding, as these microbes do not appear to behave as plant pathogens nor as enteric pathogens infecting their respective hosts. The present study provides clear evidence that *Salmonella* growth on tomato is highly regulated, and responsive to the plant environment, expressing a unique suite of genes when associating with the phyllosphere and root system, enabling us to elucidate the molecular mechanisms underlying this enteric pathogen-plant interaction. Stress-related genes involved in multidrug resistance and responding to nitrosative stimuli were significantly strongly induced in *S.* Typhimurium growing on tomato shoots and roots, indicating that the plant surface exerted various abiotic stresses on the enteric pathogen. Genes belonging to the NsrR regulon, *ygbA* and *yoaG*, were transcribed at marked higher fold changes, 3.5 and 7.7, respectively. These genes, which encode for uncharacterized cytoplasmic proteins, along with *hmp*, *hcp-hcr*, *yeaR*, *ytfE,* and STM1808 form the NsrR-regulon, controlled by the nitric oxide (NO·) sensing transcriptional repressor NsrR. Reactive nitrogen species (RNS) produced in the gastric lumen are an important defense strategy used by animal hosts against *Salmonella* infection (Henard and Vazquez-Torres, 2011). The NsrR regulon plays an important role in nitrosative stress resistance during infection and *S.* Typhimurium virulence in mammals (Karlinsey et al., 2012). Under biotic and abiotic stresses, plants also produces NO· that leads to a generation of reactive oxygen species (ROS) (Mur et al., 2013). Tomato cells activate a protein kinase pathway that is required for NO· generation upon perception of xylanase which is one of a number of pathogen-associated molecular patterns

(PAMPs) which are recognized by the innate immunity of plants (Lanteri et al., 2011). Therefore, it is possible that tomato plants produce NO· upon perception of *Salmonella's* PAMPs and, in turn, *Salmonella* switches on the machinery to detoxify this reactive nitrogen species. This explanation is supported by (Iniguez et al., 2005) in which flagella and T3SS of *S.* Typhimurium were recognized by *Arabidopsis*. Moreover, Melotto et al. (2006) showed that flagellin-derived peptide Flg22, lipopolysaccharide (LPS), and *E. coli* O157:H7 (another enteric pathogen that has caused produce-related foodborne illness outbreaks) induced stomatal closure in *Arabidopsis*, similar to the plant pathogen *Pseudomonas syringae* pv. tomato (*Pst*), but that stomatal closure was impaired when NO was inhibited, indicating an important role for this chemical in the presence of a pathogen. Although stomatal closure was less marked in *Salmonella* on lettuce compared to *Pst*, PAMPs could still trigger a burst of NO, which could have a direct bactericidal effect on the enteric pathogen.

Interestingly, genes encoding secreted effector proteins (*sseB, sseI,* and *sspH2*), which modulate the innate immunity of animal hosts during infection (Figueira and Holden, 2012), were up-regulated when the enteric pathogen was put on tomato roots. Of those genes, *sspH2* has been reported to enhance the Rx-dependent hypersensitive response in plants because this effector was recognized by the conserved host protein SGT1 which functions in plant disease resistance (Bhavsar et al., 2013). However, *sseA,* encoding secretion system chaperone SseA which functions in translocation of the effector proteins, was down-regulated on the same plant niche. *S.* Typhimurium mutants with loss of function in SseA showed reduction in the total amount of SseB

effector protein in cells and the export of SseB was prevented *in vitro* (Zurawski and Stein, 2003).

This study also has seen that the *spv* (*Salmonella* plasmid-associated virulence) genes, required for *Salmonella* to cause systemic disease, were up-regulated especially on shoots compared to LB culture. These genes are demonstrated as N (and P- and C-)-starvation-inducible (Nickerson and Curtiss, 1997; Spector, 1998) although a direct link between starvation-stress and virulence has not been established yet. Recently, Neumann et al. (2014) reported that the *Salmonella* effector protein SpvC, a phosphothreonine lyase, that is known for contributing to reduction in inflammatory response during intestinal phase of animal infection with lyase activity on host mitogen-activated protein kinases (Mazurkiewicz et al., 2008; Haneda et al., 2012), attenuated the induction of immunity-related genes of *Arabidopsis* when present in plant cells. The authors also showed that this effector protein interacted with and dephosphorylates activated *Arabidopsis* Mitogen-activated Protein Kinase 6 (MPK6), thereby inhibiting defense signaling (Neumann et al., 2014). Moreover, the requirement of *Salmonella* SpvC was shown by the decreased proliferation of the *ΔspvC* mutant in *Arabidopsis* plants. Thus, this study is in agreement with the previous observations.

To date, one study describing the genome-wide transcriptome of *Salmonella* (serovar Weltevreden) associating with a food plant, alfalfa sprouts has been published (Brankatschk et al., 2014). This study showed that genes encoding proteins involved in cellular attachment with curli, motility, and biofilim formation were induced in *Salmonella*, while fewer stress-responsive genes were up-regulated. The liquid nature

of this studied system could explain the induction of motility and chemotaxis. Chemotaxis gene expression was also reported as needed for *Salmonella* internalization of lettuce leaves via stomata (Kroupitski et al., 2009). By contrast, in the present study, expression of genes involved in flagellar assembly and bacterial chemotaxis were not significantly different than in LB culture, although exhibiting a marked downward trend (Fig. 6). This observation could be a caveat of our system, in which motility was greatly up-regulated in LB culture, thus downplaying the response in the plant system. Alternatively, it could indicate that the tomato host provides a less favorable environment than lettuce leaves and alfalfa sprouts, potentially as a result of fewer chemotactic cues, or via recognition of the pathogen. Kroupitski et al. (2013), however, also identified induction of *Salmonella* stress response genes when associating with post-harvest lettuce leaves using recombinase-based in vivo expression technology, RIVET. Of the induced genes, six were identified homologous to stress response proteins. Increased transcription of oxidative stress response genes was also detected in *E. coli* O157:H7 exposed to lysates of lettuce leaves (Kyle et al., 2010).

We have previously shown that populations of *S.* Newport and *S.* Typhimurium increased after 3 days on leaves of young tomato seedlings (Han and Micallef, 2014). In this study *S.* Typhimurium maintained its population density on leaves and roots of tomato for up to 11 days post inoculation. This indicates that there must be well-adapted strategies for *S.* Typhimurium to maintain cellular metabolic and energy fluxes, and in turn a stable population. The results show that chemotaxis was not invoked, and that *S.* Typhimurium is capable of thriving epiphytically for several

days. Whilst most of the metabolic pathways were down-regulated, two functional groups were found up-regulated. One group included genes involved in biosynthesis of phenylananine, tyrosine, and tryptophan (*aroF*, *trpB*, *trpC*, *trpD*, *trpE* and *tyrA*), and the other, genes involved in sulfur metabolism for energy (*asrC*, *cysD*, *cysN*, *metA*, *ttrA* and *ttrB*) (Fig. 5). Interestingly, the *trp* operon and its regulators (*trpECDBA*, *trpR*, and *trpS2*) involved in tryptophan biosynthesis appear to play a critical role in biofilm development in *S*. Typhimurium (Hamilton et al., 2009). Biofilm formation is known to enhance the capacity of pathogenic bacteria to survive stresses in the environment and during host infection. Therefore, the up-regulation of tryptophan biosynthesis genes identified in this study could explain the state of *Salmonella* on tomato, where biofilm formation is required to enhance survival. Sulfur metabolism was induced in our *Salmoenlla*-tomato system. Brankatschk et al. (2014) also reported that many genes involved in sulfur metabolism (sulphate and cysteine biosynthesis) were induced when *S. enterica* Weltevreden was grown with alfalfa sprouts. *Salmonella* may therefore be able to thrive on tomato in part through their unique ability of utilizing tetrathionate. The *ttrRSBCA* locus in *Salmonella* confers the ability to use tetrathionate as an electron acceptor in anaerobic respiration in the gut, conferring a growth advantage over other competing microbiota that are unable to utilize this compound, which only becomes available in the lumen of the inflamed gut in response to the pathogen (Winter et al., 2010). Plants produce thiosulfate (Brychkova et al., 2013), and localized stress responses of plants to pathogen invasion is the production of reactive oxygen species (ROS) at microsites in the phyllosphere and root system. The induction of *ttrA* and *ttrB* in *S*. Typhimurium

on tomato roots (Fig. 5) suggests that *S*. Typhimurium was utilizing tetrathionate in this niche, which could have originated from oxidation of thiosulfate by ROS. Whether *Salmonella* is able to use this strategy in both animals and plants, and whether this capability would allow *Salmonella* to better colonize plants over other epiphytes remains to be investigated.

Being a non-spore forming enteric pathogen, nutrient acquisition is critical for *Salmonella* to maintain the integrity of cellular metabolic pathways. Successful colonization and population size are usually limited by nutrient availability on the plant surface (Lindow and Brandl, 2003). Nutrient availability on leaves is highly spatially heterogeneous and this patchiness is a major determinant of bacterial colonization (Mercier and Lindow, 2000). The transcriptional responses of *E. coli* O157:H7 grown on intact lettuce leaves pointed to stress responses triggered by nutrient limitation, supporting the limiting nature of nutrient availability in the phyllosphere (Fink et al., 2012). In this study, similar transcriptomic patterns were observed in *S*. Typhimurium grown on tomato shoots and roots. The most noticeable apparent change in transcriptional regulation, in relation to growth to LB, occurred in genes involved in metabolite transport (Fig. 5). The bacterial phosphotransferase (PTS) system works as the center of a network regulating carbohydrate flux in the cell (Postma et al., 1993). Therefore, the down-regulation of the PTS system in *S*. Typhimurium on tomato could have resulted in the overall down-regulation in carbohydrate metabolism (Fig. 5). Moreover, in this study, bacterial ABC transporters, best known for their role in the import of essential nutrients including ions, amino acids, peptides, and sugars, as well as the export of toxic molecules

(Davidson et al., 2008), were repressed relative to LB, a nutrient rich medium. This down-regulation is probably a reflection of lower concentrations of nutrients on plant surfaces compared to LB, but could explain the attenuation of several metabolic pathways, reflected in the down-regulation of energy metabolism, nucleotide metabolism, and genetic information processing, compared to growth in LB.

Our RNA-seq data was validated using qRT-PCR of selected genes, on RNA isolated from a biologically repeated experiment. Validation was adequate for the most part, with the directionality of gene expression (up or down) being similar for most genes. Certain discrepancies however are expected, due to differences in method sensitivities, Moreover, during the qRT-PCR confirmation experiments, the portion of rRNA which comprises more than 95% of total RNA, was not removed from the samples, as opposed to most of the rRNA being depleted prior to RNA-seq library construction. This caveat might have resulted in a lowered resolution of the qRT-PCT, in turn explaining the partial agreement of the expression of certain genes with the RNA-seq data.

In this study, we investigated the response of *S*. Typhimurium LT2 to colonization on tomato shoots and roots at the transcriptomic level. We identified key signals that were down-regulated and up-regulated in the enteric pathogen upon interacting with tomato. This is the first study to examine the *Salmonella*-tomato interaction at a whole-genome transcriptional level. Our findings are broadly summarized in a schematic shown in Fig. 9. The proposed model depicts the cellular processes related to signals needed to preserve cell viability when multiple abiotic stresses in conjunction with low nutrient availability are encountered, while simultaneously

repressing unnecessary energy demands, or maintaining them at a level equivalent to growth in a nutritious medium. It is possible that biofilm formation, NO detoxification and S metabolism are crucial essential functions for the enteric pathogen to survive on plants, during transit in the environment to another animal host.

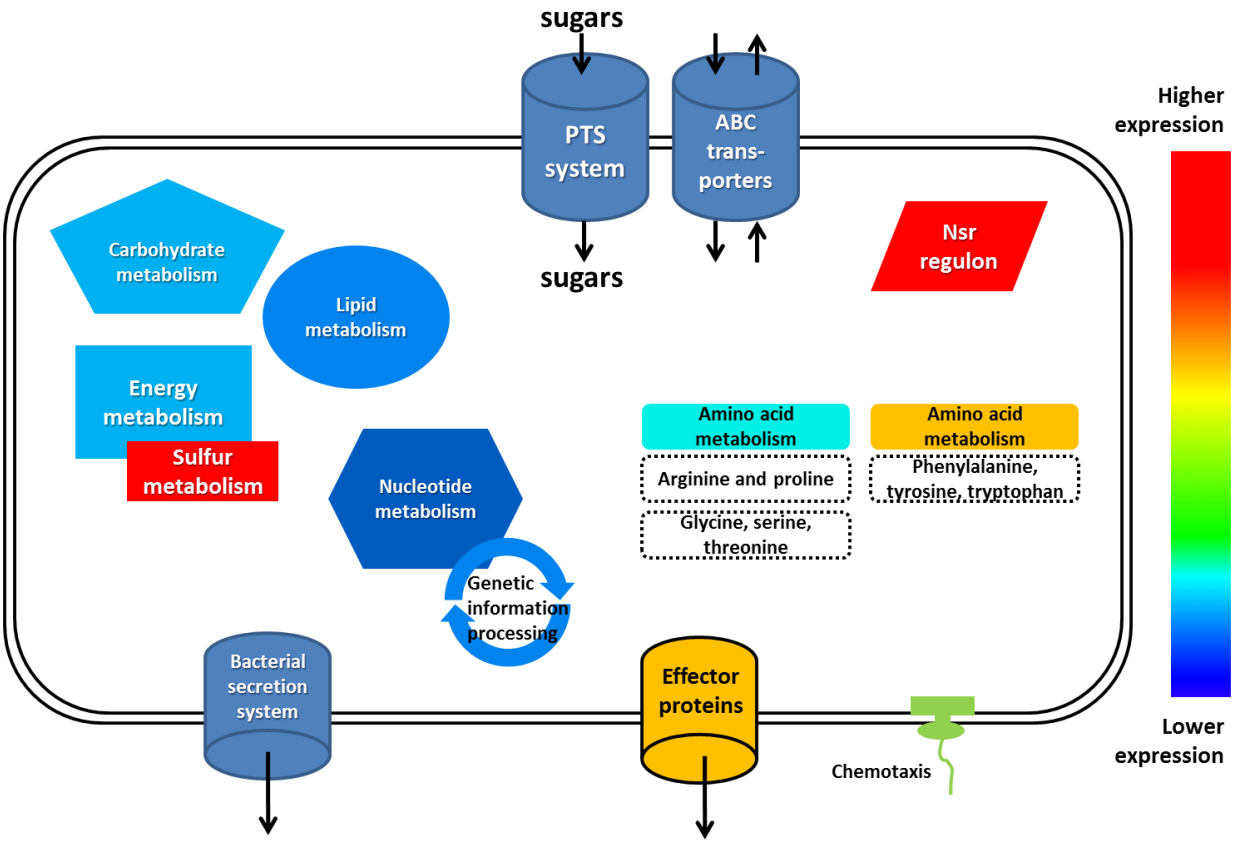


Fig. 9. Proposed model of transcriptional changes occurring during colonization of *S.* Typhimurium on tomato plants. Each symbol represents a range of genes involved in particular physiological or regulatory processes. Blue indicates decreased expression; red indicates increased expression, relative to growth in LB.

Chapter 6: Conclusions and Future Directions

Public interest in the microbial food safety of fresh fruit and vegetables has grown considerably over the past two decades. In the United States, the dissemination of the guidance for industry "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" by FDA in 1998 sparked the necessity to better understand foodborne pathogen-plant interactions. However, scientific data required to support the development of evidence-based recommendations, guidance, and policies are still not comprehensive. For instance, out of over 72,000 publications PubMed-indexed under *Salmonella*, less than 100 of them were concerning *Salmonella*-plant interactions (Brandl et al., 2013).

The results from this study indicate that the tomato plant genotype is one of the factors determining the success of *Salmonella* establishment, colonization, and persistence on various plant organs. Additionally, differences in chemical composition of metabolites leached or exuded from plant surfaces are responsible, at least in part, for the differential growth responses of *Salmonella* in the phyllosphere and root system. Here I have started to tease apart metabolite groups associated with enhanced or impaired bacterial growth. These findings can lead to other studies that are applicable to the agricultural and food industries regarding food safety. Screening natural compounds in plant exudates that inhibit or even defeat enteric pathogens completely is one example. Breeding a cultivar that is inherently less susceptible to bacterial colonization by means of exudates it releases is another. This study also shows that an *S*. Newport strain implicated in tomato outbreaks is a better fruit colonizer than *S*. Typhimurium, a laboratory strain. This finding generates more

questions regarding what bacterial traits are responsible for differences in the colonization efficiences of these two serotypes. This will improve our understanding of the mechanisms by which enteric pathogens survive outside their normal host. One of the most relevant findings from the transcriptome analysis presented in this study is that *Salmonella* may survive in the phyllosphere and root system using a specific set of genes needed to tolerate stresses. The identified differential gene expression of *Salmonella* explains that biofilm formation, nitric oxide detoxification, and sulfur metabolism could be crucial essential functions for this enteric pathogen to survive on plants, possibly during transit in the environment to another animal host. The motivation for this study was to be able to find a tomato phytochemical that inhibits the survival and persistence of *Salmonella* at the interface between the bacteria and a plant host, and consequently to analyze bacterial gene expression when being subjected to stresses established within that interface. While the survival and persistence of enteric pathogens on plants have been reported, and the effects of plant lysates on their fate have been studied, this study is the first to analyze the impact of plant-regulated exudates on bacterial fate in the phyllosphere or rhizoshpere. The standardization in collecting exudates from a living plant matrix was a challenge as I wanted to establish a protocol applicable for different tomato cultivars through different developmental stages and by different organ. Despite these difficulties, water soluble phytochemicals in exudates were collected and tested in a reproducible manner for supportive or inhibitory effects on the growth of *Salmonella*, which is described in Chapter 4. Phytochemical effects not only from those water soluble metabolites but from water insoluble or volatile compounds could be examined using

the methodology described in Chapter 3. Epiphytic growth of *Salmonella* was favored by high humidity within the studied system in an attempt to read a signal (increase or decrease of *Salmonella* populations) caused by phytochemicals exuded on the surface of plants. The methods newly developed for this study were deployed successfully to allow me to come close to finding a tomato phytochemical most influential on *Salmonella*. Unexpectedly, I found that variation in susceptibility of tomato cultivar to colonization by *Salmonella* changes as tomato plants mature due to the change in composition of metabolites in exudates. This implies that we should rather focus on tomato fruit, the edible part, rather than plant seedlings, when trying to obtain food safety data. The isolation of mRNA from *Salmonella* attaching and colonizing tomato plants was also challenging. Aside from difficulties in growing tomato plants sterilely for 6 weeks while excluding fungal contamination, no studies have ever been done to retrieve bacterial cells of enteric pathogens off of the surface of plants for an RNAseq application. Biologically relevant transcriptomic studies are impeded by the high concentration of RNA needed for reliable results. It has been shown that an inoculum level of about 8 log CFU *Salmonella* per g of sample is ideal for optimal amount and purity of RNA for down-stream applications (Sirsat et al., 2011). Since I did not want to saturate tomato plants with *Salmonella*, I had to lower the inoculum level enough to mimic a realistic situation while ensuring microbial RNA in a sample. Great amount of care was taken during rRNA depletion, purification, and RNAseq library construction with 'invisible pellets' and it seemed that no data would be collected until I actually received all the read sequences on hand. To my best knowledge, this is the first study depicting a genome-wide

transcriptome of *Salmonella* attaching and colonizing the phyllosphere and rhizosphere of tomato plants at a mature developmental stage.

Throughout this study, it becomes clear that *Salmonella* can sense subtle environmental cues brought about by the genotype or physiological state of plants and can respond with distinct patterns of gene expression. However, future work should focus on answering whether this bacterial behavior on plants results from an evolutionary adaptation to use plants as a vector to infect their host.

Appendix 1: Supplemental figures

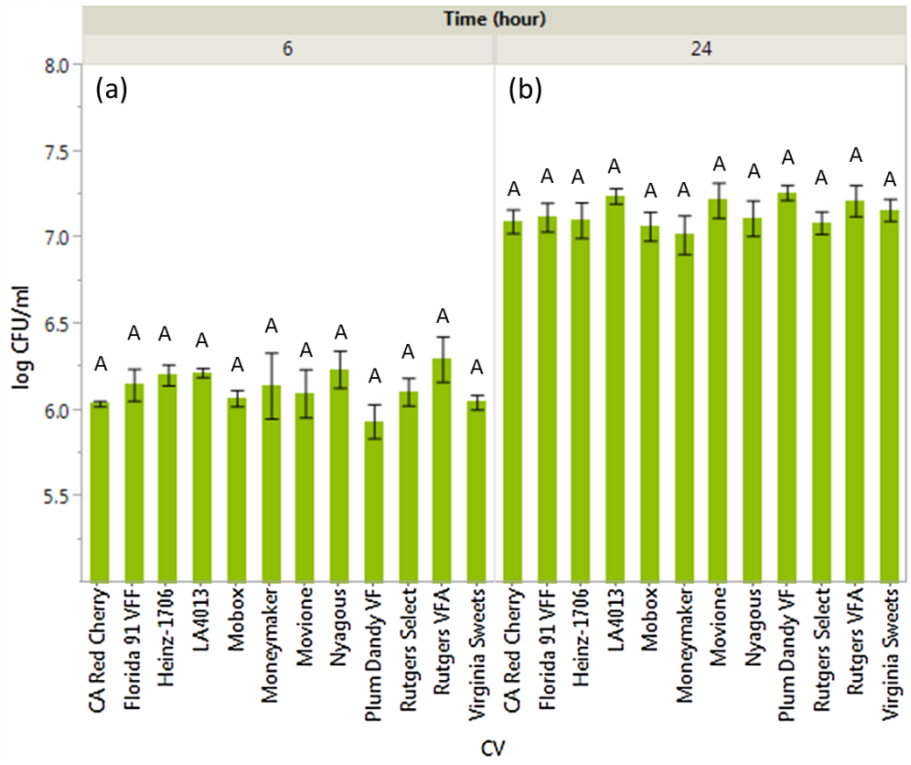


Fig. 1. Growth of *S.* Typhimurium in stem exudates; population densities measured at 6 hours (a) and 24 hours (b) post inoculation. Error bars indicate standard error of the mean; bars labeled with the same letter are not significantly different within the same time point measurement by Tukey's HSD test ($p<0.05$).

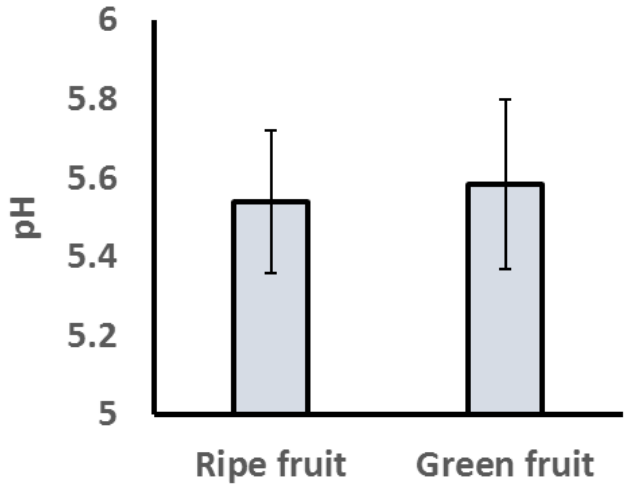


Fig. 2. pH of fruit exudates collected from cultivar ‘Nyagous’ of two different ripeness. Error bars indicate standard deviation; n=8. No significant difference by student’s t-test (p=0.65).

Appendix 2: Bacterial growth data in tomato plant exudates

Table 1. Growth of *S.* Typhimurium in 3-week old seedling shoot exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.44	0.06	5.06	0.08	6.21	0.17	7.52	0.04	8.43	0.12
Florida 91 VFF	4.49	0.05	5.10	0.04	6.52	0.06	7.49	0.07	8.31	0.03
Heinz-1706	4.41	0.05	5.26	0.06	6.56	0.02	7.60	0.04	8.54	0.04
Mobox	4.49	0.02	5.11	0.09	6.07	0.22	7.40	0.06	8.57	0.23
Moneymaker	4.30	0.00	5.11	0.05	6.28	0.04	7.35	0.06	8.58	0.05
Movione	4.51	0.02	5.07	0.08	6.06	0.14	7.24	0.09	8.38	0.13
Nyagous	4.40	0.06	5.17	0.07	6.23	0.09	7.29	0.13	8.52	0.15
Plum Dandy VF	4.46	0.00	5.33	0.05	6.40	0.03	7.56	0.01	9.74	0.18
Rutgers Select	4.62	0.03	5.20	0.02	6.40	0.03	7.33	0.06	9.87	0.02
Rutgers VFA	4.48	0.01	5.16	0.05	6.26	0.08	7.44	0.02	9.48	0.33
Virginia Sweets	4.40	0.05	5.09	0.09	6.25	0.12	7.53	0.07	8.39	0.17

Table 2. Growth of *S.* Typhimurium in 3-week old seedling root exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.68	0.09	4.89	0.10	6.16	0.15	7.07	0.18	9.15	0.35
Florida 91 VFF	4.56	0.02	5.05	0.01	6.34	0.07	7.48	0.02	9.15	0.12
Heinz-1706	4.74	0.06	4.94	0.04	6.22	0.05	7.24	0.09	9.32	0.25
Mobox	4.92	0.09	5.12	0.05	6.28	0.06	7.19	0.03	9.11	0.12
Moneymaker	4.86	0.00	4.90	0.05	5.97	0.11	6.77	0.13	9.58	0.16
Movione	4.89	0.10	5.13	0.04	6.22	0.04	7.22	0.03	8.93	0.15
Nyagous	4.78	0.06	5.12	0.05	5.99	0.03	6.91	0.08	9.13	0.22
Plum Dandy VF	5.10	0.00	5.29	0.05	6.37	0.03	7.19	0.08	9.48	0.13
Rutgers Select	4.57	0.02	5.02	0.03	6.29	0.05	7.30	0.02	8.67	0.13
Rutgers VFA	4.93	0.11	5.23	0.07	6.25	0.07	7.33	0.08	9.29	0.26
Virginia Sweets	4.76	0.05	4.98	0.05	6.18	0.05	6.74	0.15	9.10	0.19

Table 3. Growth of *S.* Typhimurium in 6-week old plant shoot exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.73	0.04	5.30	0.07	6.00	0.05	6.89	0.10	6.92	0.01
Florida 91 VFF	4.70	0.03	4.90	0.06	5.50	0.07	6.02	0.08	6.87	0.05
Heinz-1706	4.70	0.02	5.07	0.06	5.66	0.06	6.05	0.04	7.09	0.06
LA4013	4.81	0.03	5.16	0.09	5.72	0.06	6.29	0.15	7.10	0.02
Mobox	4.76	0.05	4.94	0.10	5.69	0.08	6.01	0.03	6.97	0.06
Moneymaker	4.70	0.04	5.08	0.04	5.62	0.10	6.10	0.12	6.92	0.11
Movione	4.69	0.04	4.98	0.01	5.44	0.06	6.05	0.08	7.10	0.05
Nyagous	4.73	0.03	5.21	0.07	5.77	0.05	6.35	0.03	6.98	0.06
Plum Dandy VF	4.65	0.01	4.93	0.06	5.46	0.04	5.89	0.02	6.79	0.09
Rutgers Select	4.68	0.03	4.98	0.05	5.52	0.05	6.13	0.10	7.01	0.05
Rutgers VFA	4.72	0.01	4.92	0.04	5.54	0.07	6.12	0.11	6.95	0.05
Virginia Sweets	4.76	0.04	5.22	0.04	5.81	0.05	6.38	0.10	7.17	0.07

Table 4. Growth of *S.* Typhimurium in 6-week old plant root exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.68	0.03	5.24	0.06	6.28	0.05	6.99	0.08	7.14	0.07
Florida 91 VFF	4.70	0.02	5.20	0.04	6.44	0.04	7.30	0.03	7.79	0.09
Heinz-1706	4.64	0.05	5.30	0.07	6.33	0.07	7.20	0.05	7.78	0.08
LA4013	4.62	0.03	5.37	0.02	6.28	0.06	7.23	0.10	7.46	0.04
Mobox	4.72	0.02	5.20	0.01	6.33	0.08	7.07	0.05	7.44	0.07
Moneymaker	4.71	0.03	5.25	0.06	6.22	0.05	7.14	0.03	7.65	0.09
Movione	4.61	0.02	5.16	0.05	6.21	0.07	6.98	0.13	7.79	0.10
Nyagous	4.61	0.02	5.06	0.03	6.09	0.02	7.15	0.05	7.77	0.06
Plum Dandy VF	4.65	0.03	5.40	0.01	6.36	0.05	7.31	0.11	7.40	0.11
Rutgers Select	4.58	0.07	5.07	0.04	6.18	0.10	7.17	0.05	7.61	0.07
Rutgers VFA	4.64	0.02	5.25	0.05	6.23	0.10	7.01	0.09	7.53	0.09
Virginia Sweets	4.60	0.02	5.23	0.02	6.01	0.09	6.79	0.09	7.75	0.09

Table 5. Growth of *S.* Typhimurium in 15-week old plant stem exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.72	0.04	4.82	0.05	5.45	0.05	6.04	0.02	7.10	0.07
Florida 91 VFF	4.76	0.05	5.01	0.08	5.64	0.04	6.15	0.09	7.12	0.08
Heinz-1706	4.75	0.04	4.96	0.11	5.54	0.05	6.20	0.06	7.10	0.10
LA4013	4.71	0.02	5.24	0.06	5.80	0.01	6.22	0.03	7.24	0.04
Mobox	4.76	0.02	4.84	0.08	5.42	0.03	6.07	0.05	7.07	0.08
Moneymaker	4.74	0.03	4.89	0.02	5.63	0.05	6.14	0.19	7.02	0.11
Movione	4.85	0.02	5.03	0.03	5.70	0.03	6.10	0.14	7.22	0.10
Nyagous	4.73	0.03	5.19	0.04	5.62	0.05	6.24	0.11	7.11	0.10
Plum Dandy VF	4.78	0.03	5.10	0.10	5.60	0.02	5.94	0.10	7.26	0.04
Rutgers Select	4.77	0.01	5.09	0.03	5.67	0.03	6.11	0.08	7.09	0.06
Rutgers VFA	4.75	0.03	5.10	0.05	5.70	0.07	6.30	0.13	7.22	0.09
Virginia Sweets	4.75	0.04	5.05	0.12	5.62	0.03	6.05	0.04	7.16	0.06

Table 6. Growth of *S.* Typhimurium in fruit exudates; population densities measured at 0, 2, 4, 6, and 24 hours post inoculation. SE represents standard error of the mean.

	0 h		2 h		4 h		6 h		24 h	
Cultivar	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CA Red Cherry	4.58	0.03	4.55	0.05	4.92	0.06	5.58	0.10	6.31	0.14
Florida 91 VFF	4.57	0.05	4.65	0.15	5.53	0.28	6.22	0.18	6.93	0.21
Heinz-1706	4.56	0.04	4.44	0.05	4.69	0.05	5.15	0.11	6.21	0.07
LA4013	4.55	0.05	4.74	0.14	5.59	0.34	6.13	0.25	6.96	0.25
Micro-Tom	4.62	0.04	4.75	0.02	5.32	0.04	6.33	0.20	6.82	0.11
Mobox	4.54	0.04	4.67	0.06	5.11	0.15	5.48	0.16	6.44	0.12
Moneymaker	4.54	0.04	4.69	0.06	5.31	0.10	5.83	0.12	6.68	0.11
Movione	4.56	0.04	4.58	0.02	4.93	0.07	5.68	0.20	6.26	0.12
Nyagous	4.55	0.04	4.70	0.09	5.13	0.18	5.85	0.29	6.78	0.32
Plum Dandy VF	4.56	0.03	4.43	0.05	4.58	0.06	5.21	0.15	6.14	0.09
Rutgers Select	4.55	0.05	4.76	0.07	5.27	0.10	6.03	0.13	6.82	0.14
Rutgers VFA	4.58	0.04	4.90	0.14	5.75	0.30	6.12	0.25	7.02	0.25
Virginia Sweets	4.57	0.04	4.86	0.09	5.42	0.10	6.04	0.09	6.78	0.09

Appendix 3: Metabolites in tomato plant exudates

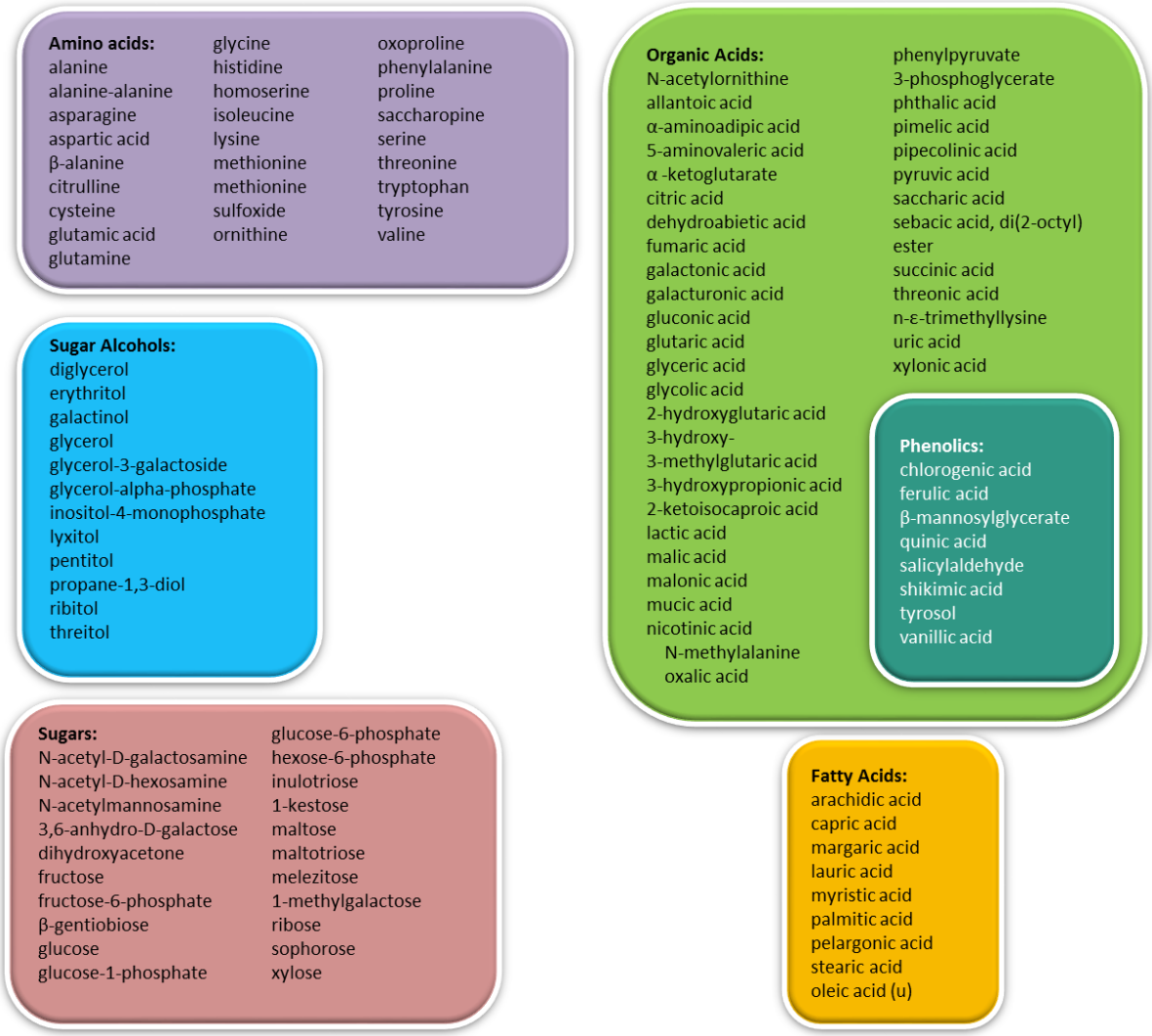


Fig. 1. Lists of metabolites identified in tomato plant exudates by GC-TOF-MS.

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