Phytopathology

SALMONELLA INTERACTIONS WITH PLANTS AND THEIR ASSOCIATED MICROBIOTA

Journal:	*Phytopathology*
Manuscript ID:	Draft
Manuscript Type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Brandl, Maria; USDA-ARS, Western Regional Research Center Cox, Clayton; University of Florida, Soil and Water Science teplitski, max; University of Florida, Soil and Water Science Department
Keywords:	Bacteriology, Ecology and epidemiology

Salmonella interactions with plants and their associated microbiota

Maria T. Brandl, Clayton E. Cox, and Max Teplitski.

First author: Produce Safety and Microbiology Research Unit, Agriculture Research Service, US Department of Agriculture, Albany, CA 94563; and second and third authors: Soil and Water Science Department, Genetics Institute, University of Florida-IFAS, Gainesville, FL

Corresponding author: M. Teplitski; E-mail address: maxtep@ufl.edu

ABSTRACT

An increase in the incidence of gastroenteritis outbreaks linked to the consumption of foods of plant origin ignited public concern and scientific interest in understanding interactions of human enteric pathogens with plants. Enteric disease caused by non-typhoidal *Salmonella* is a major public health burden, with the number of cases of illness linked to fresh produce, spices, and nuts surpassing those linked to foods of animal origin. Mounting evidence supports the hypothesis that colonization of plants is an important part of the life cycle of this human pathogen. Although plant responses to human pathogens are distinct from the more specific responses to phytopathogens, plants appear to recognize *Salmonella*, likely by detecting conserved microbial patterns, which subsequently activates basal defenses. Numerous *Salmonella* genes have been identified as playing a role in its colonization of plant surfaces and tissues, and in its various interactions with other members of the phyto-microbial community. Importantly, *Salmonella* utilizes diverse and overlapping strategies to interact with plants and their microflora, and to successfully colonize its vertebrate hosts. This review provides insight into the complex behavior of *Salmonella* on plants and the apparent remarkable adaptation of this human pathogen to a potentially secondary host.

Keywords: human pathogen, foodborne pathogen, produce, fruit, vegetable, phyllosphere, rhizosphere, enteric illness, outbreak, microbe-microbe interactions

Glossary:
Apoplast: the space outside of the plant cell membrane and between cells where water diffuses freely
Biotrophic: a plant pathogen that obtains nutrients from living cells
Curli: bacterial thin aggregative fimbriae formed by amyloid fibrils; produced by various members of the Enterobacteriaceae.
Enteric illness: human illness caused by ingestion of food that is contaminated with a pathogenic microbe or a chemical
HEP-2 cells: eukaryotic cell line derived from a carcinoma; used in studies that investigate pathogen invasion of host cells
Infectious dose: minimum number of pathogen cells required to cause disease in a host
Lumen: inner open space of an organ e.g. of the intestine
Macrophage: white blood cell that phagocytoses; act in both innate and adaptive immunity in vertebrates
MAMP: microbe-associated molecular pattern, a conserved microbial surface component recognized by the plant innate immunity
Nontyphoidal salmonellae: cause of most salmonellosis cases; include most pathogenic *Salmonella* serovars, except Typhi and Paratyphi, which cause typhoid fever
***Salmonella* fimbriae:** typically proteinaceuos appendages with multiple functions. In addition to aggregative fimbriae (structures homologous to *E. coli* curli) encoded by the *agf* genes, *Salmonella* encode *lpf, sef, pef* and *fim* fimbriae, not found in close-related lineages of enterics.
Soft rot: macerated plant tissue due to the degradation of the plant cell wall via pectinolytic activity of various bacterial plant pathogens
SPI: *Salmonella* pathogenicity island, a chromosomal cluster of genes involved in *Salmonella* virulence. The acquisition of SPI-1 separated *Salmonella* from the common ancestor with *E. coli*. Sub-species of *Salmonella* differ in the number of SPI's, some containing up to five SPI's.
SPI-1: *Salmonella* pathogenicity island 1, harbors genes encoding a type 3 secretion system required for invasion of epithelial and macrophage cells. All subspecies of *Salmonella* carry SPI-1.
SPI-2: *Salmonella* pathogenicity island 2, involved in replication in host cells, codes for a type 3 secretion system
SPI-3: *Salmonella* pathogenicity island 2, encodes genes with various functions, including *mgtCB* which are required for survival in host cells and virulence

MAIN TEXT

INTRODUCTION. Sallmonellosis caused by non-typhoidal salmonellae is the largest cause of foodborne gastroenteritis. Non-typhoidal strains of *Salmonella* are estimated to infect over 1 million people per year in the United States alone (10). The public health burden is significant, accounting for several billion dollars in medical costs and ~400 deaths per year (10).

Salmonella is the major causal agent of foodborne outbreaks for which etiological agents have been determined (26) and is ranked as the most burdensome foodborne pathogen in the US (10). The link between salmonellosis and foods of animal origin is well known and has received considerable regulatory attention. Although the number of illness cases related to the consumption of meats has declined in recent years, the overall salmonellosis outbreak rate has remained steady due to increased risk from non-traditional sources of the pathogen. These include fresh fruit and vegetables, spices, and nuts, and underscores the importance of plants as potential sources of the pathogen (10, 26, 57). From 1998 to 2007 produce was linked to more outbreaks than either beef, pork or poultry with fresh produce potentially being the riskiest food (10, 26, 57). In addition to the significant public health burden, outbreaks of gastroenteritis linked to the consumption of produce significantly reduce demand and impact the produce industry economically (77).

Despite the magnitude of the problem, relatively little is known about traits and mechanisms that allow *Salmonella* to persist outside of vertebrate animals. This paucity of information is stark: over 72,000 studies in Pubmed are indexed under "*Salmonella*", with less than 100 of them regarding *Salmonella*-plant interactions. The majority of these publications result from studies over the last two decades, highlighting a growing interest in understanding behavior of *Salmonella* outside of its animal hosts.

***Salmonella* persistence on plants.** Plants may be valuable alternate hosts for enteric pathogens by providing a refuge after excretion from the animal intestinal tract. The ability to colonize edible plants may be an effective survival strategy for *Salmonella* as it provides a direct route from its excretion in the environment back to its numerous herbivorous and omnivorous hosts (Lynch et al Epidemiol Infect 2009) (Fig. 1). Field studies revealed that *Salmonella* Typhimurium was capable of persisting in manure-amended soils for up to 231 days, and that

the pathogen was detected on above-ground parts of lettuce and parsley, and on carrots and radishes grown in these amended soils for 2-3 months (40, 41). In addition to its transfer from manure to plants, *Salmonella* deposited onto lettuce and parsley seedlings via irrigation water survived on plants until harvest (40, 41), thus corroborating the results of earlier field studies in which *Salmonella* Typhi was shown to survive on lettuce from the seedling stage to maturity after contamination with overhead water (29).

A three-step food-chain experiment by Semenov *et al.* (2010) further supports the hypothesis that plants can serve as alternative hosts for human enteric pathogens. The authors demonstrated that *Salmonella* and *E. coli* O157:H7 colonized seedlings sown into soil amended with pathogen-containing manure; cows, mice and snails who ate these seedlings shed the pathogens in their excrements; and the shed pathogens persisted in manure or soil for at least two weeks (73). Consistent with the studies of Semenov et al. (2010), Schikora *et al*. (2011) demonstrated that *Salmonella* Typhimurium inoculated into and recovered from *Arabidopsis* leaf homogenates (but not inside whole leaves) was as virulent as the inoculum grown in LB. *Salmonella* cells from leaf homogenates invaded the spleen and caused mortality in mice (72). Collectively, the above observations and the increase in salmonellosis outbreaks linked to the consumption of produce provide evidence that plant colonization by *Salmonella* can be part of its life cycle.

Early studies on the fitness of *Salmonella* in the phyllosphere revealed that this pathogen has the ability to multiply and form microcolonies on leaves, although its population sizes are often exceeded by those of plant-associated bacterial species (14). Comparative studies on lettuce leaves of different ages showed that *Salmonella* and *E. coli* O157:H7 achieved 10-fold greater population sizes on young leaves (heart) than on the older middle leaves. Given that middle leaf exudates contained less total N, but not less total C, than those of young leaves and that lower growth of the pathogens on middle leaves could be complemented by addition of N, but not of C, to the inoculum suspension, colonization of middle leaves may have been limited

by low N availability (19). Thus, it is likely that the lower fitness of human pathogens compared with that of plant-associated bacteria is partly rooted in the low abundance and restricted range of nutrients that they can assimilate on plant surfaces. However, plant surfaces are not homogenous and contain various microsites that represent oases of available nutrients (54) and which may support multiplication of human pathogens after contamination events. Because these sites are also attractive to plant-associated microbes, cells of enteric pathogens likely must interact and compete with indigenous microbial communities in order to occupy such preferred sites (17).

Laboratory studies demonstrated that *Salmonella* is capable of colonizing plants through multiple routes including wetting of leaves, contaminated soil, roots, seeds or flowers (14, 23, 36). For colonizing bacteria, aerial plant surfaces are a challenging environment, presenting stresses such as desiccation, UV irradiation, and starvation, with only patchy nutrient availability (38, 55). Human pathogens on leaves have been shown to preferentially move towards stomata and colonize the vein areas, the bases of trichomes and lesions or other surface irregularities (9, 14, 19, 47) (2, 18, 48, 49), which may provide shelter from these stresses and increased nutrient and water availability. Hence, these microsites may offer physico-chemical conditions that are conducive not only to survival but also may be exploited by *Salmonella* for growth.

***Salmonella* genes involved in plant colonization.** If the hypothesis that plants can serve as alternate hosts for enteric pathogens is correct, *Salmonella* should not behave solely as a transient immigrant with a restricted residence time in the plant habitat, but should harbor traits allowing for its interaction with plants and their colonization. Thus, it should be possible to find genotypic and phenotypic evidence of *Salmonella* adaptation to its life in and on plants.

Screens of *Salmonella* mutant libraries for those unable to attach to alfalfa sprouts or colonize tomato fruits identified 20 and 55 unique non-overlapping genes, respectively (4, 60). Even though screens in these studies were not saturating, the predicted functions of the *Salmonella* genes involved in plant colonization are distinct from those typically used by this pathogen for the infection of animal models, and were also distinct from those used by phytopathogens in plants (77). It is of note that the *Salmonella* virulence genes located on Pathogenicity Islands (SPIs) appear to have different roles during interactions with different plant species: in tomatoes, SPI mutants were as fit as the wild type (60) whereas in alfalfa and lettuce, SPI mutants have phenotypes that are distinct from those of the wild type strain (27, 39, 72). These differences in the roles for the SPI genes could be due to the differences in the interactions of *Salmonella* with plant vegetative and reproductive organs, which were sampled in these studies.

The *rdar phenotype in Salmonella colonization of plants.* The involvement of the *rdar*-like phenotype (Text Box 1) in the persistence in and on plants seems to be conserved in human enteric pathogens. Aggregative fimbriae, encoded by the *agf* operon, contribute to biofilm formation on HEP-2 cells and in the chicken intestine (53). *Salmonella agfB* and *rpoS* mutants (defective in the production and regulation of aggregative fimbriae, respectively) were deficient in initial attachment to the root surface of sprouts (4). *agf* genes also played a significant role in the colonization of the parsley phyllosphere following irrigation with *Salmonella*-contaminated water (52). Laboratory studies with isogenic non-*rdar Salmonella*

Text Box 1. *Rdar* phenotype Under laboratory conditions on agar surfaces, most wild type strains of *Salmonella* form rough and dry colonies, which can absorb Congo Red dye. This phenotype is known as "*rdar".* It requires multiple regulatory inputs, which converge to control the production of cellulose and aggregative fimbriae (curli) (67). Mutations in any of the corresponding genes result in a variety of non-*rdar* phenotypes (e.g. *bdar, saw, pdar*). Interestingly, non-*rdar* mutants are more common in collections of *Salmonella* isolates recovered from produce-related outbreaks than in those of clinical strains (or those recovered from meats) (75, 92) (Fig. 2).

mutants suggest that their fitness within tomato fruits is significantly increased compared with *rdar* strains (92). *E. coli* O157:H7 variants lacking curli fimbriae are readily recovered from *E. coli* O157:H7 populations associated with produce outbreaks (21). Carter *et al.* (2011) reported that *E. coli* O157:H7 curli-positive variants (equivalent to *Salmonella rdar*) have greater survival under low nutrient stress conditions than their curli-negative variants (equivalent to non-*rdar* *Salmonella*), whereas the opposite trend is observed for acid stress (21). Increased acid stress-resistance in curli-negative variants of *E. coli* O157:H7 is due to the presence of a functional RcsB, which positively regulates acid resistance in *E. coli* but negatively regulates curli production (21). Thus, while the molecular and physiological basis of the increased competitive fitness of *Salmonella* non-*rdar* mutants over their *rdar* strain in tomato fruit is not clear, it is possible that like in *E. coli* O157:H7, the selection for the non-*rdar* mutant cells over the wild-type cells results from enhanced tolerance of the non-*rdar* strain to acid stress in tomato fruit tissue and from a lack of nutrient limitation, which would favor the *rdar*-genotype.

Cellulose production contributes to the *rdar* phenotype along with aggregative fimbria (see Text Box 1) and is produced at different levels among *Salmonella* strains recovered from outbreaks related to fruits and vegetables (66, 92). This surface polymer, which was first identified as an important host plant attachment factor in *Agrobacterium tumefaciens* (58), and later as a fitness determinant in *Pseudomonas fluorescens* in the sugar beet rhizosphere and phyllosphere (32), also plays a role in the binding of *Salmonella* and *E. coli* to alfalfa sprouts (5, 59). In this Focus Issue, Kroupitski *et al*. (2013) report that *Salmonella* mutants in *bcsA*, *misL, and yidR,* encoding a cellulose synthase catalytic subunit, an adhesin of the autotransporter family expressed from *Salmonella* Pathogenicity Island-3, and a putative ATP/GTP-binding protein, were impaired in attachment to and persistence on lettuce leaves stored at cold temperatures (50). It is noteworthy that MisL also effects binding of *Salmonella* to fibronectin in animal hosts (28). Hence, *Salmonella* appears to use some of its virulence strategies to interact with multiple

hosts, and also relies on factors that are commonly used in phytobacteria for attachment to plants.

Motility genes. Motility factors, such as flagella, play a role in the survival of *Salmonella* on plants. Flagella (but not those involved in chemotaxis) were required for attachment of *Salmonella* serovar Typhimurium to lettuce leaves and serovar Senftenberg to basil leaves, and mutations affecting *Salmonella* motility and chemotaxis significantly inhibited its penetration into stomata (11, 47). This is consistent with the observation that *E. coli* O157:H7 genes involved in motility and chemotaxis were strongly upregulated within the first 15 minutes of exposure to lettuce leaf lysates (51). *Salmonella* also showed chemotaxis toward lettuce root exudates (44) and movement of the pathogen up the xylem in *A. thaliana* roots was eliminated and invasion decreased in flagella- and motility-minus mutants (23). However, within red ripe tomatoes, mutations in neither the *Salmonella flhDC* (master regulator of the flagellar regulon), nor *fliF* (resulting in a non-flagellated mutant with a functional motor) had an effect on competitive fitness (60). Additionally, *fljB* and the *fli* and *flg* operons, which code for flagellar synthesis in *Salmonella* were downregulated during its colonization of soft rot lesions caused by *D. dadantii* on cilantro and lettuce (34).. Therefore, motility and chemotaxis are likely to be required during the early stages of the interactions of these enteric pathogens with plants, but not once they gain entry into plant tissues where nutrients may be plentiful. Flagella may also function as microbial-associated molecular patterns (MAMPs) in induction of plant defenses since non-flagellated mutants of *Salmonella* had a reduced endophytic fitness in alfalfa roots (39).

Salmonella genes STM0278 and STM0650, which are involved in multicellular surface spreading ("swarming"), were required for colonization of seedling surfaces (8). While it is not known how these genes affect swarming, it is tempting to speculate that swarming has other roles in addition to locomotion toward the preferred colonization sites in the rhizosphere (27, 39). Differentiation of *Salmonella* into multicellular surface swarms is associated with global physiological changes (87), including increased resistance to antibiotics mediated by the *cysB* gene (82, 83). Interestingly, *cysB* was differentially regulated inside tomatoes of different varieties, with the strongest expression of *cysB* in tomatoes of cv.Hawaii 7997, which is resistant to certain races of *Ralstonia solanacearum* (60). It is tempting to surmise that this enhanced resistance to the phytopathogen is at least partly mediated by plant basal defense antimicrobials that may have upregulated *cysB*.

Text Box 2. *In planta* monitoring of *Salmonella* gene regulation: technological considerations. To better understand how *Salmonella* interacts with plants and to test whether its persistence in plants is a co-evolved part of its lifecycle, it will be important to define the factors that allow *Salmonella* to colonize its various animal and plant hosts. There is a need for sensitive tools to define *Salmonella* gene regulation *in planta* and its responses to specific plant metabolites. While mutant screens are ideally suited for the identification of genes required for a specific step in the interactions of enterics with plants (e.g. attachment to surfaces, (6)), they will likely miss more complex phenotypes, in which multiple inputs are involved in modulating a behavior. Various promoter-probe screens are better suited for the identification of the genes that are differentially regulated during attachment or persistence within plants. For example, a differential fluorescence induction (DFI) screen combined with FACS technology led to the identification of ~ 50 unique predicted fragments that induced the differential fluorescence of the reporter (60). The differentially regulated promoters functions include *Salmonella* metabolism within plants and its ability to recognize specific plant metabolites. Single mutants in the genes corresponding to the promoters identified with DFI-FACS had no fitness defect in plants, consistent with the bias of DFI screens (60).

The role of plant genotype in interactions with *Salmonella*. While scientific consensus on the issue of plant-associated gene regulation in human enteric pathogens is beginning to emerge, the role of plant genotype in *Salmonella*-plant interactions remains significantly less understood. Several research groups demonstrated that the outcomes of interactions with *Salmonella* depend on the plant species and genotype (6, 9, 42, 44, 60). Indeed, internalization

of *Salmonella* into plant tissues varies greatly not only among plant species (42), but crop colonization also differs among cultivars of a given species (6, 9, 44). There was an approximately 100-fold difference in the phyllosphere populations of *Salmonella* on four tomato varieties, with *Solanum pimpinellifolium* variety WVa700 supporting the lowest number of bacteria (9). Interestingly, WVa700 was also significantly less susceptible to bacterial speck caused by *P. syringae* pv tomato (9). Similarly, the plant genotype has an important role in the proliferation of *E. coli* O157:H7 in the lettuce phyllosphere (63). These observations suggest that either specific genetic factors pertaining to the plant response to microbial colonization drive the outcome of the interaction of enteric pathogens with plants, or that differences in physicochemical properties, such as availability of certain nutrients or surface morphology, associated with different crop genotypes impact proliferation of *Salmonella* and *E. coli* on and inside plants. These discoveries point to the potential for selection of plant genotypes with enhanced immunity to help control or reduce contamination with enteric pathogens, although the economic feasibility of breeding for resistance to these contaminants is not yet clear (77). It remains unknown whether there is a correlation between plant basal immune responses to phytopathogens and to human pathogens. Such a correlation would provide an opportunity to integrate breeding for increased basal resistance of crops to both plant and human enteric pathogens.

Evidence of *Salmonella* recognition by plants. Once *Salmonella* has gained entry into plant tissue, such as the leaf mesophyll, its presence in the plant apoplast may trigger sophisticated plant defenses aimed at inhibiting microbial multiplication and potential invasion by plant pathogens. There is increasing evidence that plants respond to *Salmonella* via basal defense pathways. In support of this hypothesis, transcriptome analysis of *A. thaliana* leaves infiltrated with *E. coli* O157:H7 revealed that the human pathogen upregulated PAMP-inducible genes (81). Suppression of plant defense functions relies partly on Type 3 Secretion System

(TTSS). Endophytic colonization of *Medicago* spp. roots by *Salmonella* was enhanced in flagella-minus and SPI TTSS-minus mutants indicating that when present, these bacterial surface components may be perceived by the plant, thereby inhibiting *Salmonella* colonization via activation of plant innate immunity (39). Shirron and Yaron (2011) suggested that an increase in the oxidative burst of tobacco protoplasts during co-incubation with a *Salmonella invA* mutant (defective in SPI-1 TTSS) resulted from a lack of suppression of the tobacco defense response (74). In a similar fashion, *Salmonella* mutants in *invA*, *prgH*, *ssaV* and *ssaJ*, all of which are defective in SPI-1 or SPI-2 TTSS structures, showed reduced colonization of *A. thaliana* leaves compared with the wild-type, possibly due to a lack of plant defense suppression (72). Recently, it was reported that SseF, a TTSS effector of *S. enterica* can induce the hyper-sensitive response after transfer into tobacco by *Agrobacterium tumefaciens* or *Xanthomonas campestris* pv. *vesicatoria,* which may indicate a non-host response by the plant basal defense (84).

The O antigen of *Salmonella* Enteritidis is implicated in attachment and colonization of alfalfa sprouts (5). Comparative studies in *A. thaliana* showed that the O antigen also plays a role in eliciting a plant response since *Salmonella* serovars expressing this antigen (e.g. Senftenberg) caused leaf chlorosis and wilting; on the contrary serovars of a different serogroup did not induce such symptoms despite considerable colonization by all of the tested serovars after their infiltration into the leaves (12). Therefore, plants may sense the presence of human pathogens with pathways additional to those involved in recognition of common plant-associated microbes.

Plant responses to *Salmonella*. Although *Salmonella* can multiply in the apoplast and was observed intracellulary in *Arabidopsis thaliana* protoplasts and in tobacco cultured cells (71, 74), its ability to infect intact cells of whole living plants has not been demonstrated. However, as implied above, there is increasing evidence that plants have the ability to recognize enteric pathogens, including their MAMPs (microbe-associated molecular patterns) with basal defense

signaling pathways. A defense-related PR1 protein was upregulated after inoculation with *Salmonella* in both *A. thaliana* and lettuce (39, 44). Salicylic acid (SA)-dependent and -independent plant defenses were triggered by flagella and components of the type 3 secretion system (39). In this Focus Issue, Roy *et al*. (2013) report that *E. coli* O157:H7 induces greater levels of expression of the plant defense gene PR1 in *A. thaliana* leaves than *Salmonella* (68). They additionally corroborate previous findings by Kroupitski *et al*. (2009) that *Salmonella* triggers weak stomatal closure in lettuce and provide evidence of a stronger stomatal immunity against *E. coli* O157:H7 in both lettuce and *A. thaliana* (48). This weaker immune response to *Salmonella* compared with that to *E. coli* O157:H7 may explain their finding that *Salmonella* has a greater ability to colonize the leaf apoplast.

Exposure of *A. thaliana* to *S.* Typhimurium 14028 and *E. coli* elicited measurable, and temporally distinct transcriptomic responses in the plant. In total, 114 plant genes (~10% of those activated in response to *Salmonella*) were activated in response to *Salmonella* at 2 and 24 hrs post-challenge with *Salmonella* (72). 160 *A. thaliana* genes were commonly up-regulated in response to *Salmonella, E. coli* K12 and *P. syringae,* however, the magnitude of specific responses to *Salmonella* or *E. coli* was significantly (50-100x) less than to *P. syringae* (72). In another study, inoculation of *A. thaliana* with *E. coli* O157:H7 elicited responses that are distinct from those elicited by the plant pathogen *Pseudomonas syringae* pv. tomato DC3000, but similar to those elicited by its attenuated mutants (81). The latter included genes belonging to hormone and stress response pathways with two exceptions: genes encoding a jasmonic acid methyl transferase and a putative anthocyanidin synthase, which were up-regulated only in response to TTSS mutants of DC3000 (81). These observations suggest that plants recognize and respond to enteric pathogens as “general” endophytes, and the responses mounted by plants in response to phytopathogens or symbionts are distinct from those elicited by these human enteric pathogens.

The proliferation of *Salmonella* and *E. coli* O157:H7 in plant tissues is not generally associated with obvious signs of plant defense responses. However, root inoculation of lettuce with 10^5 cells of *Salmonella* Dublin stunted growth of the seedlings, and led to a modest reduction in plant biomass upon extended cultivation (>12 days) (44). In *Arabidopsis*, immersion of seedlings into a dense suspension of *Salmonella* or infiltration of leaves with the pathogen can elicit chlorosis, wilting, or tissue necrosis (12, 71). Infiltration of the wild type *Salmonella* into *Arabidopsis* leaves elicited chlorosis to the same extent as the infiltration of $MgCl_2$ solution, however lesions elicited by *Salmonella* SPI-1 and SPI-2 mutants were approximately twice as large as the controls (72). The appearance of plant disease symptoms in *Arabidopsis* was associated with the ability of *Salmonella* to overcome jasmonate-mediated plant defenses (71) whereas studies by Iniguez et al (2005) implicated salicylic acid-induced defense pathways in the response of *Arabidopsis* to *Salmonella* (39). These findings suggest that the human pathogen is recognized by plants and that general host defenses are induced, although the physiological consequences of these defenses are not consistent from one study to another and need to be better understood.

Interactions of *Salmonella* with phytobacteria.

In animal studies, the ability of *Salmonella* to colonize the intestine of animals is greatly dependent on its success in becoming established within the host gut microflora, either by manipulating the host's physiology or by utilizing nutrients that are not used efficiently by the native gut microbes (80, 90). Several recent studies have also explored potential mechanisms used by *Salmonella* to interact with members of the native plant-associated microflora.

The importance of phytobacteria in the persistence of human enteric pathogens on plants first came to light from supermarket produce surveys that demonstrated that 60% of produce showing symptoms of soft rot also harbored presumptive *Salmonella* (88). Later laboratory

studies revealed that plant tissue macerated by pectinolytic pathogens such as *Dickeya dadantii* (*Erwinia chrysanthemi*) and *Pectobacterium carotovorum*, promoted growth of *S.* Typhimurium and *E. coli* O157:H7 to population densities approximately 10 times greater levels than on healthy plants; the sudden increases in proliferation of the human pathogens coincided with the appearance of soft rot symptoms (18, 34, 61, 91). Transcriptomic studies by Goudeau *et al*. (2013) revealed that *Salmonella* cells colonizing lettuce and cilantro leaf soft rot lesions caused by *D. dadantii* utilize a broad range of nutrients made available through the pectinolytic activity of the plant pathogen (34). These include fucose and rhamnose, which are substrates for the catabolism of propanediol, and ethanolamine, which originates from the plant cell membrane, both of which serve as carbon sources under anaerobic conditions (34). Propanediol utilization is required for *Salmonella* replication in macrophages and colonization of the chicken lumen (37, 45). Ethanolamine utilization confers a competitive advantage onto *S*almonella in the lumen of the inflamed intestine in the mouse colitis model (79). Commonalities between soft rot lesions and the host intestine such as anaerobic conditions and nutritional resources indicate an important overlap in ecological niche and may explain the adaptation of *Salmonella* to macerated leaf tissue (34).

Biotrophic plant pathogens, like *P. syringae* and *Xanthomonas campestris* were also shown to promote growth or survival of *Salmonella* and enterohaemorrhagic *E. coli* on plants (2, 3, 7). Formation of lesions on leaves by both these phytopathogens was associated with an increase in availability of total sugars, specifically, innositol and sucrose (3). While it is tempting to speculate that the increased leakage of these compounds favors proliferation of the human pathogens at the lesion sites, other substrates and factors may also be involved since E. coli O157:H7 is unable to utilize innositol, and most *Salmonella* serovars are unable to utilize sucrose. Furthermore, the increase in the availability of these carbon sources in plant lesions caused by *X. campestris* did not account fully for the increased proliferation of *Salmonella* and

EHEC in the lesions caused by either *X. campestris* or *Pseudomonas syringae* (3). An increase in growth similar to that observed in response to the biotrophic phytopathogens was observed on lettuce leaves that were mechanically damaged or showed symptoms of tip burn (dry lesions on leaf margins resulting from a physiological disorder) (2, 18).

Phyllosphere bacterial communities are diverse, both functionally and structurally. In addition to erwinias, xanthomonads and pseudomonads, which are ubiquitous on leaf surfaces, *Salmonella* may reside with closely related coliforms that are frequently present on plants, and more generally with β- and α- proteobacteria, Firmicutes, bacteroidetes and Actinobacteria (56, 64). Metagenomic studies revealed that the decreased abundance in ready-to-use carbon and ammonium in a biofilm composed of spinach epiphytes likely resulted in increased competition of the enteric pathogen *E. coli* O157:H7 with other spinach leaf microbes capable of converting unavailable C and N to their bio-available forms (22). Competition for nutrients with members of the Enterobacteriaceae appeared to significantly reduce the fitness of *Salmonella* and *E. coli* O157:H7 in plant-associated ecological niches (23, 24, 56). In contrast, the presence of a member of the *Burkholderiales* that utilizes different carbon sources than *Salmonella* does, modestly increased proliferation of E. coli O157:H7 in the lettuce phyllosphere (24). A similar trend was observed with other phytobacteria that stimulated growth of *E. coli* O157:H7 *in vitro* and in *planta* (56). In leaf tissue macerated by *D. dadantii*, a phytopathogen belonging to the Enterobacteriaceae, *Salmonella* underwent high growth rates and its populations sizes were highly correlated with those of the soft rot pathogen throughout disease development (34). Goudeqau et al. suggested that this apparent lack of competition with the plant pathogen stems from the extensive activity of the *Salmonella* propanediol catabolitic pathway, along with the synthesis of its co-factor, cobalamin, which are both absent in *D. dadantii* (34). Thus, although *D. dadantii* makes the necessary substrates for propanediol synthesis and catabolism available to *Salmonella* through pectinolysis, the plant pathogen itself utilizes the oligogalacturonides

released from the plant cell wall, thereby creating a nutritional environment with resources partitioned for both bacterial species.

Competition for nutrients is unlikely to be the sole mechanism by which enteric pathogens can be excluded from, or become minor members of, plant-associated bacterial communities (56). On plant surfaces, they may be exposed to phages and antibiotic-producing phytobacteria. For example, a strain of *Pseudomonas syringae* (with previously demonstrated fungicidal activities) reduced growth of *E. coli* O157:H7 on wounded apples by 10-1,000-fold (43). These discoveries led to experiments on biological control of human enteric pathogens in produce, such as those by Fett (2006) who showed that a well-characterized biocontrol strain of *P. fluorescens* (2-79) effectively reduced *Salmonella* populations on alfalfa sprouts (30). Likewise, bacteriophages can considerably reduce the contamination of various produce with enteric pathogens (76). This suggests that phages and known biocontrol bacteria may be useful as potential tools for controlling enteric pathogens throughout the produce production cycle. It is important however, to consider that zero tolerance for most human pathogens on fresh fruit and vegetables implies that even the most effective biocontrol agent would need to be integrated as one of several hurdle technologies in a general control strategy.

Besides mechanisms of metabolic cooperation or competition between phytobacteria and *Salmonella*, cell-to-cell signaling in multi-species microbial consortia on plants may also occur. The contribution of signaling via quorum sensing circuits mediated by either *N*-acyl homoserine lactones (AHL) or the autoinducer-2 (AI-2) signal to the behavior of of *Salmonella* in plant-associated bacterial communities has been tested. Even though the *Salmonella* AHL receptor encoded by *sdiA* was involved in the responses of this bacterium to AHLs from phytobacteria (see Text Box 3), *in vivo* expression technology and fitness studies conclusively demonstrated the lack of the role for SdiA and its regulon in interactions with phytobacteria *in planta* (61).

Despite the fact that the *Salmonella luxS* gene was expressed during its invasion of a soft rot, AI-2-based signaling in *Salmonella* did not appear to have an important role during its interactions with the plant pathogen *P. carotovorum* on tomato fruit, as demonstrated by Cox *et al* (2013) in this Focus Issue (25).

Salmonella has the ability to form single- and mixed-species aggregates in the phyllosphere (14, 15). As reported by Poza-Carrion *et al*. in this Focus Issue, aggregates formed by common epiphytes affect the fitness of the human pathogen in the phyllosphere. *Salmonella* cells that landed in pre-existing aggregates of *P. syringae, P. fluorescens*, and two *Erwinia* species had a greater probability of surviving dry conditions on lettuce and cilantro leaves than as solitary cells (62). These observations suggest that human pathogens may find refuge not only in particular physical microsites on plants but also in microbial conglomerates where protection from adverse conditions outweighs potential competition and antibiosis from other plant colonists.

Text Box 3. Peculiarities of *Salmonella* quorum sensing. Similarly to most Gram-negative bacteria, *Salmonella* can detect certain N-acyl homoserine lactones (AHL) and autoinducer-2. Exceptionally, the *Salmonella* AHL receptor, SdiA, does not have a cognate AHL synthase, nor does *Salmonella* (or its close relatives *E. coli, Enterobacter, Citrobacter, Chronobacter,* and *Klebsiella*) produce AHLs (1). Phylogenetic analyses revealed that SdiA likely originated from the *Pseudomonas* RhlR, which was horizontally acquired as the *rhlRrhlI* cluster by the common progenitor of enterics, including *Salmonella, Erwinia* and *Pantoea*. The *rhlRrhlI* cluster further evolved to *expRexpI* (*phzRphzI*) within this common progenitor, while *Salmonella*, *E. coli, Enterobacter, Citrobacter, Chronobacter,* and *Klebsiella* lost the AHL synthase gene (69), but retained the AHL receptor, SdiA. In all these organisms, SdiA is encoded upstream of the *gacA* ortholog. In *Salmonella*, the ability of SdiA to regulate the downstream genes in the presence of AHLs is temperature-dependent. Unlike phytopathogens, where AHL-mediated QS controls a number of genes involved in virulence, SdiA upregulates less than a dozen genes whose functions are currently unknown (1).

Salmonella possesses a second potential signaling system based on the AI-2 molecule produced via the synthase LuxS. *Salmonella* receives the signal via the *lsrACDBFG* operon, an ABC transporter with homology to the *rbs* ribose transporter of *E. coli*. The sole known function of the *lsrACDBFG* operon in *Salmonella* is the uptake and processing of AI-2. However, the primary role of *luxS* appears to be degradation of toxic intermediates in the activated methyl cycle (AMC), which makes distinguishing between metabolic changes related to the *luxS* genotype and those resulting from AI-2 signal exchange difficult. Microarray studies have shown that only a small portion of the *luxS*-responsive genes (7.9% in *Salmonella*, 1.9% in *E. coli* and 9.2% in *Streptococcus mutans*) also respond to exogenous AI-2 and an inability of exogenous AI-2 to rescue the *luxS* mutation in *Salmonella.* This underscores the complexity and uncertainties associated with role of *luxS* in AI-2 signaling in *Salmonella*.

Interactions of *Salmonella* with plant-associated protists

Within human hosts, *Salmonella* utilizes sophisticated systems to invade macrophages, and establish the *Salmonella*-containing vacuole (SCV), which it exploits for survival and replication (31). In the environment, *Salmonella* predation by protists results in its entrapment in a food vacuole where it experiences conditions that overlap with those in the SCV (65). The human pathogen *Legionella pneumophila* is known to interact with *Acanthamoeba* and survives in its cysts, a process that increases *Legionella's* infectivity (70). *Salmonella* also appears to resist digestion by *Acanthamoeba* and certain other ciliates commonly present in agricultural soils and on pre- and post-harvest vegetables, which may serve as additional environmental

reservoirs (16, 35, 78). Tolerance of *Salmonella* to digestion in *Tetrahymena* vacuoles results in its excretion in the protist's fecal pellets where the human pathogen survives at greater rates than as single cells (16). The release of fecal pellets containing intact *Salmonella* cells was observed on plants in the laboratory (35). Passage through *Tetrahymena* induces a large number of regulatory changes affecting between 989 – 1,282 genes or approximately 25% of the *Salmonella* genome (65). Many genes that were differentially regulated are involved in anaerobiosis, virulence, stress response (oxidative, osmotic, acid, and antimicrobial stress, as well as SOS response), indicating similarities in the physiology of *Salmonella* cells residing in *Tetrahymena* vacuoles to those in macrophages and epithelial cells. The acid resistance genes, *adiA* and *adiY,* were strongly upregulated and played a role in *Salmonella* resistance to digestion by *Tetrahymena* (65). Rehfuss et al. reported that the induction of acid stress response genes in *Tetrahymena* vacuoles imparts an enhanced resistance to subsequent acid stress upon *Salmonella*, and suggested that it may improve the pathogen's ability to survive the acidic stomach pH of its hosts (65). Such pre-adaptation may reduce the infectious dose of *Salmonella* in humans.

The passage of *Salmonella* through the amoeba *Acanthamoeba polyphaga* is associated with survival within contractile vacuoles, a process that relies on the *sseC, ssaU* and *phoP* genes (13, 78). These genes are part of SPI-2, which is responsible for intracellular replication in macrophages. Once established within the contractile vacuole, the bacteria entered logarithmic growth producing a population of over 200 cells which were able to persist for at least 4 days (33). The surviving *Salmonella* are subsequently able to multiple on the amoeba's waste products. Passage also induces a filamentation response which appears to provide protection from predation, although the mechanisms involved are unclear.

Fungi are prevalent members of plant microbial communities. Thus it is highly likely that *Salmonella* encounters and deals with fungi during its residence on plants. Various types of interactions between bacteria and fungi, ranging from antagonistic to beneficial, have been described (46). The attachment of *Salmonella* cells to fungal species in the cilantro phyllosphere has been observed (17). Furthermore, laboratory studies demonstrated the formation of large and dynamic *Salmonella* biofilms on *Aspergillus niger,* a common colonizer of plant surfaces, whereas *E. coli, P. agglomerans* and *P. chlororaphis* were unable to attach to the fungus and produce biofilms (20). Differences in colonization of *Aspergillus* were mirrored by differential binding of the bacterial species to chitin, an important component of fungal cell walls, and cellulose production in *Salmonella* was identified as the attachment factor mediating this relationship. It remains unclear whether *Salmonella* benefits directly from its association with *Aspergillus*, or other fungi, but it seems probable that the hyphae may vector the attached bacteria to new habitats or that their exudates provide additional nutrients to the human pathogens.

Fungi may also benefit human pathogens through habitat modification. Co-inoculation of tomato, potato and onion tissue with *Salmonella* and *Rhizopus* caused a significant increase in *Salmonella* population sizes compared with its inoculation alone (89). Similarly the post-harvest fungal pathogens *Alternaria alternata* and *Cladosporium* spp. enhanced the growth of *Salmonella* in ripe tomato fruit, likely via alkalinization of the plant tissue resulting from their proteolytic activity (85, 86). Hence it appears that the fungi provide not only enhanced access to growth substrates by degrading the plant tissue but additionally reduce environmental stress that may inhibit *Salmonella*.

CONCLUSIONS

Outbreaks of gastroenteritis linked to the consumption of fresh fruits and vegetables have provided the rationale for investigating the biology of *Salmonella* on plants. Numerous studies in this new multidisciplinary field of research have yielded important discoveries that continue to challenge the dogma that *Salmonella* is best defined as an enteric colonist. Key studies, including those in this Focus Issue, point to the ability of this human pathogen to interact with plant tissue and with the plant-associated microflora. It is clear that *Salmonella* can sense subtle environmental cues brought about by the genotype or physiological state of its plant host, and responds with distinct patterns of gene expression accordingly. Plants also recognize *Salmonella* and activate basal defenses in response to the human pathogen when at high densities and in close contact with plant cells in the apoplast. It is still unclear, however, whether *Salmonella* is a clever opportunist that shows sufficient versatility under rare conditions in the plant environment to proliferate to infectious doses, or if its behavior on plants results from an evolutionary adaptation to use plants as an important vector to infect vertebrate hosts through their dietary intake.

ACKNOWLEDGMENTS

Preparation of this manuscript was supported by USDA-NIFA AFRI grant 2011-67017-30127 to M.T., by the United States Department of Agriculture, Agricultural Research Service CRIS project 5325-42000-046, and by a USDA-NIFA post-doctoral fellowship grant 2012-67012-19708 to C.E.C., M.T.B. and M.T. We thank W.J. Zaragoza for designing Figure 2 and J.T. Noel for sharing unpublished data presented in Figure 3 of this publication.

FIGURE LEGENDS

Fig. 1. Lifecycle of enteric bacteria. Excretion from a host and subsequent colonization of plants may be part of the lifecycle of enteric bacteria. Plants germinating from cow manure are

not uncommon, as in this photograph of an alfalfa seedling growing on manure in a grazed pasture in Archer, FL. Inset: samples from the cow manure, rhizosphere and surface sterilized shoot and root tissues of the alfalfa seedling in the photograph were homogenized in PBS and plated on XLD medium (Oxoid), and incubated at 42ºC to detect fecal coliforms (yellow colonies on XLD agar). Relatively few coliforms were detected in the aged manure and in the rhizosphere, however, substantial populations of presumed fecal coliforms were detected inside the plant tissue.

Fig. 2. Phenotypes of *Salmonella* isolates on a Congo Red-containing plate. The characteristic red wrinkled appearance (*rdar* phenotype) is seen in colonies of the wild type *S.* Typhimurium 14028 (right, middle row, also see notations on the grayscale inset), *S.* Newport (from a tomato field on the Eastern Shore of Virginia, top left) and of two *S*. Braenderup isolates from clinical patients in a tomato outbreak (bottom left and top right corners, indicated with "B"). Produce isolates of *Salmonella* Agona (left, middle row; "A"), Montevideo (center, "Mo") and Michigan (middle, top row,"Mi") are non-*rdar*. Spontaneous non-*rdar* mutants can arise when *rdar* strains are passaged through tomatoes, e.g. the two non-*rdar* spontaneous mutants (92) of *S*. Typhimurium 14028 in the forefront.

Fig. 3. Soft-rot bacteria promote proliferation of *Salmonella* in plants. An increased proliferation of *Salmonella* and *E. coli* in plants infected with soft-rot bacteria has been observed in the market place (88) and under laboratory conditions (15, 18, 34, 61). In this experiment (J.T. Noel, unpublished), *Salmonella* Typhimurium 14028 (~100-500 cells) was co-inoculated with ~3 million cells of hypervirulent *P. carotovorum* SR38 by injection into the tomato pericarp, and incubated at 22ºC. For enumeration, tomatoes were macerated in PBS and dilution-plated onto XLD. The brown and green lines represent the growth of *Salmonella* with and without

Pectobacterium soft rot, respectively. Inset: appearance of representative tomatoes throughout the experiment.

LITERATURE CITED

1. Ahmer, B. M. 2004. Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. Mol Microbiol 52(4):933-45 doi:10.1111/j.1365-2958.2004.04054.x.
2. Aruscavage, D., S. A. Miller, M. L. Ivey, K. Lee and J. T. LeJeune. 2008. Survival and dissemination of *Escherichia coli* O157:H7 on physically and biologically damaged lettuce plants. J Food Prot 71(12):2384-8.
3. Aruscavage, D., P. L. Phelan, K. Lee and J. T. LeJeune. 2010. Impact of changes in sugar exudate created by biological damage to tomato plants on the persistence of *Escherichia coli* O157:H7. J Food Sci 75(4):M187-92 doi:10.1111/j.1750-3841.2010.01593.x.
4. Barak, J. D., L. Gorski, P. Naraghi-Arani and A. O. Charkowski. 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. Appl Environ Microbiol 71(10):5685-91 doi:10.1128/AEM.71.10.5685-5691.2005.
5. Barak, J. D., C. E. Jahn, D. L. Gibson and A. O. Charkowski. 2007. The role of cellulose and O-antigen capsule in the colonization of plants by *Salmonella enterica*. Mol Plant Microbe Interact 20(9):1083-91 doi:10.1094/MPMI-20-9-1083.
6. Barak, J. D., A. Liang and K. E. Narm. 2008. Differential attachment to and subsequent contamination of agricultural crops by *Salmonella enterica*. Appl Environ Microbiol 74(17):5568-70 doi:10.1128/AEM.01077-08.
7. Barak, J. D. and A. S. Liang. 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. PLoS One 3(2):e1657 doi:10.1371/journal.pone.0001657.
8. Barak, J. D., L. Gorski, A. S. Liang and K. E. Narm. 2009. Previously uncharacterized *Salmonella enterica* genes required for swarming play a role in seedling colonization. Microbiology 155(Pt 11):3701-9 doi:10.1099/mic.0.032029-0.
9. Barak, J. D., L. C. Kramer and L. Y. Hao. 2011. Colonization of tomato plants by *Salmonella enterica* is cultivar dependent, and type 1 trichomes are preferred colonization sites. Appl Environ Microbiol 77(2):498-504 doi:10.1128/AEM.01661-10.
10. Batz, M. B., S. Hoffman and J. G. Morris. 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. University of Florida, Emerging Pathogens Institute, Gainesville, FL.
11. Berger, C. N., R. K. Shaw, D. J. Brown, H. Mather, S. Clare, G. Dougan, M. J. Pallen and G. Frankel. 2009. Interaction of *Salmonella enterica* with basil and other salad leaves. ISME J 3(2):261-5 doi:10.1038/ismej.2008.95.
12. Berger, C. N., D. J. Brown, R. K. Shaw, F. Minuzzi, B. Feys and G. Frankel. 2011. *Salmonella enterica* strains belonging to O serogroup 1,3,19 induce chlorosis and wilting of *Arabidopsis thaliana* leaves. Environ Microbiol 13(5):1299-308 doi:10.1111/j.1462-2920.2011.02429.x.
13. Bleasdale, B., P. J. Lott, A. Jagannathan, M. P. Stevens, R. J. Birtles and P. Wigley. 2009. The *Salmonella* pathogenicity island 2-encoded type III secretion system is essential for the survival of *Salmonella enterica* serovar Typhimurium in free-living amoebae. Appl Environ Microbiol 75(6):1793-5 doi:10.1128/AEM.02033-08.
14. Brandl, M. T. and R. E. Mandrell. 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. Appl Environ Microbiol 68(7):3614-21.

15. Brandl, M. T., W. G. Miller, A. H. Bates and R. E. Mandrell. 2005. Production of autoinducer 2 in *Salmonella enterica* serovar Thompson contributes to its fitness in chickens but not on cilantro leaf surfaces. Appl Environ Microbiol 71(5):2653-62 doi:10.1128/AEM.71.5.2653-2662.2005.
16. Brandl, M. T., B. M. Rosenthal, A. F. Haxo and S. G. Berk. 2005. Enhanced survival of *Salmonella enterica* in vesicles released by a soilborne *Tetrahymena* species. Appl Environ Microbiol 71(3):1562-9 doi:10.1128/AEM.71.3.1562-1569.2005.
17. Brandl, M. T. 2006. Fitness of human enteric pathogens on plants and implications for food safety. Annu Rev Phytopathol 44:367-92 doi:10.1146/annurev.phyto.44.070505.143359.
18. ---. 2008. Plant lesions promote the rapid multiplication of *Escherichia coli* O157:H7 on postharvest lettuce. Appl Environ Microbiol 74(17):5285-9 doi:10.1128/AEM.01073-08.
19. Brandl, M. T. and R. Amundson. 2008. Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica*. Appl Environ Microbiol 74(8):2298-306 doi:10.1128/AEM.02459-07.
20. Brandl, M. T., M. Q. Carter, C. T. Parker, M. R. Chapman, S. Huynh and Y. Zhou. 2011. *Salmonella* biofilm formation on *Aspergillus niger* involves cellulose--chitin interactions. PLoS One 6(10):e25553 doi:10.1371/journal.pone.0025553.
21. Carter, M. Q., M. T. Brandl, J. W. Louie, J. L. Kyle, D. K. Carychao, M. B. Cooley, C. T. Parker, A. H. Bates and R. E. Mandrell. 2011. Distinct acid resistance and survival fitness displayed by curli variants of enterohemorrhagic *Escherichia coli* O157:H7. Applied and Environmental Microbiology 77(11):3685-3695 doi:Doi 10.1128/Aem.02315-10.
22. Carter, M. Q., K. Xue, M. T. Brandl, F. Liu, L. Wu, J. W. Louie, R. E. Mandrell and J. Zhou. 2012. Functional metagenomics of *Escherichia coli* O157:H7 interactions with spinach indigenous microorganisms during biofilm formation. PLoS One 7(9):e44186 doi:10.1371/journal.pone.0044186.
23. Cooley, M. B., W. G. Miller and R. E. Mandrell. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol 69(8):4915-26.
24. Cooley, M. B., D. Chao and R. E. Mandrell. 2006. *Escherichia coli* O157:H7 survival and growth on lettuce is altered by the presence of epiphytic bacteria. J Food Prot 69(10):2329-35.
25. Cox, C. E., M. McClelland and M. Teplitski. 2013. Consequences of disrupting *Salmonella* AI-2 signaling on interactions within soft rots. Phytopathology 103.
26. deWaal, C. S., X. A. Tian and D. Plunkett. 2009. Outbreak Alert! Center for Science in Public Interest.
27. Dong, Y., A. L. Iniguez, B. M. Ahmer and E. W. Triplett. 2003. Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. Appl Environ Microbiol 69(3):1783-90.
28. Dorsey, C. W., M. C. Laarakker, A. D. Humphries, E. H. Weening and A. J. Baumler. 2005. *Salmonella enterica* serotype Typhimurium MisL is an intestinal colonization factor that binds fibronectin. Mol Microbiol 57(1):196-211 doi:10.1111/j.1365-2958.2005.04666.x.

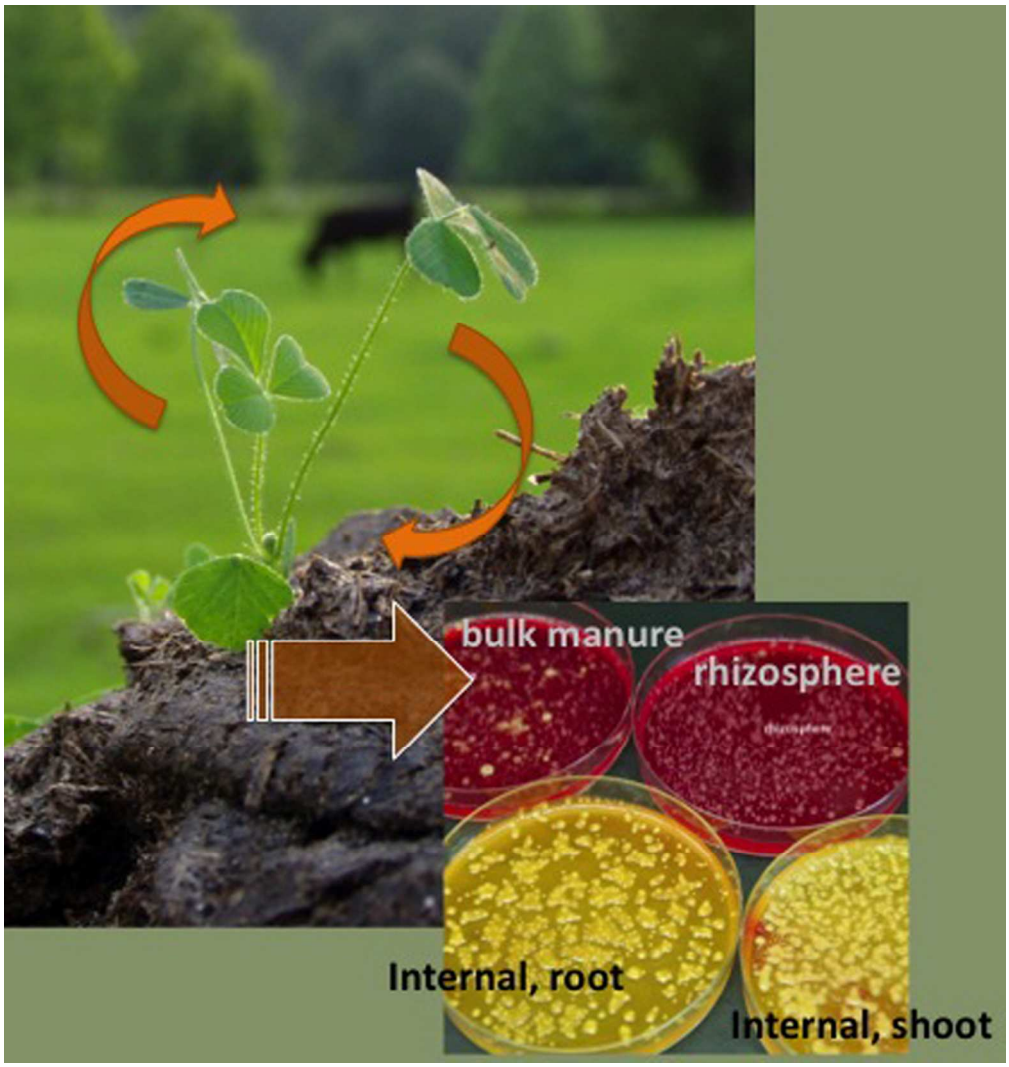
29. Ercolani, G. L. 1979. Differential survival of *Salmonella* Typhi, *Escherichia coli*, and *Enterobacter aerogenes* on lettuce in the field. Zentralbl Bakteriol Naturwiss 134(5):402-11.
30. Fett, W. F. 2006. Inhibition of *Salmonella enterica* by plant-associated pseudomonads *in vitro* and on sprouting alfalfa seed. Journal of Food Protection 69(4):719-728.
31. Foster, J. W. and M. P. Spector. 1995. How *Salmonella* survive against the odds. Annu Rev Microbiol 49:145-74 doi:10.1146/annurev.mi.49.100195.001045.
32. Gal, M., G. M. Preston, R. C. Massey, A. J. Spiers and P. B. Rainey. 2003. Genes encoding a cellulosic polymer contribute toward the ecological success of *Pseudomonas fluorescens* SBW25 on plant surfaces. Mol Ecol 12(11):3109-21.
33. Gaze, W. H., N. Burroughs, M. P. Gallagher and E. M. Wellington. 2003. Interactions between *Salmonella typhimurium* and *Acanthamoeba polyphaga*, and observation of a new mode of intracellular growth within contractile vacuoles. Microb Ecol 46(3):358-69 doi:10.1007/s00248-003-1001-3.
34. Goudeau, D. M., C. T. Parker, Y. Zhou, S. Sela, Y. Kroupitski and M. T. Brandl. 2013. The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. Appl Environ Microbiol 79 doi:10.1128/AEM.02290-12.
35. Gourabathini, P., M. T. Brandl, K. S. Redding, J. H. Gunderson and S. G. Berk. 2008. Interactions between food-borne pathogens and protozoa isolated from lettuce and spinach. Appl Environ Microbiol 74(8):2518-25 doi:10.1128/AEM.02709-07.
36. Guo, X., J. Chen, R. E. Brackett and L. R. Beuchat. 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. Appl Environ Microbiol 67(10):4760-4.
37. Harvey, P. C., M. Watson, S. Hulme, M. A. Jones, M. Lovell, A. Berchieri, Jr., J. Young, N. Bumstead and P. Barrow. 2011. *Salmonella enterica* serovar Typhimurium colonizing the lumen of the chicken intestine grows slowly and upregulates a unique set of virulence and metabolism genes. Infect Immun 79(10):4105-21 doi:10.1128/IAI.01390-10.
38. Hirano, S. S. and C. D. Upper. 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*-a pathogen, ice nucleus, and epiphyte. Microbiol Mol Biol Rev 64(3):624-53.
39. Iniguez, A. L., Y. M. Dong, H. D. Carter, B. M. M. Ahmer, J. M. Stone and E. W. Triplett. 2005. Regulation of enteric endophytic bacterial colonization by plant defenses. Molecular Plant-Microbe Interactions 18(2):169-178 doi:Doi 10.1094/Mpmi-18-0169.
40. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner and X. Jiang. 2004. Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. Foodborne Pathog Dis 1(1):27-35 doi:10.1089/153531404772914437.
41. ---. 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Appl Environ Microbiol 70(4):2497-502.
42. Jablasone, J., K. Warriner and M. Griffiths. 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a

gnotobiotic system. Int J Food Microbiol 99(1):7-18 doi:10.1016/j.ijfoodmicro.2004.06.011.
43. Janisiewicz, W. J., W. S. Conway and B. Leverentz. 1999. Biological control of postharvest decays of apple can prevent growth of *Escherichia coli* O157 : H7 in apple wounds. J Food Protect 62(12):1372-1375.
44. Klerks, M. M., E. Franz, M. van Gent-Pelzer, C. Zijlstra and A. H. van Bruggen. 2007. Differential interaction of *Salmonella enterica* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency. ISME J 1(7):620-31.
45. Klumpp, J. and T. M. Fuchs. 2007. Identification of novel genes in genomic islands that contribute to *Salmonella* Typhimurium replication in macrophages. Microbiology 153(Pt 4):1207-20 doi:10.1099/mic.0. 2006/004747-0.
46. Kobayashi, D. and B. I. Hillman. 2005. Fungi, bacteria, and viruses as pathogens of the fungal community. T. in: he fungal community: Its organization and role in the ecosystem J. Dighton., J. F. White and P. V. Oudemans, eds., CRC Press.
47. Kroupitski, Y., D. Golberg, E. Belausov, R. Pinto, D. Swartzberg, D. Granot and S. Sela. 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. Appl Environ Microbiol 75(19):6076-6086 doi:Doi 10.1128/Aem.01084-09.
48. Kroupitski, Y., R. Pinto, M. T. Brandl, E. Belausov and S. Sela. 2009. Interactions of *Salmonella enterica* with lettuce leaves. J Appl Microbiol 106(6):1876-1885 doi:Doi 10.1111/J.1365-2672.2009.04152.X.
49. Kroupitski, Y., R. Pinto, E. Belausov and S. Sela. 2011. Distribution of Salmonella typhimurium in romaine lettuce leaves. Food Microbiol 28(5):990-997 doi:Doi 10.1016/J.Fm.2011.01.007.
50. Kroupitski, Y., M. T. Brandl, R. Pinto, E. Belausov, D. Tamir-Ariel, S. Burdman and S. Sela. 2013. Identification of *Salmonella enterica* genes with a role in persistence on lettuce leaves during cold-storage by Recombinase-based *In Vivo* Expression Technology. Phytopathology 103.
51. Kyle, J. L., C. T. Parker, D. Goudeau and M. T. Brandl. 2010. Transcriptome analysis of *Escherichia coli* O157:H7 exposed to lysates of lettuce leaves. Appl Environ Microbiol 76(5):1375-87 doi:10.1128/AEM.02461-09.
52. Lapidot, A. and S. Yaron. 2009. Transfer of *Salmonella enterica* serovar Typhimurium from contaminated irrigation water to parsley is dependent on curli and cellulose, the biofilm matrix components. J Food Prot 72(3):618-23.
53. Ledeboer, N. A., J. G. Frye, M. McClelland and B. D. Jones. 2006. *Salmonella enterica* serovar Typhimurium requires the Lpf, Pef, and Tafi fimbriae for biofilm formation on HEp-2 tissue culture cells and chicken intestinal epithelium. Infect Immun 74(6):3156-69 doi:10.1128/IAI.01428-05.
54. Leveau, J. H. and S. E. Lindow. 2001. Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere. Proc Natl Acad Sci U S A 98(6):3446-53 doi:10.1073/pnas.061629598.
55. Lindow, S. E. and M. T. Brandl. 2003. Microbiology of the phyllosphere. Appl Environ Microbiol 69(4):1875-83.
56. Lopez-Velasco, G., H. A. Tydings, R. R. Boyer, J. O. Falkinham and M. A. Ponder. 2012. Characterization of interactions between *Escherichia coli* O157:H7 with

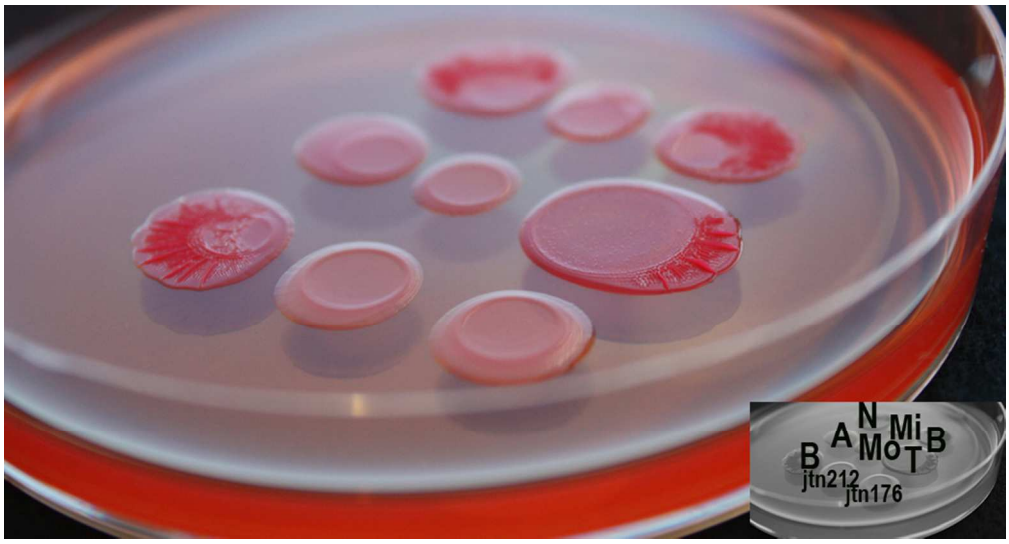
epiphytic bacteria in vitro and on spinach leaf surfaces. International Journal of Food Microbiology 153(3):351-357.
57. Mandrell, R. 2009. Enteric human pathogens associated with fresh produce: sources, transport, and ecology. in: Microbial Safety of Fresh Produce X. Fan, B. A. Niemira, C. J. Doona, F. E. Feeherry and R. B. Gravani, eds. Blackwell Publishing and the Institute of Food Technologies, Ames, Iowar.
58. Matthysse, A. G. and S. McMahan. 1998. Root colonization by *Agrobacterium tumefaciens* is reduced in *cel, attB, attD*, and *attR* mutants. Appl Environ Microbiol 64(7):2341-5.
59. Matthysse, A. G., R. Deora, M. Mishra and A. G. Torres. 2008. Polysaccharides cellulose, poly-beta-1,6-n-acetyl-D-glucosamine, and colanic acid are required for optimal binding of *Escherichia coli* O157:H7 strains to alfalfa sprouts and K-12 strains to plastic but not for binding to epithelial cells. Appl Environ Microbiol 74(8):2384-90 doi:10.1128/AEM.01854-07.
60. Noel, J. T., N. Arrach, A. Alagely, M. McClelland and M. Teplitski. 2010. Specific responses of *Salmonella enterica* to tomato varieties and fruit ripeness identified by *In Vivo* Expression Technology. PLoS One 5(8) doi:ARTN e12406 DOI 10.1371/journal.pone.0012406.
61. Noel, J. T., J. Joy, J. N. Smith, M. Fatica, K. R. Schneider, B. M. Ahmer and M. Teplitski. 2010. *Salmonella* SdiA recognizes N-acyl homoserine lactone signals from *Pectobacterium carotovorum in vitro*, but not in a bacterial soft rot. Mol Plant Microbe Interact 23(3):273-82 doi:10.1094/MPMI-23-3-0273.
62. Poza-Carrion, C., T. V. Suslow and S. E. Lindow. 2013. Resident bacteria on leaves enhance survival of immigrant cells of *Salmonella enterica*. Phytopathology 103.
63. Quilliam, R. S., A. P. Williams and D. L. Jones. 2012. Lettuce cultivar mediates both phyllosphere and rhizosphere activity of *Escherichia coli* O157:H7. Plos One 7(3).
64. Rastogi, G., A. Sbodio, J. J. Tech, T. V. Suslow, G. L. Coaker and J. H. Leveau. 2012. Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce. ISME J doi:10.1038/ismej.2012.32.
65. Rehfuss, M. Y., C. T. Parker and M. T. Brandl. 2011. *Salmonella* transcriptional signature in *Tetrahymena* phagosomes and role of acid tolerance in passage through the protist. ISME J 5(2):262-73 doi:10.1038/ismej.2010.128.
66. Romling, U., W. Bokranz, W. Rabsch, X. Zogaj, M. Nimtz and H. Tschape. 2003. Occurrence and regulation of the multicellular morphotype in *Salmonella* serovars important in human disease. International J Med Microbiol 293(4):273-285 doi:Doi 10.1078/1438-4221-00268.
67. Romling, U. 2005. Characterization of the rdar morphotype, a multicellular behaviour in Enterobacteriaceae. Cell Mol Life Sci 62(11):1234-1246 doi:Doi 10.1007/S00018-005-4557-X.
68. Roy, D., S. Panchal, B. A. Rosa and M. Melotto. 2013. *Escherichia coli* O157:H7 induces stronger plant immunity than *Salmonella enterica* Typhimurium SL1344. Phytopathology 103.
69. Sabag-Daigle, A. and B. M. Ahmer. 2012. ExpI and PhzI are descendants of the long lost cognate signal synthase for SdiA. PLoS One 7(10):e47720 doi:10.1371/journal.pone.0047720.

70. Samrakandi, M. M., D. A. Ridenour, L. Yan and J. D. Cirillo. 2002. Entry into host cells by *Legionella*. Front Biosci 7:d1-11.
71. Schikora, A., A. Carreri, E. Charpentier and H. Hirt. 2008. The dark side of the salad: *Salmonella* Ryphimurium overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle. PLoS One 3(5):e2279 doi:10.1371/journal.pone.0002279.
72. Schikora, A., I. Virlogeux-Payant, E. Bueso, A. V. Garcia, T. Nilau, A. Charrier, S. Pelletier, P. Menanteau, M. Baccarini, P. Velge and H. Hirt. 2011. Conservation of *Salmonella* infection mechanisms in plants and animals. PLoS One 6(9):e24112 doi:10.1371/journal.pone.0024112.
73. Semenov, A. M., A. A. Kuprianov and A. H. van Bruggen. 2010. Transfer of enteric pathogens to successive habitats as part of microbial cycles. Microb Ecol 60(1):239-49 doi:10.1007/s00248-010-9663-0.
74. Shirron, N. and S. Yaron. 2011. Active suppression of early immune response in tobacco by the human pathogen *Salmonella* Typhimurium. PLoS One 6(4):e18855 doi:10.1371/journal.pone.0018855.
75. Solomon, E. B., B. A. Niemira, G. M. Sapers and B. A. Annous. 2005. Biofilrn formation, cellulose production, and curli biosynthesis by *Salmonella* originating from produce, animal, and clinical sources. J Food Protect 68(5):906-912.
76. Teplitski, M., K. Warriner, J. Bartz and K. R. Schneider. 2011. Untangling metabolic and communication networks: interactions of enterics with phytobacteria and their implications in produce safety. Trends Microbiol 19(3):121-127 doi:Doi 10.1016/J.Tim.2010.11.007.
77. Teplitski, M., J. T. Noel, A. Alagely and M. D. Danyluk. 2012. Functional genomics studies shed light on the nutrition and gene expression of non-typhoidal *Salmonella* and enterovirulent *E. coli* in produce. Food Res Int'l 45(2):576-586 doi:Doi 10.1016/J.Foodres.2011.06.020.
78. Tezcan-Merdol, D., M. Ljungstrom, J. Winiecka-Krusnell, E. Linder, L. Engstrand and M. Rhen. 2004. Uptake and replication of *Salmonella enterica* in *Acanthamoeba rhysodes*. Appl Environ Microbiol 70(6):3706-14 doi:10.1128/AEM.70.6.3706-3714.2004.
79. Thiennimitr, P., S. E. Winter, M. G. Winter, M. N. Xavier, V. Tolstikov, D. L. Huseby, T. Sterzenbach, R. M. Tsolis, J. R. Roth and A. J. Baumler. 2011. Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. Proc Natl Acad Sci U S A 108(42):17480-5 doi:10.1073/pnas.1107857108.
80. Thiennimitr, P., S. E. Winter and A. J. Baumler. 2012. *Salmonella*, the host and its microbiota. Curr Opin Microbiol 15(1):108-14 doi:10.1016/j.mib.2011.10.002.
81. Thilmony, R., W. Underwood and S. Y. He. 2006. Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. tomato DC3000 and the human pathogen *Escherichia coli* O157:H7. Plant J 46(1):34-53 doi:10.1111/j.1365-313X.2006.02725.x.
82. Turnbull, A. L. and M. G. Surette. 2008. L-Cysteine is required for induced antibiotic resistance in actively swarming *Salmonella enterica* serovar Typhimurium. Microbiology 154(Pt 11):3410-9 doi:10.1099/mic.0.2008/020347-0.

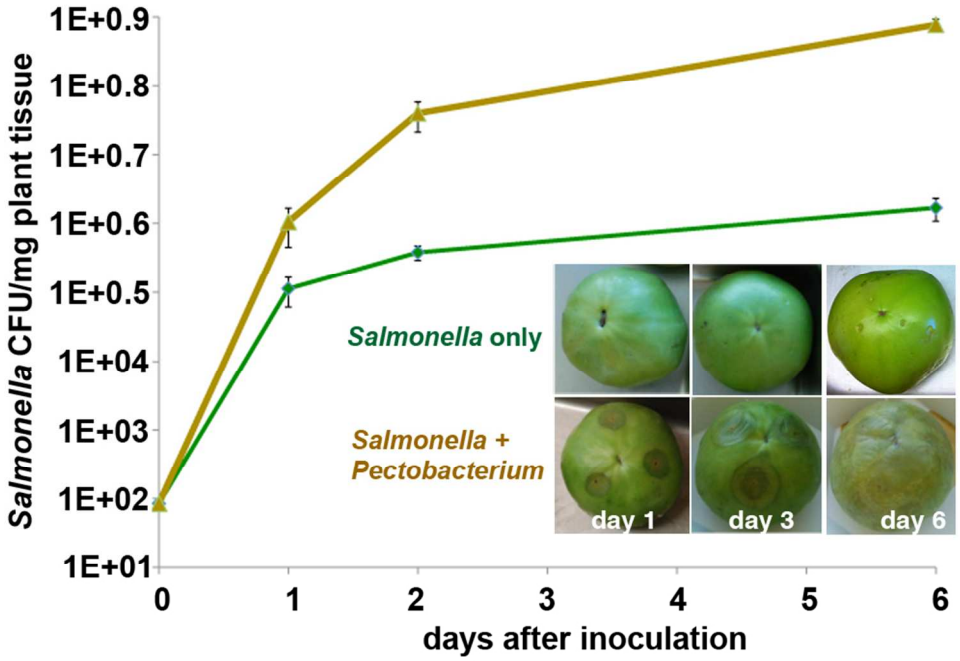
83. ---. 2010. Cysteine biosynthesis, oxidative stress and antibiotic resistance in *Salmonella* Typhimurium. Res Microbiol 161(8):643-50 doi:10.1016/j.resmic.2010.06.004.
84. Ustun, S., P. Muller, R. Palmisano, M. Hensel and F. Bornke. 2012. SseF, a type III effector protein from the mammalian pathogen *Salmonella enterica*, requires resistance-gene-mediated signalling to activate cell death in the model plant *Nicotiana benthamiana*. New Phytol 194(4):1046-60 doi:10.1111/j.1469-8137.2012.04124.x.
85. Wade, W. N. and L. R. Beuchat. 2003. Metabiosis of proteolytic moulds and *Salmonella* in raw, ripe tomatoes. J Appl Microbiol 95(3):437-50.
86. Wade, W. N., R. Vasdinnyei, T. Deak and L. R. Beuchat. 2003. Proteolytic yeasts isolated from raw, ripe tomatoes and metabiotic association of *Geotrichum candidum* with *Salmonella*. Int J Food Microbiol 86(1-2):101-11.
87. Wang, Q., J. G. Frye, M. McClelland and R. M. Harshey. 2004. Gene expression patterns during swarming in Salmonella typhimurium: genes specific to surface growth and putative new motility and pathogenicity genes. Mol Microbiol 52(1):169-87 doi:10.1111/j.1365-2958.2003.03977.x.
88. Wells, J. M. and J. E. Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. Plant Disease 81(8):867-872.
89. ---. 1999. Incidence of *Salmonella* on fresh fruits and vegetables affected by fungal rots or physical injury. Plant Disease 83(8):722-726.
90. Winter, S. E. and A. J. Baumler. 2011. A breathtaking feat: to compete with the gut microbiota, *Salmonella* drives its host to provide a respiratory electron acceptor. Gut Microbes 2(1):58-60 doi:10.4161/gmic.2.1.14911.
91. Yamazaki, A., J. Li, W. C. Hutchins, L. Wang, J. Ma, A. M. Ibekwe and C. H. Yang. 2011. Commensal effect of pectate lyases secreted from *Dickeya dadantii* on proliferation of *Escherichia coli* O157:H7 EDL933 on lettuce leaves. Appl Environ Microbiol 77(1):156-62 doi:10.1128/AEM.01079-10.
92. Zaragoza, W. J., J. T. Noel and M. Teplitski. 2012. Spontaneous non-*rdar* mutations increase fitness of *Salmonella* in plants. Environ Microbiol Reports 4(4):453-458.



87x92mm (300 x 300 DPI)



Phenotypes of Salmonella isolates on a Congo Red-containing plate. The characteristic red wrinkled appearance (rdar phenotype) is seen in colonies of the wild type S. Typhimurium 14028 (right, middle row, also see notations on the grayscale inset), S. Newport (from a tomato field on the Eastern Shore of Virginia, top left) and of two S. Braenderup isolates from clinical patients in a tomato outbreak (bottom left and top right corners, indicated with "B"). Produce isolates of Salmonella Agona (left, middle row; "A"), Montevideo (center, "Mo") and Michigan (middle, top row,"Mi") are non-rdar. Spontaneous non-rdar mutants can arise when rdar strains are passaged through tomatoes, e.g. the two non-rdar spontaneous mutants (92) of S. Typhimurium 14028 in the forefront.
87x46mm (300 x 300 DPI)



Soft-rot bacteria promote proliferation of Salmonella in plants. An increased proliferation of Salmonella and E. coli in plants infected with soft-rot bacteria has been observed in the market place (88) and under laboratory conditions (15, 18, 34, 61). In this experiment (J.T. Noel, unpublished), Salmonella Typhimurium 14028 (~100-500 cells) was co-inoculated with ~3 million cells of hypervirulent P. carotovorum SR38 by injection into the tomato pericarp, and incubated at 22ºC. For enumeration, tomatoes were macerated in PBS and dilution-plated onto XLD. The brown and green lines represent the growth of Salmonella with and without Pectobacterium soft rot, respectively. Inset: appearance of representative tomatoes throughout the experiment.
87x59mm (300 x 300 DPI)