Chapter 10

Interaction between *Salmonella* and Plants: Potential Hosts and Vectors for Human Infection

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Abstract

Fruits and vegetables are important for a healthy diet. However, when eaten raw and contaminated with human pathogens (HPs) they may cause a disease outbreak. Contamination with HPs can occur along the entire farm-to-fork production chain and *Salmonella enterica* is one of the most common foodborne pathogens. A range of biotic and abiotic environmental factors can influence the complex interactions between *Salmonella* and plants. Moreover, the outcome of experiments largely depends on the experimental design and parameters or methods employed, and on top, on the accompanying plant microbiome and the genetic equipment of the plant and the *Salmonella* strain. Particularly mobile genetic elements contribute to the diversification and adaptation of *Salmonella* to the plant environment. So far, little is known about the key processes and factors influencing the attachment and potential internalization of *Salmonella* in plants and the plant specific responses. It is therefore important to better understand the ecology of *Salmonella* in the soil and plant environment, in order to propose practicable recommendations for prevention of foodborne diseases. This also requires improved sensitivity and specificity of detection methods. In this chapter, we present the current knowledge, research needs, and methodology regarding the complex interactions between *Salmonella* and plants.

Keywords: *Salmonella enterica*, plant, biofilm, colonization mechanisms, interaction

1. Introduction

The natural microbiome of plants includes a wide diversity of microorganisms and is a key determinant of plant health and productivity, e.g., by supporting the uptake

of mineral nutrients in roots or suppressing pathogen growth and inducing the host-immune system [1–3]. Due to its relevance, the plant microbiome (totality of microorganisms associated with the plant) is even called the second plant genome. Because of the tight interplay between plants and their epiphytic and endophytic microorganisms the terms holobiont and meta-organisms are used as well. The plant microbiome is important not only for plant growth and health, but is also positively influencing human health [4]. However, besides positive effects on human health plants can also be carriers of bacterial HPs.

Salmonella is one of the major causal agents of foodborne gastroenteritis and represents a major threat to public health. It is estimated that each year 93.8 million cases of salmonellosis occur globally (86% of which foodborne), with 155,000 deaths [5]. Consumption of raw plants is more and more recognized as a source for HPs and associated with disease outbreaks in several countries. The number of outbreaks linked to fresh produce, spices, and nuts surpassed those linked to foods of animal origin [6]. Sources of HPs in the production chain and factors contributing to the contamination of fruits and vegetables include for example the application of organic fertilizers such as animal manures, contaminated irrigation water, insect and animal vectors but also the use of contaminated seeds [7]. *Enterobacteriaceae* such as *Erwinia*, *Serratia,* and *Pantoea* belong to bacteria typically associated with the phyllosphere [8–10]. However, it is not completely understood how *Salmonella* persists in the plant environment and which environmental factors trigger its survival. In this chapter, we discuss factors influencing the survival of *Salmonella* in the agricultural environment as well as adaptations that allow successful colonization of plants, such as attachment, biofilm formation, and internalization.

2. Contamination of fresh produce

Besides contaminated animal products, *Salmonella* outbreaks are increasingly associated with fruits and vegetables. Already on the field, plants may be contaminated via soil or irrigation, especially if watered with surface water [11–14]. *Salmonella* has been shown to persist in various ecological niches in soil as well as in irrigation water and fertilizers [15–17]. In this context, the watering system and the agricultural practices seem to play a key role in the prevention of contamination with human pathogens. For instance, lettuce plants were more likely to be contaminated with *Escherichia coli* when watered using overhead sprinklers when compared to subsurface drip or surface furrow irrigation [18]. Besides, even noncontaminated rain-sized water droplets could transfer HPs from contaminated soil or plants to other plants [19].

Organic fertilizers like manure, biogas plant digestates and sewage sludge offer an additional route for contamination of fresh produce. Similarly, animals like birds, game, mice, or insects can contribute to the contamination of fresh produce directly or indirectly via feces or irrigation water [7, 14, 20]. Often underestimated are soil particles, which can be carried by the wind over long distances and contribute to the transient of microbiome between plants [8]. Hence, wind-caused spread of HPs should also be considered. Contaminated plant residues might constitute additional risk if incorporated into soil before the planting of next crop.

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The infection of plants is essentially dependent on the ability of HPs to survive and persist in the agricultural environment. *Salmonella*, for example, was shown to survive in soil for more than 200 days if the soil was fertilized [21, 22]. The survival of diverse bacteria newly introduced into soil has been subject of research for many years [23], and the mechanisms that govern this process, compared often to microbial invasion, were described in many studies (recently reviewed by [24]). In order to survive in the soil, HPs need to find an adequate ecological niche in which they can establish. Furthermore, their ability to do so and to survive for extended time increased when the indigenous microbial community was reduced as a result of, for example, sterilization [25]. In addition, the survival of microorganisms that successfully invaded the soil is highly dependent on the environmental heterogeneity [26–28].

Contamination of fresh produce with HPs like *Salmonella,* can occur before the harvest and also along the whole production chain [11, 14]. Since the epidemiological investigations start very often long time after the contamination or the harvest, it is very challenging to assess whether the contamination took place in the field or occurred "post-harvest" during the processing. Consequently in the majority of cases, the information available does not necessarily reveal the real causes of contamination [29].

3. Epidemiology of *Salmonella* in agricultural systems

Fresh produce contaminated with *Salmonella* can easily trigger a salmonellosis outbreak, and despite the difficulties with identification, in the past years fresh produce were repeatedly identified as the outbreak source. Among the outbreaks in the USA, *Salmonella* is the leading cause of the fresh produce-originated foodborne diseases [30]. The available data are depending on the procedures and records in particular countries. At least 12 large, fresh produce-related *Salmonella* outbreaks have been reported since 2010, an overview of international outbreaks with more than 100 associated cases is presented in **Table 1**.

Although fruits and vegetables were identified as source of human pathogens, it is not clear whether the plants were colonized in the field or during processing. *Salmonella* may live epiphytically or be internalized through wounds, the root system, stomata, or hydathodes (see below). Additionally, *Salmonella* can be entrapped in fruits or seeds after contamination of flowers [31, 32]. Moreover, large outbreaks can be destructive to consumer's confidence which results in economic losses [33, 34]. Therefore, the research on the ecology of HPs like *Salmonella* in relation to farming and harvesting practices is very important for human health and also for the economy.

4. Factors influencing the survival of *Salmonella* in soil

Successful establishment of human pathogenic bacteria in soil depends on a variety of biotic and abiotic factors (see **Figure 1** for an overview). Numerous studies, carried out under different conditions, showed that among them are weather or atmospheric conditions like tem-

Salmonella Serovar	Vector	Year	Country	Cases/serovar confirmed	Reference
S. Newport	Tomatoes	2015	USA	115/81	[112]
S. Poona	Cucumbers	2015-16	USA	907/907	[113]
Unknown	Onions, tomatoes	2015	USA	200/0	[114]
S. Enteriditis	Sprouts, beans	2014	USA	115/0	[113]
S. Newport	Cucumbers	2014	USA	275/0	[113]
S. Typhimurium	Cantaloupe	2012	USA	261/261	[115]
S. Braenderup	Mangoes	2012	USA, Canada	127/0	[113]
S. Newport	Mung beans	2011	Germany, The Netherlands	106/32	[116]
Unknown	Produce-based salads, broccoli salad	2011	Japan	1500/0	[117]
S. Agona	Fruit, papaya	2011	USA	106/0	[113]
S. I4,[5],12:i:-	Vegetables, sprouts, alfalfa sprouts	2010	USA	140/0	[118]
S. Hvittingfoss	Vegetables, leafy greens, lettuce, fruit, tomatoes, olives	2010	USA	114/108	[119]

Only large outbreaks with more than 100 associated total or confirmed cases since 2010 are shown.

Table 1. International salmonellosis outbreaks associated with fresh produce.

perature, UV radiation, and moisture content of the soil [7, 35]. In general, temperature has an important effect on growth and decay rates of bacteria. Most studies examined the influence of temperature on survival of enteric bacteria under isothermal conditions, showing a generally reduced survival of *Salmonella* in soil with increasing temperature and, accordingly a better persistence in soil at lower temperatures [36, 37]. Semenov et al. [38] analyzed how temperature fluctuations affect *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) in cow manure and demonstrated increased decay rates with increasing amplitudes of daily oscillations. Besides temperature, water availability is a key factor for *Salmonella* to survive in the environment. Humidity in soil depends on rainfall and watering as well as on evaporation. Soil moisture also depends on soil properties like clay content or pore size. In general, it seems that survival of *Salmonella* in soil is promoted by high humidity while water shortage has a detrimental influence on persistence, probably due to drought stress [39–41]. The soil type and its physical and chemical characteristics have a strong influence on the fate of bacterial HPs. Those characteristics include texture and particle size distribution, which affect adsorption of *Salmonella* to soil particles. The soil type determines the extent of *Salmonella* leaching, if the bacteria are applied to the soil surface via contaminated slurry or manure as shown by Bech et al. [42]. In this study, percolation of *S.* Typhimurium was more pronounced in loamy

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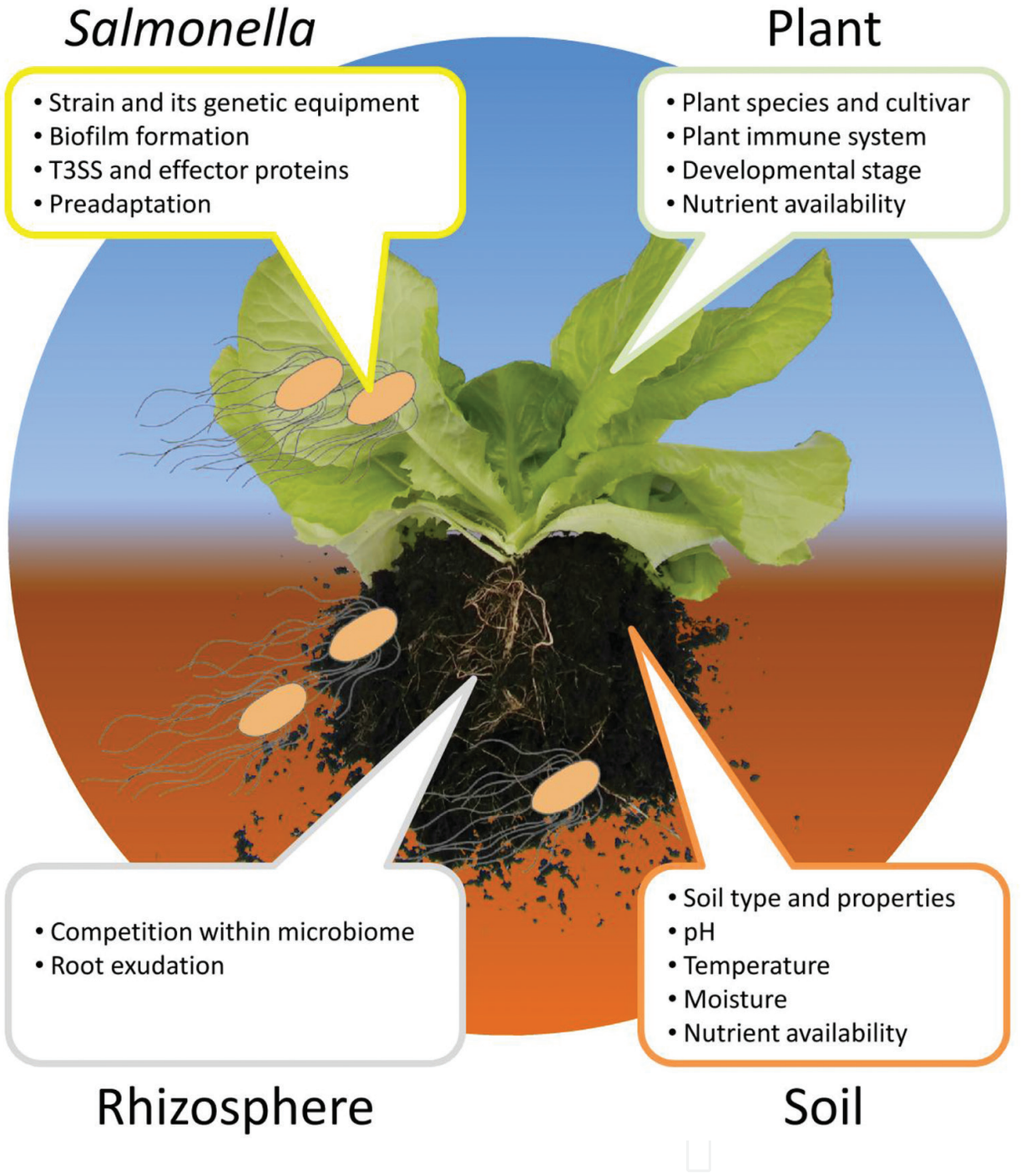

Figure 1. Factors influencing the survival of *Salmonella* in soil and its colonization of plants.

than in sandy soil with leaching bacteria reaching 1 m of depth at 10^5 CFU/ml of leachate. Transport and survival of bacterial pathogens in soil is also influenced by amendment of fertilizers probably because of the presence of organic matter [43]. Leaching of *Salmonella* through soil was observed to reach greater depths after application of slurry than of manure [44]. In the same line, the application method of fertilizers can also have an effect on *Salmonella* survival in soil since an injection of manure or slurry or clumping of the applied fertilizer

aboveground protect bacteria in the soil from desiccation, UV, and high temperatures [43, 45, 46]. Agricultural practices like tillage that have an effect on the porosity of soil determine the extent of leaching [47] and the availability of oxygen. While a detrimental influence of aeration on survival of *E. coli* O157:H7 has been demonstrated, the oxygen availability does not influence the survival of *Salmonella* [48]. Soil pH is also an important factor for *Salmonella* survival and *Salmonella* can survive in the environment with neutral to acidic pH while alkaline pH has a detrimental effect on its persistence [49]. Another important determinant of *Salmonella* survival in soil is the availability of nutrients. In this environment, nutrients can only partly be used by bacterial HPs and are generally rather scarce. *Salmonella* is chemoheterotrophic and therefore depends on carbohydrates, lipids, and protein in its environment as sources for energy, nitrogen, and amino acids. Addition of organic fertilizers improves nutrient availability by addition of readily available carbon and nitrogen sources as well as other nutrients. But amendment of fertilizers also changes the microbiological properties of soil by introducing microorganisms to the soil microbial community. Moreover, the additional nutrients stimulate growth of copiotrophic soil bacteria which might compete for the nutrient resources [50]. So far, no clear correlations between the type of fertilizer and survival in soil have been identified [51]. But when survival in manure was compared to survival in manure-amended soil, *Salmonella* usually survived better in soil [22]. This could be due to competition by the microbial flora of manure, which is more concentrated than in soil.

The soil microbial community and its composition have a great influence on the survival of *Salmonella* [52]. In the soil ecosystem, *Salmonella* has to compete with the indigenous microbial community for space and nutrients [24]. For example, it was shown that *Salmonella enterica* serovar Newport (*S*. Newport) survived about 10 weeks longer in sterilized soil compared to nonsterilized manure-amended soil [22]. Similarly, a better survival was found in γ-irradiated than in untreated soil [53]. These results indicate suppression by the native microbial community. Overall, results demonstrate the importance of the microbial community affecting the fate of *Salmonella* in soil. Plant pathogens, fungi, viruses, and animal pests present in the environment can degrade the plant material and increase the content of available nutrients or provide entry sites facilitating internalization into plants [7, 14, 54]. They may also serve as vectors [7, 55]. Effects of protists have been analyzed using protozoa showing that their presence can foster or reduce survival of different species. For example, *Salmonella enterica* serovar Thompson was accumulated in vesicles of *Tetrahymena* [56], while growth of protozoa can also decimate *S.* Typhimurium populations [37].

In addition to the environment in which *Salmonella* is introduced, the bacterial characteristics are crucial for persistence. Firstly, the genetic disposition of the strain, for example, the presence of type III secretion system (T3SS), the ability to form biofilms, chemotaxis, or motility are important. Studies using strains with mutations influencing these characteristics usually resulted in reduced survival [57–59]. *Salmonella* can also produce an *O*-antigen capsule, which improves survival under desiccation stress [60]. Furthermore, the ability to form biofilms enhances environmental persistence of some *Salmonella* serovars [61]. Similarly, a biofilm-producing *Salmonella* strain survived chlorination significantly better than the biofilm-deficient mutant [62]. The conditions under which *Salmonella* are grown before their inoculation in the environment are also important since preadaptation influences the persistence [20]. Finally,

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many studies on the survival of HPs in soil employed a relatively high inoculum, which under natural conditions occurs only locally, e.g., by fecal point contaminations. Inoculation resulted in a fast initial decline of inoculated bacterial populations with usually low numbers of *Salmonella* that survive for a long time after the inoculation.

In conclusion, studies analyzing the survival of *Salmonella* demonstrated complex interactions with the environment and a network of factors, which might play an important role in the persistence of *Salmonella*. Therefore, the very often contradictory results reflect the variability of strains, their survival strategies in a complex environment as well as differences in experimental setups used.

5. Attachment to plant surfaces and biofilm formation

Attachment and adhesion of *Salmonella* to plant surfaces are essential steps of plant colonization. Several bacterial elements such as fimbrial structures, nonfimbrial adhesins, flagella, cellulose, and lipopolysaccharides (LPS) are important bacterial factors for colonization [63, 64]. Although previous studies demonstrated that the attachment depends on plant and bacterial factors, no single factor was found to be essential, suggesting that bacteria use several parallel mechanisms to ensure attachment to different plants or to different plant cells under a wide variety of conditions [65]. Furthermore, the attachment of *S. enterica* to plant surfaces appears to be serovar-dependent [66]. For example, the strength of the attachment to basil, lettuce, or spinach leaves differed between *S. enterica* serovars. While *S.* Typhimurium, *Salmonella enterica* serovar Enteritidis, and *Salmonella enterica* serovar Senftenberg were efficient, other serovars including *Salmonella enterica* serovar Agona, *Salmonella enterica* serovar Heidelberg or *Salmonella enterica* serovar *Arizonae* showed less attachment [67]. Clear differences in attachment were also observed in leaves of different age, for example, *S.* Typhimurium showed a better attachment to older compared to younger lettuce leaves [68]. Additionally, *S. enterica* serovars were reported to actively move toward plant roots, attracted by root exudates [69]. There, they are able to efficiently attach and to form biofilms at natural openings or wounds [70, 71].

Several other studies provided evidence for biofilm formation by *Salmonella* on plant surfaces [72]. Within biofilms, bacteria are generally well-protected against environmental stresses, antibiotics, and disinfectants. The importance of biofilms for the attachment of *Salmonella* to plants and their role in the persistence in plants was recently described by Yaron and Romling [65]. Biofilm formation of *Salmonella* is influenced by environmental conditions and is reported to be maximal under reduced nutrient availability, aerobic conditions, low osmolarity, and mid temperatures [73], which are characteristic for the plant surface. In contrast, it was shown *in vitro* that *S.* Typhimurium cells grown at 37°C, the temperature in the animal host, do not produce cellulose and fimbriae [64]. Furthermore, the *red dry and rough* (*rdar*) and the *smooth and white* (*saw*) morphotypes, regulated by the *agfD* promoter and defined by a combination of traits such as the presence of thin aggregative fimbriae (tafi), cellulose, and *O*-antigen capsule, might affect the dispersal of *Salmonella* in an agricultural environment [74]. In contrast to the *saw* morphotype, the *rdar* morphotype, isolated from tomato, showed

better attachment to plant surfaces [74, 75]. Biofilm-producing *Salmonella* on parsley showed a higher resistance against disinfectants than the biofilm-deficient mutant. Furthermore, after a storage period of the plant, the cells that were able to produce the biofilm matrix were significantly more resistant to the disinfection treatment [62]. A screening of 6000 transposon mutants of *S*. Newport resulted in the identification of 20 mutants selected for reduced adherence to alfalfa sprouts [70]. Interestingly, these mutants contained insertions associated with genes, for example, for the surface-exposed aggregative fimbriae nucleator (*agfB*) and the general transcriptional regulator *rpoS*. The respective proteins have been reported to regulate the production of curly, cellulose, and other adhesins such as pili. Two other genes (STM0278 and STM0650) were identified as important factors for the colonization of alfalfa seedlings. Both play an important role in the formation of biofilms [76]. Furthermore, bacterial cellulose and curly were involved in the colonization of parsley with *S.* Typhimurium from irrigation water [77].

Although many factors influencing the colonization of plants were identified by *in vitro* experiments, a more detailed investigation of genes of *Salmonella* that are expressed during the colonization of plants is needed. New techniques for the isolation of mRNA from samples containing both plant and bacterial materials as well as for the quantitative PCR allow the analysis of the transcriptome and the identification of genes with related functions [78].

6. Internalization of *Salmonella* into plant tissues

An increasing number of salmonellosis outbreaks associated with plants shows that human pathogenic bacteria use plants as a niche for replication or as hosts and vectors for animal and human infection (**Table 1**). For a long time it was assumed that *Salmonella* rather survives on plant surfaces than colonizes the plant interior. This view has been challenged by recent reports. Today we know that *Salmonella* can actively enter and spread within the plant. Plants offer multiple entry possibilities for HPs; stomata, for example, were identified already a few years ago. Stomata are used for gas exchange between the surroundings and cells of the inner mesophyll layers, this is necessary for proper photosynthetic efficacy. They can close if a pathogen is recognized. Some pathogens, however, produce toxins (coronatine), which reopen stomata and therefore allow their use as gates for colonization of underlying tissues. *Salmonella* was shown to gather around the open stomata and enter the mesophyll tissue of lettuce leaves [58]. Similar to lettuce, a high incidence of internalization was observed in arugula leaves, while romaine and red-lettuce, as well as basil showed significantly lower internalization rates [79]. Interestingly, in this study parsley and tomato leaves showed only marginal internalization [79]. In addition to stomata, also hydratodes and trichomes allow an internalization of *Salmonella* into leaves [31, 80, 81]. Not only *Salmonella* or phytopathogenic bacteria use stomata as entry points, also other *Enterobacteriaceae,* for example *E. coli,* use similar strategies to access the plant's interior [82, 83]. Importantly to note is the fact that the preference to gather around open stomata manifests only in photosynthetically active leaves, and

an artificial opening of the stomata at night has no effect on the bacterial behavior [58]. This observation is in line with the proposed hypothesis that those bacteria are in a direct competition for C- and N-sources with the native leaf microbiome [69, 71], and suggests a chemotaxis toward the newly synthesized products of the photosynthesis.

From the consumers' point of view, not only the internalization into leaves but also the translocation within the plant, e.g., toward fruits is important. In some crop plants, e.g., tomato, such translocation was detected [81]. The authors showed internalization into the tomato fruits when the entire plant was systemically colonized. Still, the colonization rates seemed rather low [81]. Nonetheless, in light of the persistent pathogenicity in animals after the passage through a plant host [84], the internalization mechanisms are of high interest. Some detailed mechanisms were already suggested. Erlacher and coworkers proposed one of those possible mechanisms: colonization of the niche below the cuticle layer of the epidermis [9]. Obviously such a behavior protects bacteria from the harsh conditions on the leaf surface (UV light, drought, and quick changes in temperature) but also from surface sterilization agents. Another strategy would be an intracellular lifestyle, which would resemble the strategy in the animal infection model. Until now, this possibility remains unverified, two reports postulated internalization into plant cells using *Arabidopsis* and tobacco systems [84, 85]. Yet, another helpful strategy is the efficient formation of biofilms, this strategy was discussed above and was reviewed by Yaron and Romling [65]. Only recently, it was discovered that particular *Salmonella* strains may avoid the recognition by the plant immune system [86], which would make them very well adapted colonizers (see below).

Many row eaten crop plants plants associated with salmonellosis outbreaks or food poisoning are usually grown in soil (lettuce, basil, parsley, etc.). In such cases the translocation from the potentially contaminated soil (through manure or irrigation water) via roots into the harvested and consumed plant parts is of enormous importance. Several reports assessed already this possibility and pointed at a very diverse picture with regard to pathogenic *E. coli* or *Salmonella*. Here the high heterogeneity with regard to colonization in the plant population is very remarkable [69, 87], usually about 20% of the plant population is colonized, however, this range may vary from 0 to 100% and strongly depends on plant species and bacterial strain [51, 69, 77, 88–90].

7. The function of T3SS and the role of plant immune system during the interactions between plant and *Salmonella*

Bacterial pathogens use T3SS and T4SS to inject so-called effector proteins directly into the cytoplasm of host cells. Those effectors are able to manipulate the host immune system and suppress the otherwise negative effects of defense responses. *Salmonella* uses two T3SS and more than 40 effectors in order to manipulate the immune system (perception mechanisms and signaling cascades) as well as the cytoskeleton of animal cells at different stages of the infection process [91]. Recent discoveries from others and our group imply that the

mechanisms used in animal and plant hosts may resemble each other [59, 85, 86, 92–95]. The inoculation with the wild-type *Salmonella* strains and mutants in one or both of the T3SSs showed that functional secretion systems are required for efficient plant colonization [59, 85, 93]. Two observations allow such a conclusion: (1) The mutants had lower proliferation rates when compared to the respective wild type, which suggests that a functional T3SS helps with the colonization of plants; and (2) T3SS mutants induced stronger immune response of the host plant. Similar to animals or humans, plants respond to colonization of pathogenic bacteria inducing numerous immune responses, among others are oxidative burst and enhanced expression of *Pathogenesis Related* (*PR*) genes. Both were observed after inoculation with *Salmonella* and both were stronger if the inoculation was performed using mutants in T3SS [59, 85, 86]. Those results suggest that the wild-type strain is able to suppress the immune response. It is very plausible to think that this suppression is due to functional T3SS-dependent effector proteins. We know only little about their function in plant cells, since only two effectors (SseF and SpvC) were evaluated in this respect. SseF together with SseG are translocated into animal cells and are responsible for the establishment of the reproduction niche [96]. In plants, SseF induces the hypersensitive response (HR) [94]. Important is the fact that silencing of the suppressor of SGT1 eliminates the response to SseF, suggesting that this effector is recognized in R protein-dependent manner, which is the usual recognition method of pathogen effectors during the effector-triggered immunity (ETI). SpvC is a phosphothreonin lyase which dephosphorylates activated MAP kinases. Those kinases build a core compound in the signaling cascade leading from the perception of the pathogen on the cell surface to the transcriptional response at the chromatin level. Especially the trio MPK3, MPK4, and MPK6 plays an important role in plants [97], and is activated (phosphorylated) during the response to *Salmonella* [84]. SpvC interacts actively with the MPK6 and dephosphorylates this kinase, consequently abolishing the signal transduction [95]. A comprehensive overview of the reports regarding the plant immune responses to HPs was published only recently and is an excellent compendium of the current knowledge [98].

8. *Salmonella* changes its physiology in contact with plant host

During the interaction between *Salmonella* and crop plants, not only the plant reacts to the presence of the bacteria, also *Salmonella* adapts to the conditions represented by a plant organism. Recent results show that bacteria modify their physiology and motility in order to adjust to the physiological conditions occurring in plants. Several authors evaluated the transcriptional changes of bacteria when in contact with plants or plant-originated products [99, 100]. Interestingly, the analysis of the transcriptome, revealed a partial overlap between bacteria from macerated lettuce or cilantro leaves and bacteria from intestine, suggesting that those bacteria might be better adapted to the exploitation of plant material than estimated [100]. Similar results were observed for the pathogenic *E. coli* O157:H7, which seem to change its enzymatic and metabolomic equipment in order to utilize plant compounds [101, 102]. In addition, the bacteria upregulate a plethora of genes related to attachment, antimicrobial

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resistance and response to oxidative stress [101]. Very striking was the fact that although plant filtrates or root exudates contain numerous amino acids, which are available to the bacteria as C and N sources, *E. coli* induced many amino acid synthesis pathways probably to supplement the missing compounds [102].

9. Detection, characterization and quantification of *Salmonella* in environmental samples

Salmonella is rarely detected in crop plants. For example, in a previous study *Salmonella* could not be detected in more than 170 plants but in their environment [103]. This suggests that environmental factors are affecting the prevalence of *Salmonella* in the field, or that the sensitivity of the currently used detection system is not sufficient.

Traditional methods for the detection and identification of HPs often rely on cultivation-dependent techniques followed by biochemical and serological identification, which is typically time-consuming and laborious [104]. Furthermore, in response to environmental stresses *Salmonella* can enter a physiological state where the cells remain viable, but are no longer culturable on typically used growth media. *Salmonella* in this VBNC state are often only detectable by methods depending on nucleic acids. This highlights the importance of the complementary use of cultivation-dependent and -independent detection methods for the diagnosis and prevention of food contamination and foodborne diseases. In the recent decades, there have been increasing efforts to develop and improve molecular methods for the rapid detection and characterization of pathogens in animals and animal products [105–108]. These methods, which include immunological as well as biosensor- and nucleic acid-based assays (e.g., ELISA, PCR, microarrays, next generation sequencing) have an improved sensitivity and specificity but are also time-, cost-, and labor-demanding. Typically, to further increase the sensitivity of these methods nonselective or selective enrichment steps are employed. One of the most challenging problems is the sample preparation, which is strongly depending on the sample matrix, associated inhibitory compounds, and the bacterial load.

So far, knowledge is scarce regarding the specific and reliable detection of *Salmonella* in complex and often heterogenous plant- and environmental-matrices (e.g., vegetables, spices, soil samples, manure, biogas digestates) as well as the appropriate extraction and purification techniques. For iceberg lettuce, carrot- and cucumber-peelings, qPCR detection limits of 10^3 bacterial cells per gram were reported [109]. Since the infectious dose of *Salmonella* was reported to be less than 10^3 cells [110], small numbers have to be detected reliably. Besides direct extraction of total DNA from the sample material, a preceding enrichment step in the respective culture media can be performed. This enrichment has the advantage to increase the sensitivity of detection and additionally to reactivate cells in the dormant VBNC state. After extraction of DNA from the respective samples, *Salmonella* can be detected by qPCR or PCR-Southern blot hybridization, e.g., by detection of the *invA* gene [104, 111]. Alternatively or additionally to DNA-based methods, RNA-based methods

can be used. Apart from a more laborious sample preparation, RNA-based methods have the advantage that in contrast to DNA-based methods only living and active *Salmonella* are detected, i.e., *Salmonella* relevant for a potential infection of humans. Especially the detection of mRNA of pathogenicity determinants could be appropriate to prove the viability and potential virulence of HPs.

Microarrays and next-generation sequencing technologies offer intriguing possibilities regarding the rapid and accurate detection as well as genetic characterization of *Salmonella* in environmental matrices. However, the costs and technical requirements for the analysis of large data sets still limit their practicability in the day-to-day qualitative and quantitative detection. The further development of rapid, reliable, and cost-effective high-throughput detection methods will very likely contribute to the understanding of the ecology of *Salmonella* in the plant environment and consequently help to reduce or prevent infections mediated by plant-associated HPs.

10. Conclusions

Today the notion that human pathogenic bacteria such as *Salmonella* might persist on or within plants in low numbers is widely accepted. The research on the interactions between crop or model plants and *Salmonella* is obviously driven by its medical aspects and the need for better prevention methods. We already know various features of the interactions but many are still not fully understood. New techniques that use high-throughput analyses and unbiased approaches are useful. Numerous national survey agencies started to use next-generation sequencing for the epidemiological analysis of salmonellosis outbreaks and have therefore direct access to the genome sequences of particular serovars. They are also able to monitor the genomic changes, for example, reception of new plasmids or pathogenicity islands, which are important prerequisites in virulence of the bacterial strain. Similarly, the full range of "omic" approaches is being used in model systems providing very detailed data on both partners in the *Salmonella*-plant interaction at biochemical, physiological, and transcriptional levels. The study of those interactions harbors even more potential, it permits the characterization of the different infection mechanisms and the different strategies available for *Salmonella* in contact with diverse hosts. New and more efficient prevention strategies greatly depend on our understanding of these mechanisms. Therefore, the new findings might significantly improve our possibilities to diminish the number of future outbreaks.

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