Salmonella exploits the reactive oxygen species generated by the plant immune system to enhance colonization

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Abstract

Understanding bacterial interaction with the plant immune system is essential for building a sustainable food system. Discouraging pathogens enhancing beneficial bacteria and preventing food spoilage and contamination by human enteric pathogens. Immune system activation triggers a burst of reactive oxygen species (ROS). We discovered that the food-poisoning bacterium *Salmonella* exploits the plant ROS to enhance biofilm formation and colonization. Scavenging the ROS with antioxidants reduces colonization. Further, combining the antioxidant vitamin C and immune system activation with salicylic acid inhibits *Salmonella* colonization synergistically of the plant model Arabidopsis and edible plants. This safe-to-use combination is an eco-friendly method to prevent plant contamination by *Salmonella*, *Listeria* and enteropathogenic *E. coli.* The exploitation of plant immunity for enhancing colonization is not exclusive to *Salmonella*. It is exhibited by *pseudomonas* species isolated from plants, suggesting it is a general plant colonization strategy.

Introduction

The increase in the world population, global warming and the reduction in the efficiency of chemical fertilizers and pesticides necessitate the building of sustainable food systems [1]. Understanding plant microbe interaction at the molecular level is an avenue to reach this goal. Better understanding will enable us to discourage pathogens, enhance beneficial bacteria and prevent food spoilage and contamination by human enteric pathogens [2].

Food poisoning by enteric pathogens is a leading cause of morbidity and mortality worldwide, affecting one of every ten people annually [3]. The increased consumption of fresh fruits and vegetables (fresh produce), due to awareness of their importance to a healthy lifestyle and the extension of the supply chains, made it a significant source of food-borne illnesses. The Gram-negative bacteria *Salmonella* and *E. coli* and the Gram-positive bacterium *Listeria monocytogenes* are the three main bacteria contaminating fresh produce [3-5]. Chemical

disinfection of fruits and vegetables adversely affects workers' and consumers' health. Thus, eco-friendly methods to ensure food safety are highly needed [6].

Research from recent years revealed that human pathogens may utilize plants as a secondary host [5,7]. They exhibit a complex interaction with plants and activate plant immune responses. Thus, understanding their plant colonization process could inform rational approaches to fight enteropathogenic bacterial contamination. In this regard, utilizing plant immunity to fight salmonella contamination is extensively explored [6,8]. We have analyzed the molecular interaction between the enteropathogenic bacteria *Salmonella enterica* and the plant model Arabidopsis thaliana. As previously shown, the *Salmonella* activates the plant immune system [9-11]. The first level of plant immunity comprises receptors that recognize conserved microbial molecules, e.g., flagella, elongation factor Tu, prevalent in both plant pathogens and non-pathogenic bacteria. Receptor activation leads to a burst of reactive oxygen species (ROS) and a signal transduction cascade [12]. Plant pathogens employ a plethora of effectors that target plant immunity, mainly delivered through the type III secretion system. Thus, they can evade the first protection line and grow to a high number [13]. How commensal bacteria can evade immune system toxicity is much less understood [14].

Bacteria synthesize extracellular matrix to facilitate surface adhesion [15]. The extracellular matrix comprises sugar polymers, protein fibres, extracellular DNA and cell debris. Bacteria encased in a matrix are known as biofilm [16]. The matrix protects the bacteria from chemical assaults and, as such, poses a serious challenge for antibiotic treatment [17]. Biofilm formation is essential for root colonization [15,18]. However, the effect of the biofilm matrix on bacteria dealing with the plant immune system needs to be explored.

Our research revealed that biofilm formation protects Salmonella from the reactive oxygen species (ROS) produced upon plant immune system activation. Further, the bacteria exploited the ROS to enhance biofilm formation and colonization. Thus, immune system recognition paradoxically enhances root colonization.

Revealing the importance of ROS for facilitating biofilm formation and colonization enabled us to outsmart the bacteria. Scavenging the ROS with antioxidants reduced bacterial colonization and sensitized the bacteria to the plant immune system activity. The combination of the antioxidant ascorbic acid (vitamin C) and immune system activation by the plant hormone salicylic acid (SA) exhibited synergistic inhibition of bacterial contamination. Finally, the combination of vitamin C and SA inhibits plant colonization of various salmonella serovars,

listeria monocytogenes and enteropathogenic E. coli, both in Arabidopsis and in edible plants, making it a promising method to ensure food safety.

Results

As previously shown [9], *Salmonella* (We used *Salmonella enterica Serovar Typhimurium 14028s* throughout the paper unless otherwise stated) stimulated immune system activation in Arabidopsis through its flagellar epitope (Figure 1A), WT bacteria stimulated pFrK1 promoter expression, a downstream target of the FLS2 receptor [19], while *ΔflgB* bacteria lacking flagella [20], failed to do so (Figure 1A). To understand how the plant immune system modulates bacterial colonization, we inoculated bacteria into *fls2* roots, perturbed in flagellar perception [21]. Surprisingly, bacteria exhibit a significant reduction in root colonization on *fls2* roots and the triple mutant bbc (*bak1,bkk1,cerk1*) lacking pattern-triggered immunity [22] (Figure 1B). Immune system activation triggers a burst of ROS production to kill the invading pathogen [23,24]. Inoculating *Salmonella* into *rbohd rbohf* plants perturbed in ROS production [25] also revealed a reduction rather than an increase in root colonization (Figure 1B), [compare for example to[24]]. It has been previously shown that *Salmonella* colonizes the endophyte of Arabidopsis [11]. However, we failed to detect any colonization of surface-sterilized roots in our system (data not shown)

Next, we monitored root colonization by GFP-expressing bacteria. On WT roots, bacteria appeared in long strips (Figure 1C). However, on *rbohd rbohf* plants, bacteria failed to form strips (Figure 1C). Thus, our results suggest that immune system activation and ROS production are necessary for efficient root colonization by *Salmonella*.

Biofilm formation is crucial for bacteria to adhere to plants. We examined the colonization of mutant and constitutively expressing strains of CsgD, a central regulator of biofilm formation in *Salmonella* [26]. We found that *ΔcsgD* cells exhibited a significant reduction in root colonization, while PcsgD cells constitutively expressing CsgD [26] exhibited a significant increase (Figure 2A), confirming the importance of biofilm formation for root colonization. Notably, ROS enhances biofilm formation in vitro (Figure 2B). Thus, we hypothesized that plant ROS promotes root colonization by stimulating biofilm formation and failing to induce ROS production on *rbohd rbohf* and immune receptor mutant plants reduces *Salmonella* colonization. Consistently, the colonization of *ΔcsgD* cells isn't reduced when colonizing *rbohd rbohf* plants (Figure 2C). On the contrary, *ΔcsgD* cells exhibited a significant increase in the

colonization of *rbohd rbohf* and bbc plants (Figure 2C), suggesting that the ability to form biofilm protected the WT bacteria from ROS.

The results so far suggested that ROS positively affect salmonella colonization. Thus, we wondered if chemical ROS scavengers will inhibit colonization. Indeed, diverse chemical scavengers, including vitamin C, rutin, KI, TEMPOL and GssG reduced root colonization (Figure 2D). We further concentrated on vitamin C, which is a common antioxidant and is considered safe for human consumption even at high doses. Of note, the effect of vitamin C was independent of its acidity as we titrated the pH back to normal. Further, other scavengers like KI and rutin have neutral pH. Vitamin C did not affect biofilm formation *in vitro* (Figure 2B), but it can at least partially abolish the increase observed upon H_2O_2 addition (Figure 2B).

The results so far indicate that Salmonella exploits plant immunity to enhance biofilm formation. Nevertheless, stimulating plant immunity using the stress hormone salicylic acid (SA) significantly inhibits salmonella colonization (Figure 3A) [6]. SA did not affect the colonization of pCsgD, consistent with the ability of the biofilm matrix to protect the cells from the immune system (Figure 3A). We next examined the application of vitamin C and SA together. Applying both chemicals exhibits synergistic inhibition of salmonella colonization (Figure 3B) (The observed reduction is significantly lower than the theoretical combination of vitamin C and SA, $P<0.05$). Neither the compounds nor their combination affect bacterial growth in vitro (Figure 3C), indicating that their effect is plant-specific. We reason that vitamin C inhibited biofilm formation by scavenging ROS and thus exposed the bacteria to the SA-mediated immune system activity independent of ROS, leading to a synergistic inhibition of root colonization.

The genus Salmonella harbours more than two thousand different serovars, with a few dozen that can cause disease in humans. The combination of vitamin C and SA significantly inhibited the colonization of 11 different serovars isolated from human patients (Figure 4A), with inhibition efficiency ranging from 60% (*S. virginia*) to 95%-99% in most other serovars. We wanted to know if our results applied to other human enteric pathogens associated with plant food contamination. Examination of root colonized by enteropathogenic *E.coli* (EPEC serotype O127:H6) [27] and the gram-positive pathogen *Listeria monocytogenes* (strain 10403s) revealed a synergistic effect for vitamin C and SA for inhibiting bacterial colonization (Figure 4B-4C). Next, we explored the effect of vitamin C and SA on salmonella colonization of edible plants. Using sterile alfalfa sprouts and Basil leaves (Ocimum basilicum), we confirmed the

effect of vitamin C and SA on salmonella contamination of these economically important foods (Figure 5A-5B). Thus, the effect is extended beyond Arabidopsis to edible plants and also beyond the root to the stems and the leaves, highlighting its general effect. Interestingly, neither vitamin C nor SA inhibits leaf colonization alone, but the combination efficiently inhibits colonization (Figure 5B).

The experiments so far were done on sterile plants grown in axenic conditions without resident microbiota. Finally, we examined the effect of vitamin C and SA on Salmonella while colonizing non-sterile alfalfa sprouts. Notably, the colonization of nontreated plants was reduced by two orders of magnitude, suggesting that competition with the resident microbiota interferes with the pathogen's colonization (Figure 5C). Nevertheless, vitamin C and SA further inhibited the colonization (Figure 5C). Similar inhibition was observed for *Listeria monocytogenes* (Figure 5D).

Discussion

Salmonella E.coli and *Listeria* are the three main bacteria causing contamination of fresh produce, leading to enormous health and economic burdens. In order to develop sustainable and environmentally friendly methods to control fresh produce contamination by enteric pathogens, we need a mechanistic understanding of their interaction with plants. Our research revealed that Salmonella exploits the plant immune system activation, using plant-produced ROS to stimulate biofilm formation and root adhesion. Interestingly, biofilm protects the bacteria from the plant ROS, suggesting a feedback loop where 1. colonization stimulates immune system activation 2. The immune system produces ROS to inhibit the bacteria. 3. Bacteria are able to utilize ROS to enhance biofilm formation 4. Biofilm protects bacteria from the immune system and further enhances colonization. 5. Vitamin C attenuates ROS production, thus reducing root colonization; and 6. Sensitize the bacteria to the immune system activity (Figure 6A). Understanding the interaction of beneficial bacteria with plants is essential for sustainable agriculture. We hypothesize that the feedback loop between the plant immune system and the biofilm matrix is utilized by diverse plant resident microbiota to deal with the plant immune system. Of note, we monitored root colonization by a collection of bacteria from the Pseudomonadota phylum isolated from diverse environments. We found isolates that exploit the plant immune system and exhibit a reduction in colonization on bbc triple mutant roots (Figure 6B). Interestingly, all the 3 isolates that seem to exploit the plant immune system were isolated from the leaf niche and may evolve to adapt

to the plant environment. However, a larger sample size and a careful comparison are necessary to support such a conclusion. We have previously demonstrated that immune system activation enhances *Bacillus veleznesis* root adhesion [24]. The current work further highlights the complex interaction between bacteria and the immune system, beyond merely bacterial killing or growth inhibition.

Notably, our work demonstrates how mechanistic understanding of the complex interaction between bacterial colonization and plant immunity opens up a new avenue for dealing with food contamination. We discovered that safe-to-use antioxidants like vitamin C can inhibit plant colonization by enteric pathogens and synergize with immune system activation by salicylic acid. The results from the root of the model plant Arabidopsis were also applied to basil leaves and alfalfa sprouts, even under non-sterile conditions in the presence of resident microbiota. Vitamin C and low doses of salicylic acid are safe for human consumption and have other health benefits on their own [28,29]. Further exploring the utilization of this combination on fresh produce is a promising avenue for sustainable food safety.

1 **Figures**

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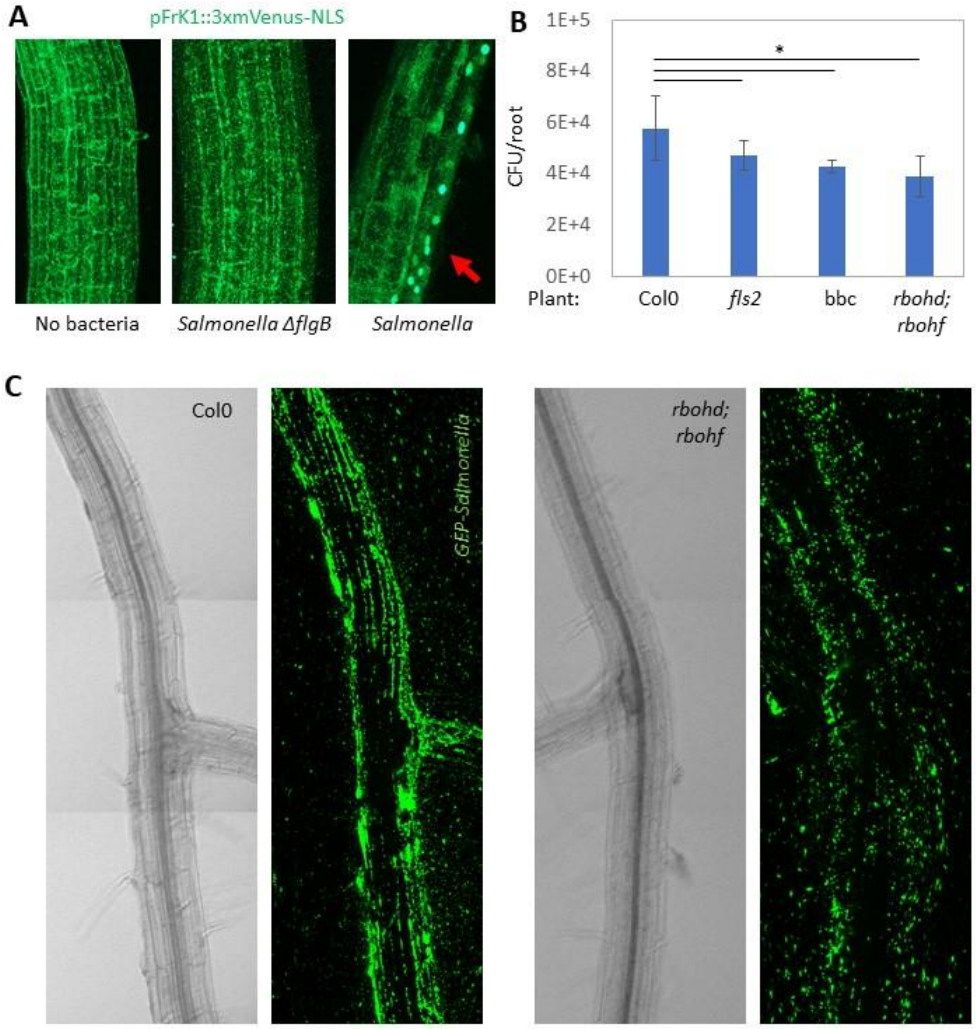
Figure 1



23 **Figure 1. Immune system activation enhances root colonization by *Salmonella***

24 **(A)** 7 days old Arabidopsis seedlings (pFrk1::3xmVenus-NLS) were inoculated with bacteria on
25 MS agar plates. mVenus fluorescence was monitored after 18 hrs using confocal microscopy.
26 Shown are representative maximal projection x20 fluorescent images out of 4 roots from each
27 treatment. WT but not *ΔflgB* bacteria induces pFrk1 promoter expression (red arrow).

28 **(B)** Seedlings of the indicated genotypes were inoculated with *Salmonella*, and the number of
29 colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations
30 (SD) of bacterial colonization from at least 3 independent experiments with 3 seedlings in
31 each. * = $P < 0.05$. The bacteria exhibit a small but significant decrease in colonization of
32 immune system mutant roots.

(C) Salmonella constitutively expressing GFP were inoculated into seedlings of the indicated genotypes for 48 hrs. Root colonization was monitored by confocal microscopy. Shown are representative maximal projection x20 fluorescent images and the complementary brightfield images of the same roots, out of 4 roots from each treatment. Bacteria colonizing WT roots (Col0) appeared in long stripes, while on roots mutant in ROS production (*rbohd;rbohf*) they did not form these strips.

Figure 2

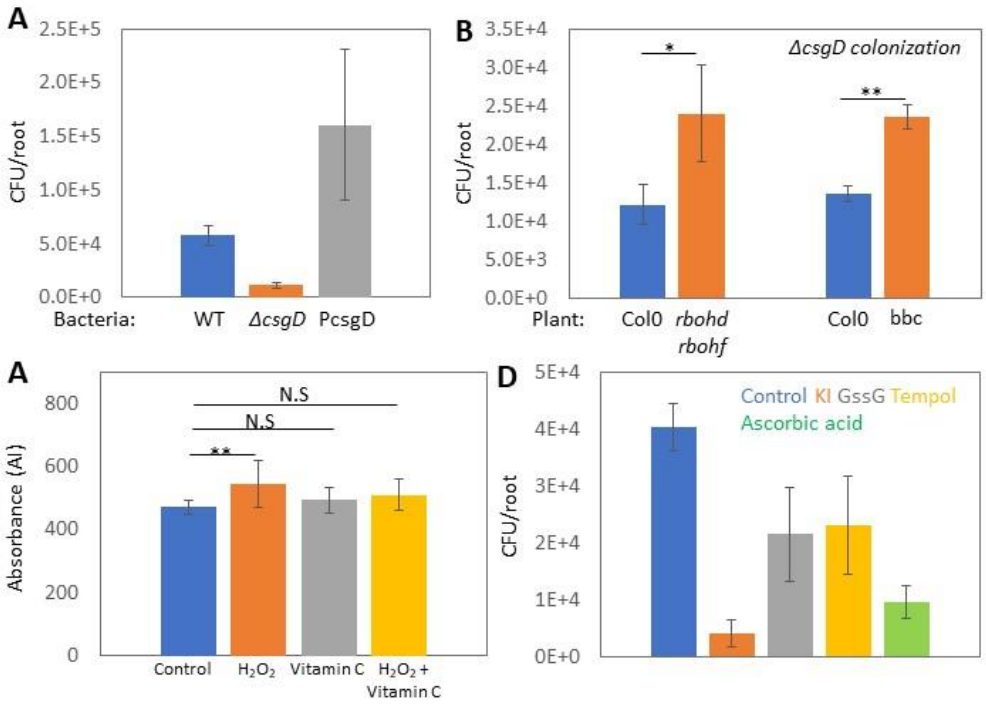


Figure 2. ROS enhances biofilm formation on the root

(A) 7 days old seedlings were inoculated with the indicated *Salmonella* strains on MS agar plates, and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 seedlings.

(B) Seedling of WT and the indicated immune mutant plants were inoculated with ΔcsgD bacteria, and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from at least 2 independent experiments with 3 seedlings in each. * = $P < 0.05$, ** = $P < 0.01$. ΔcsgD bacteria exhibited a significant increase in colonization on immune system mutants, in contrast to the decrease exhibited by WT bacteria.

(C) Bacteria inoculated into LB with no NaCl with the addition of 100µM H_2O_2, 1mM vitamin C, a combination of both or no addition, in a multi-well plate. The plate was kept with no shaking at 23° for 5 days, and adhesion to the well was monitored by crystal violet staining as an indicator of biofilm matrix formation. Shown are average and SD of at least 6 wells, * = $P < 0.05$.

(D) Seedlings were inoculated with bacteria on control plates or plates with 1mM of the indicated chemical antioxidants and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from at least 3 seedlings.

Figure 3

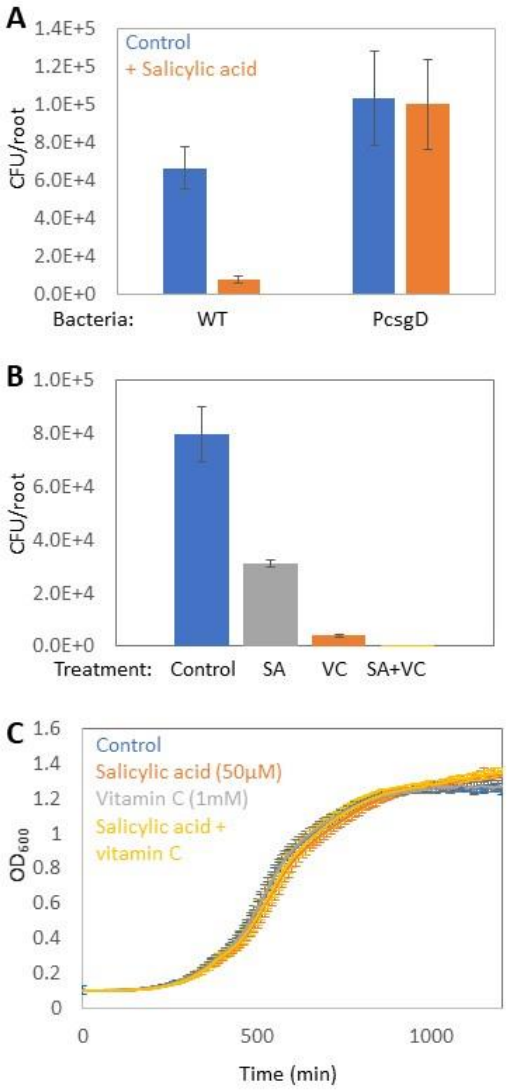


Figure 3. Salicylic acid and vitamin C synergistically inhibit root colonization

(A) 7 days old seedlings were inoculated with the indicated *Salmonella* strains on MS agar plates with or without 50µM salicylic acid, and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. ***=P<0.005.

(B) seedlings were inoculated bacteria on MS agar plates with the indicated chemicals: 50µM salicylic acid, 1mM vitamin C and a combination of both, and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. ***=P<0.005.

(C) Bacteria were grown in LB with the addition of the indicated chemicals and OD_{600} measured. Shown are averages and SD of 5 independent cultures for each treatment.

Figure 4

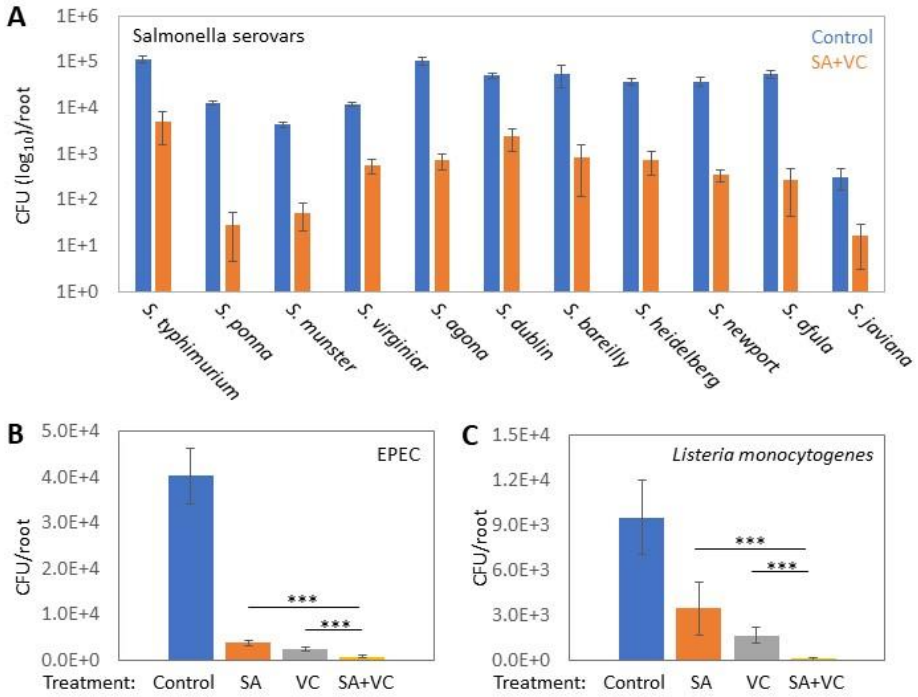


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Figure 4. SA and VC affect root colonization by diverse human pathogens

(A) 7 days old seedlings were inoculated with the indicated *Salmonella* serovars on MS agar plates with or without a combination of 50µM salicylic acid and 1mM vitamin C. The number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard

deviations (SD) of bacterial colonization from at least 4 seedlings for each serovar. For all the serovars, the P<0.005, except for *S. Bareilly* and *S.* Havana, where P<0.05.

(**B-C**) Seedlings were inoculated with either enteropathogenic *E. coli* (O127:H6) (B) or *Listeria monocytogenes* (10403s) with the indicated chemicals: 50µM salicylic acid, 1mM vitamin C and a combination of both, and the number of colonizing bacteria determined after 48hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. ***=P<0.005.

Figure 5

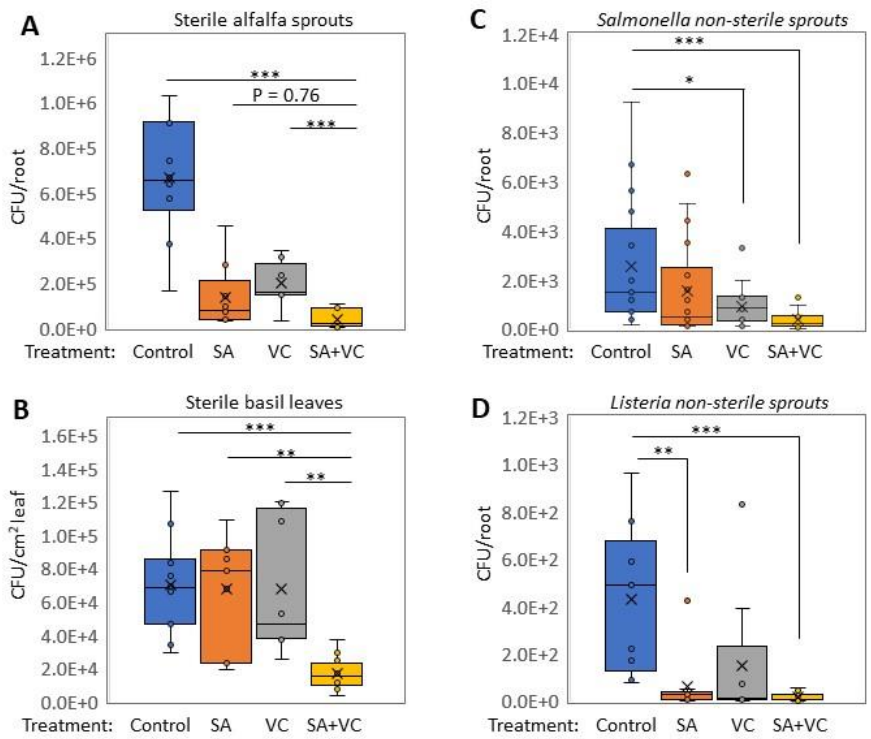

Figure 5. SA and VC affect basil leaves, and alfalfa sprout colonization

(A) 5 days old alfalfa seedlings were inoculated bacteria on MS agar plates with the indicated chemicals: 50µM salicylic acid, 1mM vitamin C and a combination of both, and the number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. ***=P<0.005.

(B) Leaves excised from 5 sterile grown 5 weeks old basil and placed on MS agar plates with the indicated chemicals as described in (A) and the number of colonizing bacteria determined after 48hrs. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. **=P<0.01, ***=P<0.005.

(**C-D**) nonsterile alfalfa sprouts were inoculated with GFP expressing *Salmonella* (C) and *Listeria* (D) with the indicated chemicals as described in (A), and the number of colonizing bacteria was determined after 48 hrs after plating on semi-selective plates and examination under the microscope. Shown are averages and standard deviations (SD) of bacterial colonization from 3 independent experiments with 3 seedlings in each. *=P<0.05, **=P<0.01, ***=P<0.005.

Figure 6

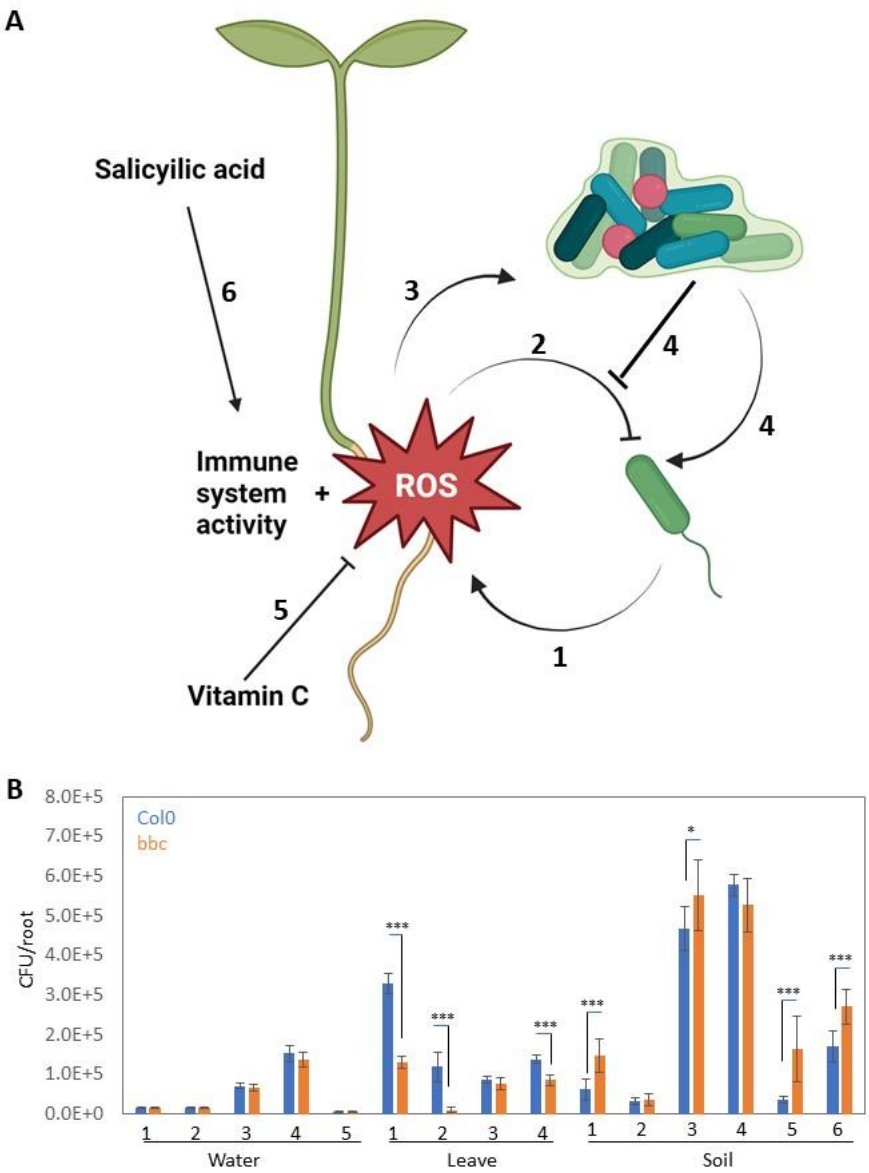

Figure 6. A model describing how Salmonella exploit the plant immune system and how we can outsmart him

(A) The plant immune system recognizes the Salmonella and activates ROS production and downstream events (1) to inhibit bacterial colonization (2). Salmonella directs the ROS for enhancing biofilm formation and promoting root colonization (3). The biofilm formation further protects the bacteria from the immune system activity (4). Reducing ROS by antioxidants like VC blocks this biofilm trigger (5) and, in turn, makes the bacteria vulnerable to the plant immune system activated by SA (6). Thus, VC and SA exhibit synergistic activity in preventing plant contamination by Salmonella and other pathogens.

(B) 7 days old Col0 and bbc seedlings were inoculated with bacteria from the Pseudomonadota phylum isolated from 3 different habitats (see Table S1 for the bacterial identity as determined by 16s sequencing). The number of colonizing bacteria was determined after 48 hrs. Shown are averages and standard deviations (SD) of bacterial colonization from at least 4 seedlings for each bacterium.

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In memory of Prof. Philip Benfey a great mentor and person.

Material and methods

Plant and bacterial strains are listed in Table S1

Plant growth

Arabidopsis seedlings and sterile alfalfa seedlings were grown on 0.5 MS media containing 1.1 gr Murashige and Skoog basal salts (in 500 ml ddH2O), 1% sucrose, 1% agar and 5 ml (in 500 ml ddH2O) MES (50 gr/l, pH=5.8 with NaOH). Arabidopsis seeds were stratified for 2 d in a 4C dark room and grown vertically for 7 days under long-day light conditions. Sterile alfalfa seedlings were grown vertically for 5 days under long-day light conditions. Non-sterile sprouts were bought in the local market. Basil plants were grown in a sterile box on 0.5 MS media for 5 weeks under long-day light conditions and then leaves were excised for the bacterial inoculation experiments.

Monitoring bacterial growth and plant colonization

Fresh bacterial colonies were grown at 250 RPM, shaking overnight in LB media at 23°. The next day, the culture was diluted 1:500 in fresh LB and grown at 37° for OD_{600}=1. We washed the bacteria in 10mM $MgCl_2$ buffer and then diluted the culture 1:100 in 10mM $MgCl_2$. 2µL of the diluted culture was gently applied on the tip of the root/stem (alfalfa)/cut leaf surface (basil) and placed on an ½ MS agar plate with no sucrose. The square plates were kept in a vertical position during the incubation time at 23° under long-day light conditions (16 h light/8 h darkness) in a plant growth chamber. For bacterial CFU counting and microscopy, plants were incubated with bacteria for 48 hrs. Then, the inoculated plant roots were cut and washed three times in sterile water. The seedlings were transferred to a tube with 1 ml of $MgCl_2$ and vortexed vigorously for 20 seconds, then serial dilutions were plated on LB plates for CFU counting or observed under confocal microscope Zeiss LSM980. The whole root and stem were taken from Arabidopsis and alfalfa, while a circle cut of 5mm in diameter was taken from the basil leaves. For colonization of non-sterile sprouts, we used GFP *Salmonella* and GFP *Listeria* and plated the samples on Kanamycin plates for the Salmonella and Listeria selective enrichment supplement containing plates (Sigma) for the Listeria. The identity of the bacteria was validated by monitoring the GFP expression under a binocular (Leica). When indicated, the following chemicals were added to the MS media: salicylic acid, KI, ascorbic acid, rutin, GssG, and TEMPOL—all the chemicals purchased from Sigma Aldrich. The pH of the MS was titrated back to normal after the addition of salicylic acid and ascorbic acid with NaOH.

Bacterial growth in the presence SA and vitamin C was measured in 48 well plates. Overnight cultures were diluted into fresh media with or without the chemicals, and OD_{600} was measured every 10 minutes for 12 hours in Tecan spark 10M.

Biofilm formation assay

Quantification of biofilm formation was done as described previously [30]. Briefly, the overnight culture of bacteria was diluted into LB with no NaCl in a multi-well plate. The plates were incubated at 23° for 5 days without shaking. After 5 days the media was poured, and a solution of 0.1% crystal violet was added and incubated for 10 min. Next, the wells were washed 3 times with tape water and dried on the bench. A solution of 33% acetic acid was added to dissolve the crystal violet bound to the wall, and the OD_{570} was measured.

Pseudomonas isolation

We isolated bacteria from the phylum pseudomonade from the local area (Kiryat Shmona, north of Israel) from 3 different niches: Local soil, local stream, and mint leaves. The soil was vigorously shaken in LB. The leaf was ground in LB. The samples were plated on Pseudomonas isolation agar (Sigma) for 48 hours at 30°. Colonies were re-streaked on Pseudomonas isolation agar to get pure colonies, and the bacterial identity was determined by 16S sequencing (Table S1).

Data analysis and presentation

Statistical analysis (student T-tset) was done in Excel. Data representation in graphs was done in Excel. Figure 6A was done in Bio-Render agreement number: UA261QD9SP.

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1 **Table S1. Bacterial and plant strains**

Bacterial strains		
Strain Name	**Description**	**Reference and Comments**
Salmonella strains		
Salmonella enterica 14028s	Salmonella enterica subsp. enterica ATCC 14028s	Kindly provided by Prof. Edward Miao Duke university
Salmonella enterica 14028s *ΔflgB*	*flgB::*Tn10 [31]	Kindly provided by Prof. Edward Miao Duke university
Salmonella enterica 14028s GFP	Wild type S. enterica 14028s strain with constitutive GFP expression	Kindly provided by Prof. Edward Miao Duke university
Salmonella enterica 14028s *ΔcsgD*	SLN112 [26]	Kindly provided by Prof. Ferric Fang Washington university
Salmonella enterica 14028s pCsgD	SLN135 [26]	Kindly provided by Prof. Ferric Fang Washington university
Other bacterial strains		
E2348/69 (*wt*)	EPEC-*wt* isolate, serotype O127:H6, NA	J. Kaper, [27]
Listeria monocytogenes 10403s	WT isolate	Kindly provided by Porf. Anat Herskovits Tel Aviv university
GFP *Listeria monocytogenes 10403s*	Wild type L. monocytogenes 10403s strain with	Kindly provided by Porf. Anat Herskovits Tel Aviv university

	constitutive GFP expression	
Isolated pseudomonases		
Aeromonas sp. strain VNR183	Water isolate 1	Determined by 16s RNA sequencing
Stenotrophomonas maltophilia strain A1-1	Water isolate 2	Determined by 16s RNA sequencing
Pseudomonas alcaliphila strain SS NBRI 36	Water isolate 3	Determined by 16s RNA sequencing
Pseudomonas punonensis strain JB11	Water isolate 4	Determined by 16s RNA sequencing
Stenotrophomonas rhizophila strain S242	Water isolate 5	Determined by 16s RNA sequencing
Pseudomonas wadenswilerensis strain RD2	Leaf isolate 1	Determined by 16s RNA sequencing
Pseudomonas sp. JNR-02	Leaf isolate 2	Determined by 16s RNA sequencing
Stenotrophomonas sp. strain NJ1024	Leaf isolate 3	Determined by 16s RNA sequencing
Pseudomonas putida strain PBS6	Leaf isolate 4	Determined by 16s RNA sequencing

Pseudomonas alkylphenolica strain 48A-P9	Soil isolate 1	Determined by 16s RNA sequencing
Pseudomonas sp. strain VNP47	Soil isolate 2	Determined by 16s RNA sequencing
Pseudomonas sp. strain huaiR14	Soil isolate 3	Determined by 16s RNA sequencing
Pseudomonas donghuensis strain Tn-1	Soil isolate 4	Determined by 16s RNA sequencing
Pseudomonas sp. EHE_1_3	Soil isolate 5	Determined by 16s RNA sequencing
Pseudomonas fulva strain XL1	Soil isolate 6	Determined by 16s RNA sequencing
Plants		
Arabidopsis		
Col0	WT Arabidopsis line	Lab stock
fls2	[21]	Lab stock
rbohd: rbohf	[25]	ABRC
bbc	*bak1: bkk1: cerk1* [22]	Kindly provided by Prof. Sheng Yang He Duke university
pFrK1::3xmVenus	[19]	Kindly provided by Prof. Niko Geldner university of Lausanne

Other plants		
Alflafa	*Medicago sativa*	Lab stock
Basil	*Ocimum basilicum*	Lab stock

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