Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria

Graphical abstract

Highlights
- The auxin-secreting bacterium *B. velezensis* triggers an immune response in *Arabidopsis*
- Bacterial auxin counteracts the plant immune response and ROS toxicity
- In a feedback loop, ROS induces auxin and enhances root colonization
- Immune modulation by *B. velezensis* enhances plant protection from fungal infection

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In brief
How beneficial bacteria interact with the plant immune system is poorly understood. Here, Tzipilevich et al. report that activation of the plant immune system is necessary for efficient bacterial root colonization. A feedback loop is established between bacteria and the plant immune system, promoting the fitness of both partners.
Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria

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SUMMARY

Although plant roots encounter a plethora of microorganisms in the surrounding soil, at the rhizosphere, plants exert selective forces on their bacterial colonizers. Unlike immune recognition of pathogenic bacteria, the mechanisms by which beneficial bacteria are selected and how they interact with the plant immune system are not well understood. To better understand this process, we studied the interaction of auxin-producing Bacillus velezensis FZB42 with Arabidopsis roots and found that activation of the plant immune system is necessary for efficient bacterial colonization and auxin secretion. A feedback loop is established in which bacterial colonization triggers an immune reaction and production of reactive oxygen species, which, in turn, stimulate auxin production by the bacteria. Auxin promotes bacterial survival and efficient root colonization, allowing the bacteria to inhibit fungal infection and promote plant health. Thus, a feedback loop between bacteria and the plant immune system promotes the fitness of both partners.

INTRODUCTION

Plant roots interact with a plethora of bacteria in the surrounding soil. Extensive efforts have characterized the diversity of these bacterial species (Bai et al., 2015; Lundberg et al., 2012). Bacteria can have pathogenic, beneficial, or neutral effects on plants. Bacterial diversity is reduced when moving from bulk soil to the root surface (rhizosphere) and further into the root interior (endosphere), indicating that plants exert selective forces on their colonizing bacteria. An early filter used by plants to recognize and respond to bacteria and other organisms is its immune system (Couto and Zipfel, 2016), which utilizes receptors to recognize bacterial molecules called MAMPs (microbe-associated molecular patterns). These include flagella, peptidoglycans, bacterial elongation factor TU, and others (Jones and Dangl, 2006; Zipfel, 2014). Recognizing these molecules leads to a cascade of molecular events. The earliest stages of this response include an efflux of calcium ions and a burst of reactive oxygen species (ROS) (Zipfel, 2009). This is followed by phosphorylation events that lead to the induction of immune-related genes (Spoel and Dong, 2012). Plant immune system recognition and activation have been extensively characterized in the context of pathogenic bacteria (Dodds and Rathjen, 2010; Xin et al., 2018). However, MAMP receptors recognize molecules found in all bacteria, and beneficial bacteria can induce an immune response similar to the pathogenic ones (e.g., Colaianni et al., 2021; Stringlis et al., 2018). The influence of the immune system on the healthy root microbiome and how beneficial bacteria respond to the plant immune system is an active area of research, and much remains to be learned (Chen et al., 2020; Hacquard et al., 2017; Teixeira et al., 2019). To better understand this process, we studied the interaction of auxin-producing Bacillus velezensis FZB42 with Arabidopsis roots and found that activation of the plant immune system is necessary for efficient bacterial colonization and auxin secretion. A feedback loop is established in which bacterial colonization triggers an immune reaction and production of reactive oxygen species, which, in turn, stimulate auxin production by the bacteria. Auxin promotes bacterial survival and efficient root colonization, allowing the bacteria to inhibit fungal infection and promote plant health. Thus, a feedback loop between bacteria and the plant immune system promotes the fitness of both partners.
Figure 1. Bacterial auxin stimulates plant lateral root formation and bacterial root colonization
(A) Seedlings were inoculated with either WT, ΔysnE bacteria, or buffer (mock) on agar plates for 7 days and the number of lateral roots was counted. (n ≥ 20) (*p < 0.05, ANOVA followed by post hoc Tukey Kramer).
(B and C) Arabidopsis DR5::GFP reporter lines were inoculated with the indicated bacterial strains for 48 h on agar plates.
(B) 100x maximal projection confocal images of GFP fluorescence from DR5::GFP expression.

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with the salicylic-acid signaling pathway (Kunkel and Harper, 2018). However, it is unclear if the production of auxin by bacteria has a direct effect on their colonization capacity (Duca et al., 2014; Patten and Glick, 1996). We found that a positive feedback between the plant immune system and bacterial auxin secretion occurs during root colonization. Immune system modulation by bacteria triggers ROS production by the plant, which, in turn, activates auxin secretion by the bacteria. This secreted auxin is necessary for bacterial survival in media containing elevated ROS levels, and for colony formation on the root. An efficient colony formation enables the bacteria to fight pathogenic fungi and enhance plant health. Thus, our work reveals that bacterial auxin directly impacts its capacity for root colonization and uncovers a positive influence of the plant immune system on bacterial colonization with beneficial effects for the plant.

RESULTS

Bacterial auxin plays a dual role during root colonization
To characterize the interaction between bacteria and plant roots, we inoculated B. velezensis onto Arabidopsis seedlings growing on agar plates. Consistent with previous results (Fan et al., 2011; Idris et al., 2007), after 7 days of incubation, colonized plants exhibited reduced primary root growth and increased lateral root emergence (Figures 1A, S1A, and S1B) in comparison with seedlings treated with the mock (mock), a response consistent with bacterial auxin secretion (e.g., Spaepen et al., 2014). Plants expressing an auxin response reporter (DR5::GFP Liao et al., 2015) revealed increased auxin response in roots inoculated with B. velezensis (Figures 1B and 1C). The root phenotype was dependent on bacterial auxin secretion, as plants inoculated with a strain of B. velezensis deficient in auxin production (JysnE (Idris et al., 2007), see Figure S1C) did not exhibit primary root growth inhibition or lateral root stimulation (Figures 1A, S1A, and S1B) and had reduced DR5::GFP fluorescence (Figures 1B and 1C).

JysnE bacteria failed to colonize the root as efficiently as WT bacteria, indicating that bacterial auxin not only triggers a root developmental response but is also necessary for efficient root colonization (Figures 1D–1F). Coexpression of YsnE in trans (JysnE::amyE::Pamy::YsnE) restored auxin production (Figure S1C) and root colonization (Figure 1D). Similarly, the addition of exogenous auxin (IAA) also restored JysnE bacterial colonization (Figure 1D). Similar results were obtained with two other mutants in the auxin biosynthesis pathway, jtrpAB and jtrpED (Idris et al., 2007) (Figure 1D). JysnE bacteria exhibited normal growth in vitro (Figure S1D), normal swarming motility (Figure S1E), and biofilm formation (Figure S1F), indicating that these processes, which can influence root colonization (Chen et al., 2013; Dietel et al., 2013), and are not responsible for the reduced colonization capacity. In an attempt to elucidate the underlying cause, we performed time-lapse microscopy of root colonization by WT and JysnE GFP-expressing bacteria (Fan et al., 2011). Although most of the WT bacteria replicated and formed colonies over the root (Figure 1G), JysnE bacteria failed to replicate (Figure 1H). We conclude that bacterial auxin is necessary for B. velezensis to survive and replicate on the root.

Auxin is necessary for B. velezensis to antagonize the plant immune reaction
The reduced ability of JysnE bacteria to colonize the root (Figure 1H) led us to hypothesize that B. velezensis is able to trigger a plant immune response (see also Xie et al., 2017). Auxin produced by pathogenic bacteria has previously been shown to reduce the plant immune response (McClerklin et al., 2018). RNA sequencing of whole roots after 48 h of bacterial colonization (Table S1) revealed that gene categories related to immune system activation, such as camalexin synthesis and callose deposition, were enriched in the root transcriptome compared with buffer-inoculated roots (mock) (Figure 2A; Table S2). These early response results were corroborated by increased expression of immune-related promoters (pPER5, pFRK1) fused to fluorescent reporters (Zhou et al., 2020) (Figures 2B and 2C), as well as callose deposition (Figures S2A and S2B), indicating that B. velezensis colonization elicits an immune reaction. Monitoring plant ROS production also revealed a significant response (Figure 2D).

To identify the pathway by which the immune response is triggered, we measured the ROS response in plants deficient in three different MAMP receptors—fbs2, mutant in the receptor for bacterial flagella (Zipfel et al., 2004), efr2, mutant in the receptor for bacterial elongation factor TU (Zipfel et al., 2006), and lym1, lym3, mutant in the receptor for bacterial peptidoglycan (Willmann et al., 2011). We found that efr2 and fbs2 exhibited a significant reduction in ROS production (Figure 2B). These results are consistent with the similarity between elf18 and fgs2 and their respective epitopes in the B. velezensis genome (70.6% and 66.7% identity, respectively, Figure S2C). efr2 also exhibited a reduction in callose deposition (Figures S2A and S2B). We hypothesized that if bacterial auxin is necessary to antagonize the plant immune response, then the colonization of bacteria deficient in auxin production would be restored on mutant plants with compromised immunity. Consistent with

(C) Quantification of GFP fluorescence from maximum intensity projection images. Shown are averages and SD n = 5, each circle represents one root. (**p < 0.005, two-tailed t test with Bonferroni correction) Scale bar, 50 μm

(D) Seeds were inoculated with the indicated bacterial strains with or without 5 μM IAA for JysnE bacteria for 48 h on agar plates and the number of colonizing bacteria was counted. Shown are averages and SD (log10 transformed); each circle represents an average of 3 technical replicates from the same root. (**p < 0.005, two-tailed t test with Bonferroni correction).

(E and F) Seedlings were inoculated with either WT or JysnE bacteria expressing GFP (amyE::Pamy::gfp) for 48 h on agar plates. Shown are 200 x maximal projection confocal images of DIC (differential interference contrast) from roots (left panels) and GFP fluorescence from bacteria (right panels) for WT (E) and JysnE (F) bacteria. Scale bars, 50 μm.

(G and H) Seedlings were inoculated with either WT (G) or JysnE (H) bacteria expressing GFP (amyE::Pamy::gfp) and followed by time-lapse confocal microscopy for 12 h. Shown are 400 x maximal projection overlay images of DIC from roots (gray) and GFP fluorescence from bacteria (green), taken at the indicated time points. Yellow arrows highlight bacterial growth arrest, probably culminating in cell death. Red arrows highlight JysnE bacteria replicating away from the root plane. Scale bars, 10 μm.
 Auxin antagonizes ROS toxicity

To identify the components of plant immunity perturbed by bacterial auxin, we monitored \textit{JysnE} bacterial colonization on mutants in immune response genes. Mutations in SALK\_068675, an additional \textit{efr} mutant allele, restored \textit{JysnE} bacterial colonization. Moreover, a \textit{bak1-5} mutant, defective in multiple MAMP receptor activation, as well as \textit{crf3}, mutant in a gene essential for EFR receptor function (Li et al., 2009), restored \textit{JysnE} bacterial colonization (Figure S3A).

Neither perturbation in the indoleglucosinolate and camalexin synthesis pathway (\textit{myb51} Freitagmann et al., 2014 and \textit{cyp71a13}, Mucha et al., 2019) nor the defects in plant stress hormone effectors (\textit{npr1-5}, \textit{ein2-5}, \textit{jar1-1}) affected \textit{JysnE} bacterial colonization (Figure S3A). The lack of SA response (\textit{npr1-5} Ding and Ding, 2020) is notable, as auxin is known to antagonistically interact with the SA pathway to enhance the colonization of \textit{P. syringae} (McCl Erin et al., 2018; Robert-Seilaniantz et al., 2011). In contrast, the growth of auxin-deficient bacteria was restored on \textit{rbohd rbohf} plants, which are defective in immune triggered ROS production (Figure 3D) (Torres et al., 2005). Moreover, significant recovery of \textit{JysnE} bacterial colonization was obtained when ROS production was chemically inhibited by DPI (Tsukagoshi et al., 2010) (Figure S3B). These results suggest that bacterial auxin antagonizes plant ROS production to enable root colonization.

On \textit{rbohd rbohf} plants, \textit{B. velezensis} caused a negative effect with a significant increase in root colonization (Figure 3D), reduced the number of lateral roots and smaller plants (Figures S3C and S3D). We hypothesize that \textit{efr2} plants, although perturbed in \textit{B. velezensis} triggered immunity are still capable of eliciting a sufficiently strong immune response with ROS production (Figure 2B) to keep the bacteria from overgrowing the plant.

ROS are toxic molecules utilized by the plant to kill invading pathogens and to signal cells neighboring infection sites to induce defense pathways (e.g., Fones and Preston, 2012). NADPH oxidase enzymes, such as RbohD and RbohF, produce...
superoxide (O$_2^-$) ions, which can further be converted into other ROS, such as H$_2$O$_2$ (Wang et al., 2018). O$_2^-$ was highly toxic to B. velezensis in vitro (Figure 4A), whereas H$_2$O$_2$ killed bacteria only at a high concentration (500 µM) (Figure S3E). O$_2^-$ was significantly less toxic to WT bacteria in comparison with auxin-deficient bacteria (Figure 4A). Exogenous IAA enhanced the survival of both bacteria (Figure 4A). These results suggest that auxin enables bacteria to survive the toxic effects of ROS.

To gain a deeper understanding of the effect of auxin on bacterial interaction with ROS, we examined global gene expression changes in WT and JysnE bacteria in culture after the addition of O$_2^-$. In WT bacteria, 371 genes were upregulated and 374 genes downregulated (Figure 4B), whereas JysnE bacteria exhibited a weaker response (Figures 4B and 4C), with only 153 genes upregulated and 184 downregulated (Figure 4B; Table S3). Enriched GO categories for upregulated genes in WT bacteria included SOS response and DNA repair, whereas the DNA repair category was missing in JysnE (Figure 4D; Table S4). The katA and ahpF genes are important for ROS detoxification (Engelmann and Hecker, 1996; Poole, 2005), and the recA gene is important for DNA repair (Alonso et al., 2013). All three were upregulated in response to ROS treatment (Table S3, all three were induced to a greater extent in WT bacteria.). The expression of these genes in JysnE bacteria under an IPTG-inducible promoter significantly enhanced root colonization (Figure 4E). Interestingly, GO categories related to iron homeostasis were enriched in the transcriptome of WT bacteria but not in JysnE bacteria (Figure 4D and Table S4). Ferrous (Fe$^{2+}$) iron is known to interact with hydrogen peroxide in a Fenton reaction to produce a toxic hydroxyl radical (Cornelis et al., 2011), potentially amplifying the toxicity of the short-lived O$_2^-$ molecules. Thus, iron sequestration can protect cells from the toxic effects of ROS. The expression of the siderophore bacillibactin or heme synthesis operons in JysnE bacteria under IPTG-inducible promoters enhanced their ability to colonize the root (Figure S4A). Lowering the iron content of MS media by 50% also improved root colonization by JysnE bacteria (Figure S4B). Of note, auxin was able to protect B. velezensis from iron toxicity in vitro (Figures S4C and S4D). Among the significantly depleted gene categories in WT bacteria were the TCA cycle and carbohydrate and amino acid transport, although none of these categories was depleted in JysnE bacteria (Table S4). We speculate that WT bacteria enter a growth arrest that can protect them from ROS toxicity, whereas JysnE bacteria fail to induce growth arrest are killed. Thus, our results establish ROS as a major limiting factor during root colonization and auxin as a key bacterial effector to mitigate ROS toxicity. The addition of IAA to bacteria without ROS had negligible effects on transcription (Table S3), suggesting that auxin alone is not sufficient to explain these transcriptional changes and that other factors induced during stress are necessary for auxin to have its effect.

Given our findings that auxin plays a major role in mitigating ROS toxicity, we hypothesized that ROS exposure leads to auxin accumulation in bacteria. To test this hypothesis, we fused YsnE to GFP and observed that it accumulated upon ROS treatment in vitro (Figures S4E and S4F). We collected the supernatant from bacterial cultures treated with ROS and applied it to DR5::GFP-expressing plants, which led to a greater increase in DR5::GFP fluorescence as compared with plants treated with the supernatant from JysnE bacteria or from untreated WT bacteria (Figure 4F). Consistent with these results, efr2 roots colonized by bacteria failed to exhibit...
**Figure 4. Bacterial auxin counteracts ROS toxicity**

(A) Bacterial cultures grown to OD<sub>600</sub> = 1 were treated with O<sub>2</sub>• in the presence or absence of 5 μM IAA for 30 min, and CFU were counted. Shown are averages and SD (log<sub>10</sub> transformed) n = 3. Each circle represents an average of 3 technical replicates from the same culture. (*p < 0.05, ***p < 0.005, two-tailed t test with Bonferroni correction).

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lateral root stimulation and had normal primary root length (Figures 4G and S4G). Furthermore, bacteria colonizing efr2 plants harboring DR5::GFP induced significantly less GFP expression than DR5::GFP WT plants (Figure 4H), suggesting that EFR-induced ROS production by the plant is necessary to trigger efficient bacterial auxin production.

**Auxin promotes bacterial adhesion and colony formation on the root**

Although the growth of ΔysnE bacteria is restored on efr2 roots (Figures 2E and S2C), these bacteria do not adhere to the root in the same way in which WT bacteria adhere to Col-0 roots (Figure 3A). Interestingly, similar inefficient adhesion occurred when WT bacteria colonized efr2 roots (Figures 5A, 5B, S5A, and S5B). The quantification of root adhesion from time-lapse microscopy revealed that, on average, 83% of bacteria colonizing WT Col-0 roots remained adhered to the root during a 12-h experiment (Figure 5A), while only 32% did so on efr2 roots (Figures 5A and SSA). The macrostructure of bacteria colonizing a root after 48 h revealed large clusters on Col-0 roots (Figures 5B and S5B), while bacteria colonizing efr2 roots were in small patches (Figures 5B and S5B), probably reflecting the same phenomenon of perturbed adhesion and colony formation. This suggests that efficient ROS response, perturbed in efr2 roots, is necessary for tight root adhesion and colony formation. The addition of exogenous IAA stimulated colonization on Col-0 as well as on efr2 plants (Figure 5C). Exogenous IAA can stimulate root colonization on mutant plants impaired in auxin perception (Figures S5C and S5D), suggesting that auxin, at least in part, affects the bacterial ability to adhere to the root rather than the root’s response to bacteria, although root response cannot be excluded.

To elucidate the mechanism by which auxin promotes root adhesion and spreading, we screened an array of colonization-related mutant bacteria, impaired in motility, adhesion, and biofilm formation genes (Chen et al., 2007), for auxin-enhanced colonization (Figure 5D). Bacteria with a mutated lipoteichoic-acid synthase gene, Δyfh1, lost their ability to colonize the root, irrespective of IAA addition. Bacteria lacking a flagellar apparatus, Δhag, colonized the roots similar to WT bacteria. However, they failed to exhibit enhanced colonization following IAA addition (Figures 5C and S5D). Auxin-induced flagellar formation was also suggested by the transcriptome analysis (Table S3, IAA-induced hag gene expression logFC = 0.79) and in vitro motility assay (Figure S5E). Δhag bacteria also failed to induce lateral root formation (Figure 5E). Interestingly, similar results were obtained with ΔawnA bacteria, a regulator of flagellar synthesis. However, ΔmotA bacteria, harboring intact but nonmotile flagella, were still able to respond to IAA addition (Figure S5F), suggesting that the presence of flagella, but not its movement, is what is important for root adhesion. We conclude that auxin-induced flagella production is able to enhance root colonization necessary for lateral root stimulation.

**The plant immune system stimulates root colonization and auxin secretion by diverse bacterial species**

To determine if bacterial auxin secretion and plant immunity interact in a similar manner for other bacteria, we analyzed the colonization capacity of *Paenibacillus polymyxa* (*P. polymyxa*), a Gram-positive bacteria known to secrete high amounts of auxin and stimulate plant growth (Jeong et al., 2011). *P. polymyxa* stimulated lateral root formation, shorter primary roots, and DR5::GFP expression in roots on agar plates (Figures 6A–6C). *P. polymyxa* also stimulated plant ROS production in an FLS2-dependent manner (Figure 6D). On fss2 plants, *P. polymyxa* failed to stimulate lateral root production (Figure 6B) and had longer primary roots as compared with Col-0 (Figure 6C), despite the bacteria reaching a higher CFU on fss2 plants (Figure 6E), suggesting that immune system activation and ROS production are necessary for bacterial auxin production. Furthermore, exogenous IAA stimulated root colonization by *P. polymyxa* (Figure 6F). Finally, IAA induced FLS promoter expression (Figure 6G). Thus, auxin produced by *P. polymyxa* and plant immunity interact with each other, despite being modulated by a different immune receptor than *B. velezensis*. Arthrobacter MF161 is another Gram-positive auxin-secreting bacterium isolated from Arabidopsis roots (Levy et al., 2017). Inoculation by this bacterial strain stimulated lateral root formation and DR5::GFP expression (Figures S6A and S6B), as well as triggering the immune response in an FLS2-dependent manner (Figure S6C). Arthrobacter MF161 failed to enhance lateral root formation and had longer primary roots on fss2 plants (Figures S6B and S6D). No difference in root colonization was observed between Col-0 and fss2 plants (Figure S6E). Finally, exogenous IAA further stimulated root colonization by this bacterial strain (Figure S6F). Auxin did not stimulate root colonization of auxin-secreting *Pseudomonas* species 65 (Kamilova et al., 2006) and WCS374 (Zamioudis et al., 2013) (Figure S6G), suggesting...
that auxin-stimulated colonization is not a general phenomenon but is bacterium specific.

Our results indicate that ROS production by the plant immune system is necessary for efficient root adhesion. However, this phenomenon is not manifested in differences in bacterial load (Figure 5). Given that bacteria in nature compete with many other species to inhabit the same plant root niche (Bai et al., 2015; Lundberg et al., 2012), we hypothesized that differences in B. velezensis root adhesion ability would become evident during competition with other bacteria. To test this hypothesis, we co-inoculated P. polymyxa and B. velezensis on Col-0 and efr2 plants. P. polymyxa colonization was only modulated by the FLS receptor but not by EFR (Figure 6D), whereas B. velezensis colonization was mainly modulated by the EFR receptor (Figure 2D). After co-inoculation, B. velezensis outcompeted P. polymyxa on Col-0 (Figure 6H). However, on efr2 plants, we observed a significant increase in P. polymyxa colonization, concomitant with a reduction of B. velezensis colonization (Figure 6H). B. velezensis inoculated onto Col-0 and efr2 plants for 48 h and then transferred into nonsterile soil also exhibited enhanced colonization of Col-0 plants (Figure 6I), indicating that immune modulation helps the bacteria to compete with the soil microbiota. Co-inoculation of B. velezensis and Arthrobacter MF161 on Col-0 and efr2 plants had no significant effect on either bacteria (Figure S7A). The inspection of colonization sites revealed that B. velezensis and P. polymyxa heavily colonize the elongation and maturation zones of the root (Figures S7B1 and S7B2), whereas Arthrobacter MF161 is largely absent from these regions and colonizes differentiated parts of the root (Figures S7B3 and S7B4). Thus, our results suggest that immune system enhanced colonization affects B. velezensis and P. polymyxa competition, as both compete for the same niche, but not B. velezensis and Arthrobacter MF61 competition, as they colonize different niches. Root colonization by Arthrobacter MF161 inside a synthetic community of 34 bacteria was previously characterized (Castrillo et al., 2017; Teixeira et al., 2021). To explore the possibility that immune modulation of Arthrobacter MF161 by FLS affects its root colonization inside the community, we performed 16S RNA sequencing of the 34 bacterial community, colonizing...
Figure 6. Plant immunity interaction with bacterial auxin secretion in *P. polymyxa*

(A) An Arabidopsis DR5::GFP reporter line was inoculated with *P. polymyxa* for 96 h on agar plates. Shown are 100× maximal projection confocal images of GFP fluorescence from the DR5::GFP reporter line.

(B) Col-0 or *fis2* seedlings were inoculated with *P. polymyxa* or buffer (mock) on agar plates for 7 days and the number of lateral roots was counted, n ≥ 20. (p < 0.05, ANOVA followed by post hoc Tukey Kramer).

(C) Col-0 or *fis2* seedlings were inoculated with *P. polymyxa* or buffer alone (mock) on agar plates for 7 days and the length of the primary root was measured, n ≥ 20. Each circle represents one root. (***p < 0.005, two-tailed t test with Bonferroni correction).

(D) Leaf discs from 28-day-old plants taken from the indicated plant genotypes were incubated with bacteria adjusted to OD 0.1, and the ROS burst was measured. Shown are averages and SD, n ≥ 10.

(E) Col-0 or *fis2* seedlings were inoculated with *P. polymyxa* for 48 h on agar plates and the number of colonizing bacteria was counted. Shown is an average and SD of two independent replicates (log10 transformed), with n = 3 for each. Each circle represents an average of 3 technical replicates from the same root. (***p < 0.005, two-tailed t test).

(F) Seedlings were inoculated with *P. polymyxa* for 48 h on agar plates with or without 5 μM IAA and the number of colonizing bacteria was counted. Shown are averages and SD of 2 independent replicates (log10 transformed), with n = 3 for each. Each circle represents an average of 3 technical replicates from the same root. (***p < 0.005, two-tailed t test).

(G) Seedlings of pFLS::NLS-3xmVENUS, pUBQ10::RCI2A-tdTomato were inoculated with WT bacteria, grown in the presence of 5 μM IAA, or buffer for 48 h. Shown are 400× representative overlay images of pUBQ10::RCI2A-tdTomato (cell wall, red) and pFLS::NLS-3xmVENUS (FLS-expressing cells, green) from five roots for each condition. Scale bar, 25 μm.

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either Col-0 or efr2 plants. However, the results were inconclusive (Figure S7C), suggesting that the community context is important for Arthrobacter colonization.

B. velezensis produces secondary metabolites that can inhibit the growth of plant fungal pathogens (Fan et al., 2018). We asked if plant immune system activation, triggering B. velezensis colony formation, enhances its ability to inhibit plant pathogen infection. We colonized Col-0 and efr2 plants with B. velezensis and infected the plants with the fungal pathogen Rhizoctonia solani (Dean et al., 2012). B. velezensis inhibits the growth of R. solani in vitro (Figure 7A) and is able to protect plants from fungal infection (Figure 7B) (Chowdhury et al., 2013). Of note, plant protection was significantly better on Col-0 plants, as measured by plant weight, although efr2 has no effect on fungal infection per se, (Figure 7C R. solani alone). Monitoring the fungal load reveals a significant reduction on Col-0 plants in comparison with efr2 plants (Figure 7D). EFR activation modulates B. velezensis colony formation but may also enhance plant survival through induced systemic resistance (ISR) (Pieturse et al., 2014). To further differentiate between these effects, we measured plant protection by Δhag B. velezensis (Figure 7C). Δhag bacteria failed to protect the seedlings from R. solani infection (Figure 7C), despite inducing an immune response in the plant, similar to WT bacteria (Figure 7E). Thus, we conclude that the enhanced colony formation of B. velezensis on immune competent plants enables it to better protect the plant from fungal infection.

### DISCUSSION

Our results are consistent with the presence of a feedback loop between the plant immune system and bacterial auxin secretion (Figure 7F). Root colonization by bacteria triggers an immune response and ROS production. ROS, in turn, elicits bacterial auxin production to mitigate ROS toxicity. Auxin promotes bacterial spreading over the root and colony formation, while also inducing the expression of plant immune receptors, further accelerating the feedback loop. This enhanced colonization promotes the ability of B. velezensis to inhibit plant pathogenic fungi. Thus, a feedback loop between bacteria and the plant immune system promotes the fitness of both partners.

Recent work has elucidated the role of the plant immune system in shaping the normal root microbiota, in addition to fighting pathogens (Haccquard et al., 2017; Teixeira et al., 2019). In these studies, an immune reaction was viewed as a negative factor for root colonization, shaping the microbiota by preventing bacterial overgrowth. Consistent with this view, our results show that B. velezensis modestly overgrows on rbohd rbohf plants completely lacking ROS production (Figure 3D). However, bacteria grow on efr2 plants with partially perturbed immunity, demonstrating that plant immune system activation also plays a positive role for bacterial colonization, triggering the induction of auxin production by bacteria necessary for efficient root adhesion and colony formation. We hypothesize that bacteria exhibit significantly higher growth on rbohd rbohf but not efr plants due to the fact that ROS is still produced in efr2 plants (Figure 2B), probably through activation of other immune receptors. Consistently, bacteria overgrew bak1-5 plants, defective in the activation of multiple receptors (Figure S5A). In addition, rbohd rbohf plants also exhibit non-immune-related phenotypes that may affect bacterial colonization (e.g., Song et al., 2021).

Our results suggest that immune system activation interacts with bacterial auxin secretion to enhance bacterial colonization, irrespective of the specific immune receptor, as we provide evidence that a similar feedback loop exists during P. polymyxa and Arthrobacter MF161 colonization, despite being modulated by the FLS2 receptor rather than the EFR2 receptor. Thus, we uncovered a unique aspect of bacterial interaction with the immune system.

A prevalent view of mutualistic interactions is that symbiosis evolved through exploitative interactions that became attenuated over evolutionary time (Cao et al., 2017; Delaux and Schornack, 2021; Sachs et al., 2011). Parallels were found between the immune system signaling pathway and the symbiotic association between plants and specialized mutualists, such as the interaction between legumes and rhizobia (Cao et al., 2017; Tóth and Stacey, 2015), as well as the association between plants and arbuscular mycorrhizal fungi (Miya et al., 2014). Our results reveal a more widespread relationship between plant immunity and colonization of beneficial bacteria, including nonspecialized auxin-secreting beneficial bacteria, potentially representing an earlier stage of the evolution of mutualism.

Auxin is a key plant hormone that plays a wide range of roles in plant development (Teale et al., 2008). Many bacterial species, including pathogens such as Agrobacterium tumefaciens and Pseudomonas syringae, as well as beneficial bacteria such as Azospirillum brasilense, are known to synthesize auxin and manipulate the plant through auxin secretion (Costacurta and Vanderleyden, 1995; Kunkel and Harper, 2018; Spaepen et al., 2007). However, despite decades of research on bacterial auxin production and how it affects plants, the role played by auxin on bacterial physiology is poorly understood. Previous studies found a bacterial transcriptional effect for auxin, but only at concentrations far above those that modify plant physiology (Bianco et al., 2006; Djami-Tchantchou et al., 2020; Van Puyvelde et al., 2011). Our results suggest that auxin primarily affects the producer bacteria, acting as a stress-related signal to protect them from ROS. Mutations in the auxin synthesis pathway lead to profound transcriptional effects following ROS treatment. However, we failed to observe a substantial role for exogenous IAA under nonstressed conditions. This suggests that auxin may not be sufficient by itself to induce a significant response in bacteria similar to its effect on plants. Rather, auxin needs other factors that are induced during stress to have its effect. Further research will be necessary to elucidate the role played by auxin in bacterial symbiosis.
by auxin in bacterial physiology and stress adaptation for beneficial, as well as pathogenic bacteria. Plants interact with a wide variety of bacterial species in nature. The composition of the plant microbiome is affected by factors such as soil geochemistry, bacterial diversity, the amount and composition of exudates, immune system activation, and by bacterial interaction with other bacteria, with phages, and with other organisms. Understanding the effect of each of these components will enable rational manipulation of the plant microbiome to the benefit of the plant. Bacterial auxin production is highly prevalent among root-colonizing bacteria (Zhang et al., 2019), and the effect of auxin-secreting and -degrading bacteria in the root microbiome on plant physiology in a complex microbiome was recently explored (Finkel et al., 2020). Here, we have shown that auxin-secreting bacteria interact with the plant immune system to promote their association with the plant and their competition with other bacteria.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

- **Bacteria**
- **Fungi**
- **Plants**

**METHOD DETAILS**

- **Bacterial genetic manipulation**
- **Monitoring bacterial growth on plant roots**
- **Microscopy**
- **Measurement of plant ROS production**
- **RNA extraction library preparation and computational analysis**

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2021.09.005.

**ACKNOWLEDGMENTS**

We thank G. Wachsman for help in RNA sequence analysis. We are grateful to S.Y He (Duke), X. Dong (Duke), B. Kunkel (Washington University), T. Nolan, R. Shahan, and C. Winter from the Benfey lab, for critical reading of the

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**Figure 7. Immune system modulation by *B. velezensis* enhances plant protection from fungal infection**

(A) *B. velezensis* and *R. solani* were spotted on PDA plates and allowed to grow for 72 h. Shown is a representative plate from 3 plates.

(B) 6-day-old seedlings were inoculated with *B. velezensis* or buffer for 48 h on agar plates. Then, the plates were inoculated with *R. solani* and incubated for an additional 7 days. Untreated plants were used as a control. Shown are representative plates from at least 5 plates for each treatment.

(C) *Col-0* or *efr2* seedlings were inoculated with WT or Δhag (only Col-0) *B. velezensis*, or buffer for 48 h on agar plates. Then, the plates were inoculated with *R. solani* and incubated for an additional 7 days and plant weight was measured. Untreated plants (neither bacteria nor fungi) were used as a control. Shown are averages and SD, n ≥ 20. (*p < 0.05, two-tailed t test with Bonferroni correction).

(D) 6-day-old seedlings were inoculated with *B. velezensis* or buffer for 48 h on agar plates. Then, the plates were inoculated with *R. solani* and incubated for an additional 3 days. After 3 days, seedlings were thoroughly washed and transferred to new plates and the number of attached mycelia was counted under the microscope after 24 h. Control plants were completely covered by fungi, precluding detailed quantification. (**p < 0.005 two-tailed t test). *(E)* *pPER5::NLS-3xmVENUS, pUBQ10::RCI2A-tdTomato* seedlings were inoculated with either WT or Δhag *B. velezensis* or buffer alone (mock) for 48 h. Shown are 400× overlay images of pUBQ10::RCI2A-tdTomato (red) and *pPER5::NLS-3xmVENUS* (green) from 5 roots from each condition. Scale bars, 25 μm.

(F) Model describing the feedback loop between plant immune system activation and bacterial auxin secretion.
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AUTHOR CONTRIBUTIONS
E.T. and P.N.B. conceived the project. E.T. performed the experiments. D.R. designed and analyzed experiments. J.L.D. donated strains. E.T. wrote an initial draft. P.N.B., J.L.D., and D.R. reviewed and edited the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES


### Key Resources Table

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(Continued on next page)
RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Philip N. Benfey (philip.benfey@duke.edu)

Materials availability

Bacterial mutants and Arabidopsis lines generated in this study are available upon request.

Data and code availability

Arabidopsis Raw sequence reads were deposited in the SRA accession number: PRJNA718879. B. velezensis raw sequence reads were deposited in the SRA accession number: PRJNA718895. 16S raw sequence reads were deposited in the SRA accession number: PRJNA742484. Original/source data for the paper is available in Mendelely data [https://doi.org/10.17632/8zyrz7ccbh.1] (Zhou et al., 2020)

Software and algorithms

DADA 2 version 1.16 (Callahan et al., 2016)
BlueBee Genomics Platform (https://www.bluebee.com/lexogen)
Kallisto (Bray et al., 2016)
EdgeR (Robinson et al., 2010)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacteria

B. velezensis Fzb42 bacteria and its mutant derivatives ΔysnE, Δspf, ΔtrpAB, ΔtrpED and Paenibacillus polymyxa ATCC842 were purchased from the Bacillus genetic stock center (http://www.bgsc.org/). Arthrobacter MF161 was described previously (Levy et al., 2017). B. velezensis amyE::pSpac-GFP was purchased from NORDREET company (https://www.nordreet.de/). Other B. velezensis mutant strains including: ΔepsH, ΔtasA, ΔbslA, ΔdegU, Δhag, ΔpznL, Δyfnl, ΔynfF, Δaslin, ΔysnE amyE::PysnE::ysnE-gfp. IPTG inducible genes including: PiptGrecA, PiptGkatA, PiptGahpF, PiptGhemA, PiptGdhbA, and ysnE-gfp were generated in this study. The bacteria were cultivated routinely on Luria broth (LB) medium. When needed, the medium was solidified with 1.5% agar. For biofilm formation, bacteria were inoculated into MSgg medium and incubated without shaking for 4 days at 25°C as described in (Branda et al., 2001). For experiments with IPTG inducible promoters (Figures 3B and S4B), 0.5mM IPTG was added to the growth media.
30 min before root inoculation, and later bacteria inoculated onto roots, on plates containing 0.5 mM IPTG. For O$_2^{-}$ treatment, bacteria were grown to OD$_{600}$ = 1, then 0.5 mM xanthine added and 5 µl xanthine oxidase enzyme (Sigma) (Figures 3A, 3H, and S5A) or 0.5 enzyme for the RNA sequencing experiments.

**Fungi**

*Rhizoctonia solani* isolate was kindly provided by Prof. Marc Cubeta (NCSU). Fungi were routinely grown on PDA plates (Sigma).

**Plants**

The Arabidopsis (Arabidopsis thaliana) SALK, SAIL and CS series of transfer DNA insertion lines of ein2-2 (CS3071), npr1-1 (CS3724), fts2_SAIL, efr (SALK_068675), lym1 lym3 (CS2103242), rbohd (CS68747), rbohd (CS68748), rbohd rbohd (CS68522), tir1-1 abf4-8 abf2-3 (CS69646), jar1-1 arxl-3 (CS67934), myb51 (CS421816), arx5-1 (CS16234), cyp71a13 (CS769462), and ctn3 (CS2103723) mutant alleles were purchased from the Arabidopsis Biological Resource Center (http://www.arabidopsis.org/). efr-2 (Zipfel et al., 2006) and bak1-1 (Schwessinger et al., 2011) are from Dangl lab stock. pFRK1::NLS-3xmVENUS pUBQ10::RCI2A-tdTomato, pPER5::NLS-3xmVENUS pUBQ10::RCI2A-tdTomato, and pEFR::NLS-3xmVENUS pUBQ10::RCI2A-tdTomato, and pFLS::NLS-3xmVENUS pUBQ10::RCI2A-tdTomato, were kindly provided by Prof. Niko Geldner (University of Lausanne). DR5::GFP; efr:2 line was generated in this study. All plants 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). Plants were stratified for 2 d in a 4°C dark room and grown vertically for 4-10 days under long-day light conditions.

**METHOD DETAILS**

**Bacterial genetic manipulation**

The media and growth conditions used for DNA transformation of *B. velezensis* were described in (Idris et al., 2007). Gene deletions were performed by PCR amplification of 1000bp upstream and downstream of a given gene, the gene flanking regions were fused to an antibiotic resistance cassette using NEB builder (NEB) according to manufacturer's instructions. The reactions were amplified by PCR for 30 cycles and transformed into *B. velezensis*. amyE::P$_{pyr}$-ysnE performed by PCR amplification of ysnE + 300bp upstream of ysnE, the PCR product fused to the upper and lower half of the amyE gene amplified by PCR using NEB builder (NEB) according to manufacturer's instructions. The reactions were amplified by PCR for 30 cycles and transformed into *B. velezensis*. Bacteria with IPTG inducible genes were generated by PCR amplification of 1000bp on either side of a gene and cloned with an antibiotic resistance cassette, pHyperSpac promoter [from pdr111 plasmid Pdr111 and antibiotic resistance cassette kindly provided by Prof. David Rudner (Harvard)] and the gene. The 4 fragments were fused together using NEB builder (NEB). The reaction was amplified by PCR for 30 cycles and transformed into *B. velezensis*. ysnE-gfp bacteria were generated by PCR amplification of ysnE without a stop codon, the GFP coding region from AR16 (Rosenberg et al., 2012), an antibiotic resistance cassette, and 1000bp downstream of ysnE. The 4 fragments were fused together and transformed into *B. velezensis*.

**Monitoring bacterial growth on plant roots**

Bacteria from fresh colonies were grown in LB medium to an OD$_{600}$ = 1.0 and then diluted 1:100 in PBSx1 for CFU measurements and microscopy, or 1:10$^5$ for lateral roots and primary root measurements, yielding approximately 1×10$^5$, or 1×10$^6$ cfu/ml respectively. Six-day old seedlings were transferred onto square Petri dishes containing 0.5 MS but without sucrose. 2 µL of bacterial dilution were put right above the root tip and left to dry for 2 min. The square plates were kept in a vertical position during the incubation time at 22°C 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. For CFU counting the seedlings were transferred to a tube with 1 ml of PBSx1 and vortexed vigorously for 20 seconds, then the serial dilution was plated on LB plates. To asses the effect on EFR on *B. velezensis* colonization in the presence of the normal microflora (Figure 6H), seedlings were inoculated with *B. velezensis* amyE::pSpac-GFP (erm) for 48 hrs and then transferred for non-sterile potting soil (Sun Gro horticulture) for 7d. 8 plants for each genotype, normalized for approximate rosette size were picked. The roots were excised, and normalized to 20 gr +/- 5%. The roots were washed 3 times, transferred to a tube with 10 ml of PBSx1 and vortexed vigorously for 20 seconds, then the serial dilution was plated on erm (1 µg/ml) plates, and the number of GFP expressing bacteria was counted. Measuring callose deposition was done as described in (Schikora, 2015). For fungal infection, 6 day old seedlings grown on 0.5 MS plates were inoculated with 10$^3$ CFU/ml of *B. velezensis* or buffer for 48 hrs, then a 5 mm mycelial plug from the fungal culture was placed on the bottom of the plate and allowed to spread for an additional 7 days, after which, plant weight was measured. For estimation of fungal load, plant were treated as described above, after 3 days of fungal infection, seedlings were thoroughly washed for 20 times, and then transferred to a new agar plate. 24 hrs later the number of mycelia attached to the plant was quantified under the microscope. Seedling infected with fungus alone, without *B. velezensis* colonization were completely covered, precluding detailed quantification of fungal load (see Figure S9B). For symcom analysis the 34 bacteria were grown for over night at 30°C, Streptomyces species were grown for 48 hrs. Then bacteria were adjusted to OD$_{600}$ = 1, mixed together, centrifuged and resuspended in PBSx1. The mixed was diluted 1:100 in PBSx1 and inoculated as describes above. After 7 days the roots were excised, and treated and described above, then the PBSx1 was centrifuged and the supernatant frozeed in -80°C. Syncm DNA was extracted using PowerSoil DNA extraction kit (Qiagen). Library preparation and sequencing were done as described previously (Gohi et al., 2016). For sequence
analysis Sequences were filtered and agglomerated into amplicon sequence variants (ASV) by DADA 2 version 1.16 (Callahan et al., 2016). *Arthrobacter MF161*’s ASV was identified based on 100% identity.

**Microscopy**

Roots were observed using a Zeiss LSM 880 laser scanning confocal microscope with the indicated lenses. Lateral root number was counted under a Zeiss Axio Zoom.V16 fluorescence dissecting scope at 10× magnification. Fluorescent intensity and length measurement were done using ImageJ.

**Measurement of plant ROS production**

Leaf discs were cut with a 4 mm biopsy punch from 4 week-old plants and placed on sterile water with their adaxial side up in a white 96-well microtiter plate (Costar, Fisher Scientific) containing 150 μl H2O and then incubated overnight at 22°C in continuous light for 20 to 24 hours to reduce the wounding response. Immediately prior to elicitation, H2O was removed from each well and 100 μl of the elicitation solution (100μg/ml HRP (sigma), 1μM luminol (sigma) and bacteria adjusted to OD600=1) were added. Elicitation solution without bacteria was used as a control. Plates were analyzed every 1 min for a period of 45 min using a TECAN Infinite 200 PRO microplate reader with signal integration time of 0.5s. Statistical comparison between different plant genotypes was performed by Student t-test on maximal luminescence intensity values.

**RNA extraction library preparation and computational analysis**

For plant RNA, plant roots were cut and immediately frozen in liquid nitrogen. RNA prepared using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s instructions. RNA-seq libraries were prepared using QuantSeq 3’ mRNA-Seq Library Prep Kit (Lexogen) according to the manufacturer’s instructions. Illumina NextSeq 500 High-Output 75bp single reads were aligned to the *Arabidopsis thaliana* genome, and differentially expressed genes analyzed on the BlueBee platform (https://www.bluebee.com/lexogen) with default parameters. GO annotation was analyzed on (http://geneontology.org/) with default parameters.

For bacterial RNA preparation, bacteria treated with O2/C0 for 30 min were precipitated and bacterial pellets immediately frozen in liquid nitrogen. Pellets were then resuspended in 500μl lysis buffer (30 mM Tris, 10 mM EDTA, 10 mg/mL lysozyme) for 30 min in 37°C. RNA was prepared using the RNAzol reagent according to the manufacturer’s instructions. rRNA was removed using NEBNext® rRNA Depletion Kit (Bacteria) according to the manufacturer’s instructions. RNA-seq libraries were prepared using KAPA RNA HyperPrep Kit (Roche) according to the manufacturer’s instructions.

Illumina MiSeq v2 150bp PE reads were aligned to *B. velezensis Fzb42* using Kallisto (Bray et al., 2016). Differentially expressed genes with logFC>0.5 and p-value < 0.01 were identified using the edgeR package. The full code was described in (Wachsman et al., 2020). Genes were annotated based on homology to the genome of *B. subtillis* 168, and GO annotation analyzed on (http://geneontology.org/) with default parameters with *B. subtillis* 168 based annotation. At least 72% of the differentially expressed genes from each comparision had homologs in the *B. subtillis* 168 genome.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All data analysis, and graphs were executed in Excel, except for RNA sequence analysis (see RNA extraction library preparation and computational analysis section below.) Two tailed t-test was applied for statistical comparison, with Bonferroni correction for multiple comparisons when relevant, or one way ANOVA followed by posthoc Tukey Kramer, as indicated in the relevant figure legends.
Supplemental information

Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria

Elhanan Tzipilevich, Dor Russ, Jeffery L. Dangl, and Philip N. Benfey
Supplemental information

Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria

Elhanan Tzipilevich, Dor Russ, Jeffery L. Dangl, and Philip N. Benfey
Supplemental information

Plant immune system activation is necessary for efficient interaction with auxin secreting beneficial bacteria

Elhanan Tzipilevich, Dor Russ, Jeffery L. Dangl, Philip N. Benfey
Figure S1 Bacterial auxin effects on the plant roots and bacterial physiology. Related to figure 1.

(A) Seedlings were inoculated with either WT, ΔysnE bacteria, or buffer (mock) on agar plates for 7 days. Shown are representative plates from each of the conditions tested.

(B) Seedlings were inoculated with either WT, ΔysnE bacteria or buffer alone (mock) on agar plates for 7 days and the length of the primary root was measured, n ≥ 20. Each circle represent one root. (***) = P < 0.005, two tailed t-test with Bonferoni correction).

(C) The indicated bacterial strains were grown in Landy medium at 23° for 72 hrs with low shaking (25 RPM), and IAA concentration measured using Salkowski method. Shown are averages and SD, n = 3. Each circle represents the average of 3 technical repeats from one culture. (*) = P < 0.05, two tailed t-test with Bonferoni correction).

(D) WT or ΔysnE bacteria were grown at 23° and OD_{600} measured. Shown are averages and SD, n = 3.

(E) WT or ΔysnE bacteria were inoculated in the middle of 0.7% agar plates and incubated at 37° for 18 hrs. Shown are representative plates from 3 plates for each genotype.

(F) WT or ΔysnE bacteria were inoculated into MSgg medium in 6 well plates and incubated at 23° for 96 hrs. Shown are representative wells from 3 for each genotype.
Figure S2

A

Mock +Bacteria Mock +Bacteria

B

# Callose deposits

0 2 4 6 8 10 12 14 16

Plant: col0 efr2

Mock Bacteria

C

efl8 peptide 1 SKEFERTKPHWNTGTIG 18
KEKF+R K H N+GTIG

B. valezensis tuf 2 AKEKFDRSKSHANIGTIG 19

flg22 peptide 1 QRLSTGSRINSAXDDAQLQI 21
+ LS G RIN A DDAAGL I

B. valezensis hag 28 EKLSSGLRINRAGDDAQLAI 48

D

col0 WT col0 ΔysnE efr2 ΔysnE lvm1 lvm3 ΔysnE fls2 ΔysnE
Figure S2. Bacteria induced an immune response in the root, in an EFR dependent manner. Related to figure 2.

(A-B) Seedlings of Col-0 or efr2 were inoculated with bacteria or buffer alone (mock) for 48 hrs, then cleared with ethanol overnight and stained with aniline-blue dye. Shown are 200x confocal DAPI images (C) and average and SD from quantification of the number of callose deposits (D). n ≥ 4. Each circle represent one root. (*) = P < 0.05, two tailed t-test). Scale bar 25μm.

(C) Blast2seq analysis of elf18 and flg22 peptides against the respective B. valezensis proteins, Tuf, and Hag.

(D) Seedlings from the indicated genotypes were inoculated with the indicated bacterial strains expressing GFP for 48 hrs on agar plates. Shown are 200x maximal projection confocal images from roots (left panels) and GFP fluorescence from bacteria (right panels). Scale bar 50μm.
Figure S3 Bacterial auxin increases bacterial survival of plant immune system response. Related to figure 3.

(A) Seedlings of the indicated genotypes were inoculated with WT or ΔysnE bacteria for 48 hrs on agar plates and the number of colonizing bacteria was counted. Shown are averages and SD (log₁₀ transformed), n ≥ 3. Each circle represents an average of 3 technical replicates from the same root. (*** = P < 0.005, two tailed t-test).

(B) Seedlings were inoculated with WT or ΔysnE for 48 hrs on agar plates containing 5µM DPI and the number of colonizing bacteria was counted. Shown are average and SD of 2 independent experiments, n = 3. Each circle represents an average of 3 technical replicates from the same root. (* = P < 0.05, two tailed t-test).

(C-D) Seedlings of rbohd rbohf plants were inoculated with bacteria or buffer (mock) on agar plates for 7 days and the number of lateral roots was counted. Shown are average and SD (n ≥ 20) (C) and representative plates (D). (*** = P < 0.005, two tailed t-test).

(E) Bacterial culture grown to OD₆₀₀=1 were treated with the indicated concentrations of H₂O₂ for 30min and CFU were counted. Shown are averages and SD (log₁₀ transformed), n=3. Each circle represents an average of 3 technical replicates from the same root. (* = P < 0.05, two tailed t-test with Bonferoni correction).

(F) Bacterial cultures grown to OD₆₀₀=1 were treated with O’ through enzymatic reaction with xanthine and either 5µL xanthine oxidase enzyme (similar to Figure 3A) or 0.5µL xanthine oxidase enzyme (the concentration used for the RNA sequencing experiments, Figure 3C) for 30min and CFU were counted. Shown are averages and SD (log₁₀ transformed), n=3. Each circle represents an average of 3 technical replicates from the same root.
Figure S4 ROS induced genes are important for host survival of plant immune system response.

Related to figure 4.

(A) Seedlings were inoculated with the indicated bacterial strains in the presence or absence of 0.5 mM IPTG for 48 hrs and bacterial CFU counted. Shown are averages and SD of 2 independent experiments (log$_{10}$ transformed), $n \geq 3$. Each circle represents an average of 3 technical replicates from the same root. ($** = P < 0.01$, $*** = P < 0.005$, two tailed t-test).

(B) Seedlings were inoculated with WT or ΔysnE bacteria for 48 hrs on 0.5 MS agar plates without iron, supplemented with either 27.8 mg/l FeSO$_4$ (full Fe) or half of that amount and the number of colonizing bacteria was counted. Shown are averages and SD (log$_{10}$ transformed), $n \geq 3$. Each circle represents an average of 3 technical replicates. (* = P < 0.05, two tailed t-test).

(C-D) The indicated bacterial strains were spread on LB agar plates with or without 5μM IAA. 5μL from 1M FeSO$_4$ was spotted over the bacterial lawn, plates were incubated overnight at 37° and the growth inhibition zone around the spot was measured. Shown are images from ΔpfeT bacteria (C), which are mutant in the iron efflux pump (Guan et al., 2015). Grown in the presence (upper) or absence (lower) of 5μM IAA. Average and SD from quantification of growth inhibition zone of at least 6 FeSO$_4$ spots are shown in D. Each circle represents one spot. (** = P < 0.005, two tailed t-test). Scale bar 0.5mm

(E-F) Ysne-GFP expressing bacteria grown to OD$_{600}$=1 were treated with O for 30min and observed under the microscope. Shown are 400x DIC image (left) and GFP fluorescence from YsnE (right) (E), and quantification of GFP (F). Bacteria treated with the substrate without the enzyme (Mock) were used as control. Scale bar 3µm. (GFP exposure time = 520 ms).

(G) Seedlings of Col-0 or efr2 were inoculated with bacteria or buffer alone (mock) on agar plates for 7 days and length of the primary root was measured, $n \geq 20$. Each circle represents one root.
Figure S5. ROS induced auxin enhances root colonization through stimulation of bacterial flagella.

Related to figure 5.

(A) Col-0 or efr2 seedling were inoculated with GFP expressing bacteria (amyE::Pspac-gfp) and then followed by time lapse confocal microscopy for 12hrs. Spots of colonizing bacteria were counted at t = 2hr and followed until t =12. Bacteria that remained attached during this time course were counted as successful colonization events (see Figure 4A for quantification). Shown are 400x overlay images of DIC from roots (grey) and GFP fluorescence from bacteria (green), taken at the indicated time points from efr2 roots. Red arrows highlight bacteria counted as having undergone a successful colonization. Yellow arrows highlight bacteria counted as having undergone an unsuccessful colonization. Scale bar 10µm

(B-C) Seedlings of the indicated genotypes were inoculated with WT bacteria expressing GFP, for 48 hrs on agar plates. In the absence (B) or presence of 5µM IAA (C). Shown are 200x maximal projection confocal images of DIC from the roots (left panels) and GFP fluorescence from bacteria (right panels). Scale bar 50µm

(D) Seedlings of the indicated genotypes were inoculated with WT bacteria in the presence or absence of 5µM IAA for 48 hrs on agar plates and the number of colonizing bacteria was counted. Shown are averages and SD from 2 independent experiments (log₁₀ transformed) with n ≥ 3 for each. Each circle represents an average of 3 technical replicates from the same root. (*** = P < 0.005, two tailed t-test).

(E) Bacteria were inoculated in the middle of 0.7% agar plates with 5µM IAA or without (mock) and incubated at 37°C for 9 hrs. Shown are representative plates from 3 plates for each condition. Scale bar 2mm.

(F) Seedlings were inoculated with the indicated bacterial strains with or without 5µM IAA for 48 hrs on agar plates and the number of colonizing bacteria was counted. Shown are averages and SD (log₁₀ transformed), n = 3, each circle represents an average of 3 technical replicates from the same root. (*) = P < 0.05, *** = P < 0.005, two tailed t-test).
Figure S6

A

D5-GFP

Mock

Arthrobacter

B

#Lateral roots

Plant: col0 fls2 col0 fls2

Mock

Arthrobacter

C

Luminescence (AU)

Time (min)

0 5 10 15 20 25 30 35 40 45

Arthrobacter

co0 fls2 efs2

D

Root length (mm)

Mock +Arthrobacter

E

CFU/root

col0 fls2

mock +IAA

F

CFU/root

Mock +IAA

G

Bacteria: P. fluorescens wcs365 P. defensor wcs374

CFU/root

1E+5

1E+4

1E+3

1E+2

1E+1

1E+0

n.s

mock +IAA

1E+5

1E+4

1E+3

1E+2

1E+1

1E+0

n.s

mock +IAA
Figure S6. Plant immunity interaction with bacterial auxin secretion in *Arthrobacter Mf16*. Related to figure 6

(A) Arabidopsis DR5::GFP reporter lines were inoculated with *Arthrobacter Mf161* or buffer alone (mock) for 48 hrs on agar plates. Shown are 100x maximal projection confocal images of GFP fluorescence from DR5::GFP reporter. Scale bar 50µm.

(B) Col-0 or *fls2* seedlings were inoculated with *Arthrobacter Mf161* or buffer alone (mock) on agar plates for 7 days and the number of lateral roots was counted, n ≥ 20. (* = P < 0.05, ANOVA followed by posthoc Tukey Kramer).

(C) Leaf discs from 28 day-old plants, taken from the indicated genotypes were incubated with bacteria adjusted to OD 0.1, and ROS production was measured. Shown are average and SD (n ≥ 10).

(D) Col-0 or *fls2* seedlings were inoculated with *Arthrobacter Mf161* or buffer alone (mock) on agar plates for 7 days and the length of the primary root was measured. (n ≥ 20). Each circle represent one root. (*** = P < 0.005. * = P < 0.05, two tailed t-test with Bonferroni correction).

(E) Col-0 or *fls2* seedlings were inoculated with *P. polymyxa* for 48 hrs on agar plates and the number of colonizing bacteria counted. Shown are averages and SD of 2 independent replicates (log_{10} transformed) with n = 3. Each circle represent an average of 3 technical replicates from the same root.

(F) Seedlings were incubated with *P. polymyxa* for 48 hrs on agar plates in the presence or absence of 5µM IAA and the number of colonizing bacteria counted. Shown are averages and SD of 2 independent replicates (log_{10} transformed) with n = 3. Each circle represent an average of 3 technical replicates from the same root. (*** = P < 0.005, two tailed t-test).

(G) Seedlings were incubated with *Pseudomonas fluorescence WCS365* or *Pseudomonas defensor WCS374* for 48 hrs on agar plates in the presence or absence of 5µM IAA and the number of colonizing bacteria counted. Shown are averages and SD (log_{10} transformed), n = 4. for each treatment. Each circle represent an average of 3 technical replicates from the same root.
Figure S7

A

B

C

B. victorinii
Arthrobacter nif

Relative Abundance

Col-0
fts2

Col-0
fts2

1E+0
1E+1
1E+2
1E+3
1E+4
1E+5

Plant:

col0

EFR2

mono-culture
coculture
Figure S7. Immune system activation enhances bacterial colonization in face of competition. Related to figure 6

(A) Seedlings of Col-0 or efr2 were inoculated with either *Arthrobacter Mf161* or *B. valezensis* alone (monoculture) or in a mixture (1:1 ratio, co-culture) for 48 hrs on agar plates and the number of colonizing bacteria from each strain was counted. Shown are averages and SD of 2 independent replicates (log$_{10}$ transformed) with n=3. Each circle represent an average of 3 technical replicates from the same root.

(B) Seedlings were inoculated with the indicated bacterial strains for 48 hrs on agar plates. Shown are 400x confocal images of DIC from roots incubated with *B. valezensis* (1), *P. polymyxa* (2), and *Arthrobacter Mf161* (3-4). The images on the left are magnifications of the black frames on the right images, in which the rod-shape *B. valezensis* (1) and *P. polymyxa* (2) and the small rod / cocci shape of the *Arthrobacter Mf161* (4) can be seen. Yellow arrows highlight the small cells of the root elongation zone. Red arrow highlights the root hair of a trichoblast cell in the differentiated part of the root. Scale bar 10µm.

(C) Seedlings of Col-0 or fls2 were inoculated with a community of 34 bacteria (Teixeira et al., 2021) including *Arthrobacter Mf161* for 7 days on agar plates and bacterial abundance was measured by 16S RNA sequencing. Shown are averages and SD of the relative abundance of *Arthrobacter Mf161* from 2 independent experiments with n ≥ 6 for each. Each circle represents one root. The relative abundance difference in the experiment in the left panel is statistically significant (*P* < 0.005), But not in the right panel.