

Special Issue: Unraveling the Secrets of the Rhizosphere

## Review

## Beneficial Microbes Affect Endogenous Mechanisms Controlling Root Development

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Plants have incredible developmental plasticity, enabling them to respond to a wide range of environmental conditions. Among these conditions is the presence of plant growth-promoting rhizobacteria (PGPR) in the soil. Recent studies show that PGPR affect *Arabidopsis thaliana* root growth and development by modulating cell division and differentiation in the primary root and influencing lateral root development. These effects lead to dramatic changes in root system architecture that significantly impact aboveground plant growth. Thus, PGPR may promote shoot growth via their effect on root developmental programs. This review focuses on contextualizing root developmental changes elicited by PGPR in light of our understanding of plant-microbe interactions and root developmental biology.

## Beneficial Microbes Can Induce Plant Growth by Modifying Root Development

In the early 1900s, Hiltner made the key observation that the soil around the plant root contains more microorganisms than the surrounding soil [1]. Soil has since been documented to have exceptional microbial diversity [2], containing fungi, invertebrates, archaea, and bacteria [3]. Among the soil bacteria are PGPR (see Glossary) [4,5]. Unlike obligate symbionts, these bacteria can interact with numerous host plants and improve plant growth and health via various mechanisms that can be direct, such as nitrogen fixation [6,7], or indirect, including competition with pathogens [8,9]. PGPR are capable of modulating the root system architecture (i.e., the spatial configuration of the root system), which is a significant determinant of crop yield [10–12]. The potential of PGPR to affect plant growth and root architecture was excellently addressed in two recent reviews [13,14]. By contrast, the mechanisms by which PGPR influence cell division, and alter the balance between proliferation and differentiation in the primary root and lateral root initiation sites, remain largely unknown.

In this review, we focus on the ability of PGPR to affect post-embryonic root development. It has become clear that PGPR affect post-embryonic root development by altering cell division and differentiation within the primary root as well as affecting root hair formation and lateral root development. We highlight recent findings demonstrating that bacteria modulate endogenous root developmental mechanisms to establish these effects. This review has three main sections, describing root–bacterial interactions in progressive detail. We begin by describing root–bacterial interactions in the **rhizosphere** and the determinants of microbial community structure in association with plant roots. Subsequently, we describe the current knowledge of the effects of soil bacteria on root development at the cellular level. Finally, we discuss the current understanding of bacteria on plant regulatory mechanisms. We have selected a few bacterial

## Trends

Interaction between plant roots and the beneficial bacteria within their rhizosphere shapes the bacteria community composition, and enhances plant growth and plant pathogen defense.

Plant growth-promoting rhizobacteria (PGPR) affect cell division and differentiation leading to changes in root system architecture, which contributes to enhanced shoot growth. These modifications are established by changing plant endogenous signaling pathways.

While several PGPR can produce phytohormones, many effects on plant developmental pathways are exerted by other molecules.

Several fungi have the same effects on root system architecture as PGPR, indicating that growth-promoting mechanisms might be conserved across kingdoms.

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species to highlight those that alter cell division and differentiation in the *Arabidopsis* root. While our focus is on PGPR, we conclude our review by addressing how fungi affect root development to draw attention to the similarities between these two plant–microbe interactions.

Future studies integrating the fields of plant–microbe interactions and plant developmental biology will lend insight into how soil microbes affect root development. This work will enhance our understanding of these complex cross-kingdom interactions and increase our knowledge of root developmental biology and bacterial signaling. Ultimately, this knowledge will foster development of sustainable plant growth-promoting technologies that have the potential to dramatically increase crop yield and food security.

### Plant–Microbe Interactions in the Rhizosphere

When Hiltner observed the increased number of microorganisms around roots compared with bulk soil in the 1900s, he assumed that this increase was due to nutrient secretion by the plant and termed it ‘the rhizosphere effect’ [1]. Since then, the rhizosphere has been defined as the soil around plant roots influenced by the root and its exudates, whereas the rest of the soil is referred to as bulk soil (Figure 1) [15]. Soil properties themselves are exceptionally diverse, with abiotic factors that influence the bacteria in the bulk soil including pH, moisture, and nutrient content [2,16]. The extent to which plants and soil characteristics influence the microbial communities in the soil has been elegantly reviewed [17–20]. Recently, studies using deep-sequencing techniques found that soil type has a more dramatic effect on rhizosphere microbial communities than plant genotype [21–23]. These results suggest that the soil composition plays a pivotal role in shaping the bacterial communities in the soil and rhizosphere.

As Hiltner speculated in addition to soil type, bacterial communities in the rhizosphere are likely dependent on the type and composition of root exudates secreted by the plant [24,25]. Root exudates include sugars, amino acids, organic acids, fatty acids, phenolics, enzymes, and flavonoids [26]. The ability of bacteria to thrive in the rhizosphere depends on their ability to move toward these plant-derived carbon sources (for more on chemotaxis, see [27,28]) and to use them and other root-derived rhizodeposits such as sloughed-off root cells or lysates as energy sources [29–31]. The rhizosphere effect has been documented by numerous groups since Hiltner's initial observations [32]. Correlated with differences in root exudates, *Arabidopsis* accessions, or natural variants, differ in their root-associated microbial community [33]. Additionally, a single mutation in the gene encoding an ATP-binding cassette transporter in *Arabidopsis* changes the microbial community around the plant root [34]. Plant-derived compounds, including polysaccharides and, rosmarinic acid, affect **quorum sensing** in soil bacteria [35,36], suggesting that plants excrete certain compounds to influence bacteria in the rhizosphere. Microbes within the rhizosphere can in turn modify root exudate composition [37–40], enhance growth [41–43], and induce systemic resistance to subsequent pathogen attack [44–47]. These examples reveal a rich language of chemical communication between plants and rhizosphere-inhabiting microbes resulting in altered microbial community structure and plant growth and health.

Bacteria found within plant roots are referred to as **endophytic** bacteria and comprise a much less diverse community than what is found in either the rhizosphere or the bulk soil [21,22,48,49]. The plant immune system [50] likely mediates the decreased bacterial diversity. **Phytohormones** used in plant defense, including jasmonic acid, **ethylene**, and salicylic acid, have been shown to influence rhizosphere composition in certain soils [51]. Salicylic acid was recently shown to alter the colonization of certain bacterial families within roots [52]. Additionally, mutant plants defective in multiple phytohormone signaling pathways had lower survival rates and distinct endophytic microbial colonization compared with wild-type plants [52]. These results reflect the importance of plant defense mechanisms in regulating bacterial colonization within the root.

### Glossary

**Auxin:** phytohormone that, among other plant processes, is involved in cell division and specification in the root meristem as well as formation of lateral root primordia.

**Casparian strip:** a waxy cell-wall thickening in the root endodermis that restricts the flow of solutes and water into and out of the central vasculature. This barrier also restricts bacteria and fungi from entering these cells. The Casparian strip is a hallmark of differentiated endodermis.

**Cytokinin:** phytohormone that often functions antagonistically of auxin. In root development, cytokinin induces differentiation of cells as the move shootward.

**Endophytes:** microorganisms living within plant tissue without causing harm to the plant.

**Ethylene:** phytohormone involved in regulation of cell size, aging, and fruit ripening.

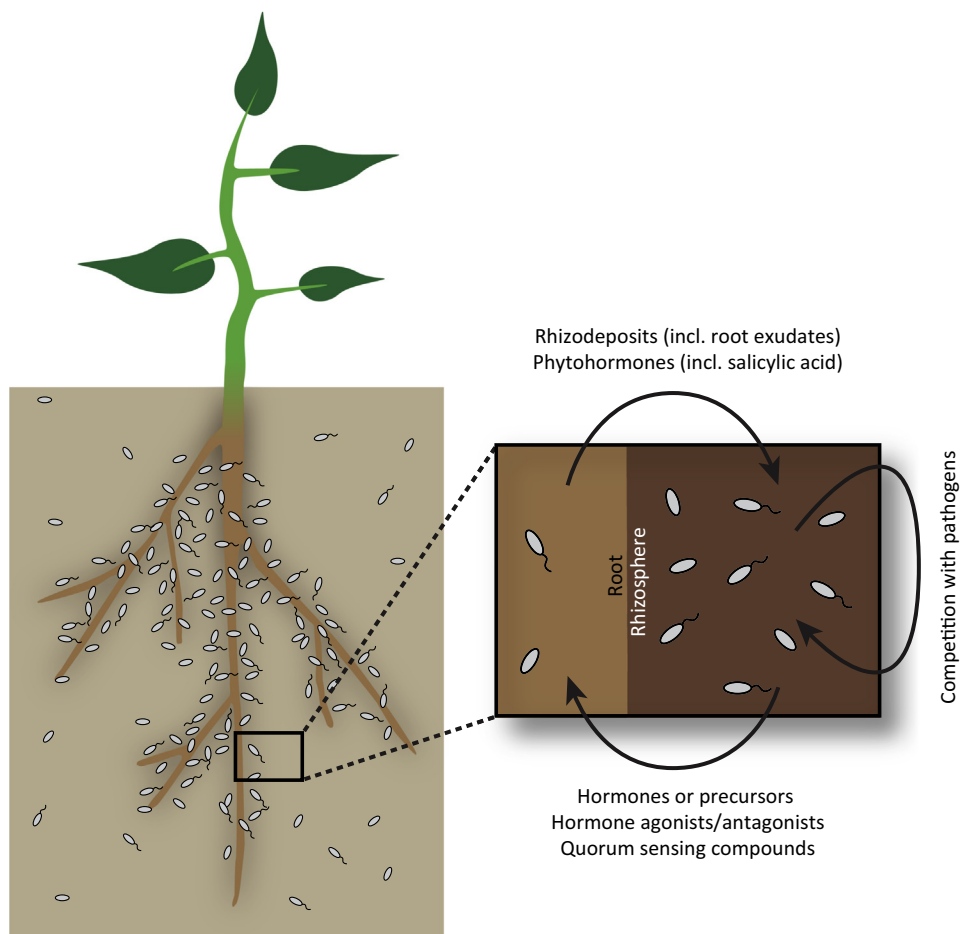
**Phytohormone:** signaling molecule produced by the plant that regulates a broad range of cellular processes from cell division and plant defense to aging. Examples include auxin, cytokinin, and ethylene.

**Plant growth-promoting rhizobacteria (PGPR):** bacteria found in the rhizosphere that promote plant growth or health either directly or indirectly.

**Quorum sensing:** a process by which bacteria measure their density and modify their behavior accordingly, i.e., to form biofilms, produce antibiotics, or coordinate virulence.

**Rhizosphere:** the thin layer of soil around plant roots that is influenced by the root and its exudates. The rhizosphere harbors a more numerous, but less diverse, group of microorganisms than the surrounding bulk soil.

**Stem cell niche:** the group of cells near the root tip that contains the initials, or stem cells, and quiescent cells. Together these cells supply cells that enable primary root elongation and root topology. A stem cell niche is established in the tip of lateral roots during their formation.



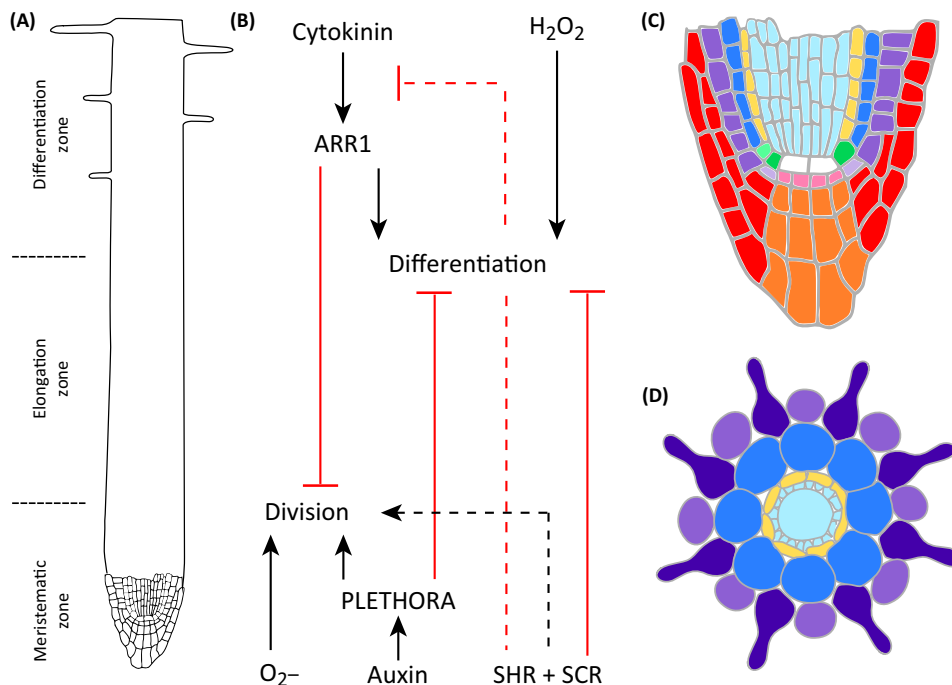
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**Figure 1. Plants Affect the Bacterial Community Composition within the Rhizosphere and the Root.** The bacterial community within the rhizosphere – the thin layer of soil around a plant's root system that is affected by the roots and their exudates (dark brown) – is more numerous than the community in bulk soil (tan). Plants appear to influence the rhizosphere microbiome composition via their root exudates, although this effect varies between studies. In return, bacteria affect plant growth via many compounds, including hormones and hormone agonists and antagonists and quorum-sensing compounds. In addition, they indirectly affect plant growth by competing with pathogens for both space and nutrients. A select group of bacteria is found within the *Arabidopsis* root (inset). The plant–bacteria interface provides many possibilities for communication.

## Post-embryonic Root Development Is Affected by PGPR

### Post-embryonic Primary Root Development in *Arabidopsis*

The *Arabidopsis* root architecture comprises a primary root with iteratively branching lateral roots. The primary root can be divided into three developmental zones: the meristematic, elongation, and differentiation zones (Figure 2A). The root tip, which contains the meristematic zone, is surrounded and protected by a group of gravity-sensing cells called the root cap. The root cap deposits mucilage, cell debris, and whole cells into the rhizosphere as the root grows [53]. The meristematic zone contains the **stem cell niche**, the progenitor cells that give rise to distinct cell types, and the dividing daughter cells [54]. A quiescent center located at the center of the niche comprises four rarely dividing cells [55] that repress differentiation of the surrounding initials, or stem cells [56]. There are four sets of initials: columella, lateral root cap/epidermis, cortex/endodermis, and stele or vasculature (Figure 2C) [55,57]. The transition zone, or basal



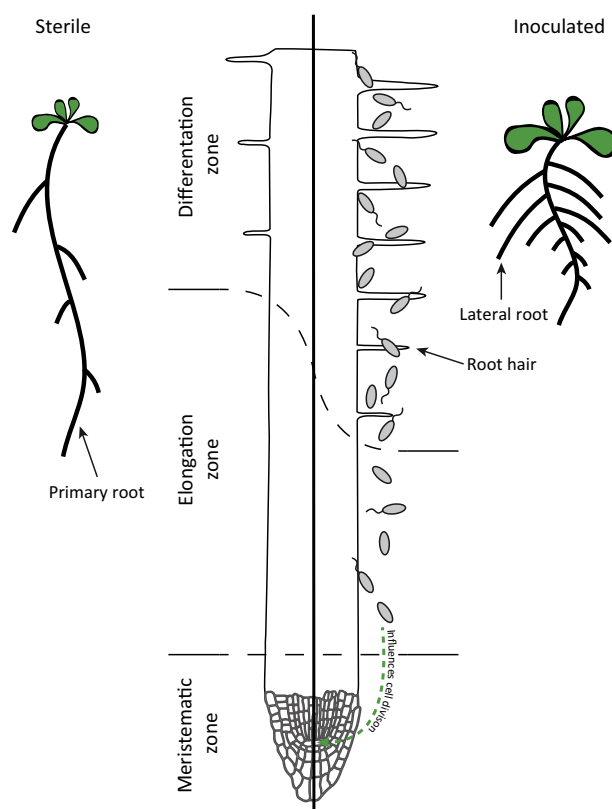
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**Figure 2. Arabidopsis Root Structure.** (A) Longitudinal diagram of the root. The root can be divided into three developmental zones: the meristematic zone, where the stem cell niche and rapidly dividing cells are located; the elongation zone, where cells increase in length; and the differentiation zone, where cells acquire their unique features. (B) In the root tip, the high concentration of auxin induces division and inhibits differentiation. Scarecrow (SCR) and shortroot (SHR) inhibit differentiation within the quiescent center specifically by inhibiting the cytokinin response via their downstream effector *WOX5*. More shootward, the decreasing concentration of auxin and the increasing concentration of cytokinin ultimately result in a hormonal balance that shifts in favor of differentiation. The antagonistic action of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide ( $\text{O}_2^-$ ) is also involved in determining the transition point between division and differentiation, independent of auxin and cytokinin. (C) Longitudinal section of the root meristem. The quiescent center (white) is surrounded by stem cells: the cortex/endodermal initial (green); the epidermal/lateral root cap initial (violet); and the columella initials (pink). (D) Transverse section of the root in the differentiation zone reveals the radial symmetry of the outer cell types around the pericycle and vasculature (light blue), root hair epidermis (dark purple), non-root hair epidermis (light purple), cortex (blue), and endodermis (yellow).

meristem, is located shootward of the meristematic zone. The cells in this zone do not divide and cell lengthening is slow. In the neighboring elongation zone, cells elongate up to 300% within 3 h [54]. Finally, cells acquire their mature characteristics in the differentiation zone, which extends shootward. The differentiation zone can be distinguished by the emergence of root hairs, which emerge from epidermal cells overlying two cortical cells (Figure 2D) [58,59]. Another defining feature of the differentiation zone is the formation of the **Casparian strip** in the endodermis [60,61].

#### Effect of PGPR on Primary Root Development

PGPR-induced root phenotypes have been described in the literature. The most common is an inhibition of primary root growth coupled with proliferation of lateral roots and root hairs [62,63] leading to increased shoot biomass. Another phenotype is an increase in plant biomass coupled to an increase in primary root growth [42,64]. These effects are dependent on both the bacterial density and the distance from the plant root at which the bacteria are applied [41,65]. Although growth promotion phenotypes have been well described [4,5], few molecular mechanisms underlying these effects are known. Recently, several PGPR have been shown to induce root



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**Figure 3. Bacteria Influence Overall Plant Physiology and Primary Root Development.** Typically, beneficial bacteria enhance lateral root formation and shoot growth and inhibit primary root growth. Within the meristem cell division is affected either positively or negatively depending on the species and growth conditions. In addition, differentiation is induced closer to the root tip and root hair density and length are increased.

developmental changes in cell division and differentiation at both the root meristem and sites of lateral root formation (see below). These cellular level changes alter the overall root system architecture of the plants.

Colonization of the *Arabidopsis* root by many PGPR species, including *Pseudomonas simiae* WCS417 (formerly *Pseudomonas fluorescens* WCS417 [66]) or *Bacillus megaterium* UMCV1 influences the transition from proliferation to differentiation in the root [67–72]. In the meristematic zone, *P. simiae* increases cell division [67] while *B. megaterium* decreases cell division [68]. These PGPR species decrease primary root length when applied directly to the roots by decreasing cell elongation in the elongation zone by 40% and 70%, respectively (Figure 3) [67,68]. Possibly as a result of premature differentiation, root hairs emerge closer to the root tip in colonized plants [68]. In addition, root hair density increases upon colonization due to a higher number of cortical cells around the radial axis. The higher number of cortical cells increases the number of root-hair-forming cells; that is, epidermal cells overlying two cortical cells. In addition, root hairs grow longer and root hair formation is accelerated by an as yet undefined mechanism [67].

#### Lateral Root Development in *Arabidopsis*

Besides affecting primary root development, PGPR also influence lateral root formation. Lateral root development has been elegantly studied by several groups [73–76]. In brief, there are two steps to forming a lateral root. First, pairs of cells in the pericycle (the cell layer in the vasculature

neighboring the endodermis) called lateral root founder cells must become competent to form a lateral root. Second, lateral root founder cells are activated, divide multiple times, and differentiate to form the lateral root primordia, which ultimately emerge from the primary root in the differentiation zone [77]. Endodermal cells overlying the lateral root primordia separate to accommodate the emerging lateral root, [78] change shape, reduce their size, and form small holes in the Casparian strip [79]. It remains to be seen whether these breakpoints in the Casparian strip can account for the abundance of bacteria reported at lateral root emergence sites [21,49,80]. Further divisions and differentiation of the lateral root cells after emergence ultimately result in a lateral root anatomy identical to the anatomy of the primary root, including a meristematic zone that controls the lateral root's growth rate [77].

#### Effect of PGPR on Lateral Root Development

*P. simiae* WCS417 and *B. megaterium* UMCV1 increase the number of both lateral root primordia and lateral roots [67,68], indicating that the number of lateral-root-competent sites and lateral root outgrowth are affected by colonization. It is unknown whether PGPR also influence lateral root founder-cell specification. Interestingly, the induction of lateral root formation and shoot growth can occur without the aforementioned observed primary root growth inhibition: volatile organic compounds produced by *P. simiae* WCS417 stimulate lateral root formation but do not inhibit primary root growth [67].

### PGPR Affect Endogenous Root Developmental Programs

#### Plant Endogenous Mechanisms Regulating Root Development

PGPR probably induce the above root development phenotypes by modulating plant endogenous mechanisms regulating root development. As described above, primary root development is controlled in the stem cell niche, which is established during embryogenesis [81]. Post-embryonically, positioning and maintenance of the niche requires the plant hormone **auxin** and its downstream PLETHORA (PLT) transcription factors that form a gradient with a maximum within the niche [82–84]. The transcription factors SHORTROOT (SHR) and SCARECROW (SCR) act in parallel with and independent of the PLTs to maintain stem cell niche identity [83,85,86]. SHR is expressed in the stele and moves to the nuclei of the adjacent cell layers to activate SCR expression [87]. In the quiescent center, SCR inhibits differentiation and maintains the identity of the surrounding stem cells [88] by suppressing **cytokinin** perception [89]. In the transition zone, the suppression of cytokinin perception is relieved and cytokinin negatively regulates expression of the auxin efflux transport proteins known as PINs. This leads to auxin redistribution and induces differentiation (Figure 2B) [90].

Reactive oxygen species (ROS) also regulate the transition from proliferation to differentiation independent of auxin and cytokinin [91]. Two transcription factors, UPBEAT1 and MYB36, have been found to regulate reactive oxygen homeostasis [92,93]. Repression of certain peroxidases from the elongation zone shootward increases hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels and decreases superoxide (O<sub>2</sub><sup>•−</sup>) levels, resulting in differentiation. ROS potentially enhance differentiation by stopping the cell cycle and modifying cell walls to allow cell expansion (Figure 2B) [92]. Thus, PGPR may alter ROS balance within the root resulting in growth and developmental changes.

As mentioned, lateral roots form from pericycle cells in the differentiation zone. Local auxin synthesis has been shown to induce lateral root primordia [94]. Until recently, it was thought that an auxin maximum in the lateral root founder cells dictates lateral root formation (reviewed in [75]). Recently, however, it was shown that competence to form a lateral root is induced by periodic gene oscillations in the transition and elongation zones and that auxin is not sufficient to generate these gene oscillations [95,96]. However, an auxin response is activated in the pericycle founder cells before the first division [97]. In addition, its downstream targets PLT3, PLT5, and PLT7 prevent clustering of lateral root competence sites and are essential

for subsequent lateral root emergence [98]. Thus, auxin is required for lateral root formation but periodic gene oscillations generate lateral root competence sites [76].

As in primary root development, cytokinin functions antagonistically with auxin: cytokinin inhibits lateral root formation [99–101]. Interestingly, cytokinin levels are not decreased in the transition zone. Instead, only the response to cytokinin seems to be downregulated [101], similar to the inhibition of the cytokinin response in the quiescent center by SCR. Thus, the phytohormones auxin and cytokinin are the major opposing players regulating both primary and lateral root growth, with SCR and SHR inhibiting the cytokinin response in the quiescent center specifically.

#### Effect of PGPR on Endogenous Root Developmental Programs

Several PGPR are known to produce cytokinin [102–104] and inoculation of lettuce plants with cytokinin-producing *Bacillus subtilis* strains increases plant growth and plant cytokinin content [104]. Moreover, the growth-promoting effects of *B. megaterium* strains are dependent on functional cytokinin receptors in Arabidopsis [105]. Similarly, the amount of auxin produced by PGPR has been shown to correlate with the ability to induce plant growth for several bacterial species and isolates from the rhizosphere [106–110]. Moreover, the increased cell division in the Arabidopsis meristem induced by *P. simiae* is accompanied by increased auxin-responsive gene expression [67] and the decrease in cell division caused by *B. megaterium* is accompanied by a decreased auxin response in the primary root tip as measured with the auxin-responsive marker construct *DR5:uidA* [68]. Since auxin effects are dose dependent, these different auxin levels could explain how bacteria elicit different growth effects or affect growth along the length of the root distinctly.

Although a functional auxin response within the plant is essential for several of *P. simiae*'s effects on root development, *P. simiae* does not make auxin [67]. Instead, it might produce an auxin mimic, as has been shown for *P. aeruginosa* [111]. Additionally, bacterial quorum-sensing molecules may be involved. Quorum sensing is the process by which bacteria assess the population density to coordinate behavior like virulence or biofilm formation. Diketopiperazines (DKPs) compounds are produced by many bacterial species that are involved in quorum sensing. DKPs have a planar structure containing a heterocyclic system also found in auxin and activate auxin-inducible gene expression, potentially by binding to the auxin receptor itself [111]. The auxin-responsive gene expression subsequently induces lateral root growth but does not inhibit primary root growth [111].

Indole, a compound used by bacteria for a wide range of functions such as biofilm formation and virulence, can also affect auxin signaling within a plant. When applied to plants, indole enhances lateral root primordium development. Polar auxin transport is required for this effect but is not influenced by indole application. Moreover, although indole can be converted into auxin it does not significantly change auxin levels within the plant and impedes the ability of plants to respond to exogenous auxin. This suggests that indole is converted into an auxin antagonist within the plant [112].

*N*-Acyl-homoserine lactones (AHLs) are another group of quorum-sensing molecules produced by soil bacteria that influence root development independent of auxin. At high concentrations, AHLs induce lateral root growth and inhibit primary root growth possibly by modifying the cytokinin response instead of the auxin response [113]. Lower concentrations of short-chain AHLs increase primary root elongation by increasing meristematic cell division and cell elongation through a mechanism involving G protein signaling and calmodulin [43,64]. Interestingly, the virulence factor pyocyanin (PCN), regulated by quorum sensing, has been shown to induce the root phenotype independent of either auxin or cytokinin. This molecule may affect root development by manipulating ethylene levels, subsequently leading to changing ROS levels in the

primary root tip [114]. Interestingly, *Bacillus* sp. B55 is able to rescue ethylene-insensitive mutants by means of the volatile compound dimethyl disulfide [115]. Still other PGPR, including *B. megaterium* affect root system architecture independent of either auxin or ethylene by an unknown mechanism [68]. These examples illustrate the importance of signaling between bacteria and plants.

In summary, the cellular effects of PGPR on root system architecture are generally accompanied by changes in plant endogenous responses. While auxin and cytokinin homeostasis are most often affected in the plant [63,67,111,113], changes in ethylene levels and its downstream effectors the ROS have also been observed [114]. Since several PGPR have been shown to produce these hormones, it is tempting to suggest that these changes may be induced by these hormones directly. However, so far, most evidence indicates that molecules involved in essential bacterial processes such as quorum sensing function as phytohormone mimics or indirectly influence plant phytohormone homeostasis. It remains to be determined whether bacteria-induced changes in root development and growth are beneficial to the bacteria; for example, by increasing carbon sources in the form of plant root exudates.

#### Plant–Fungi Interactions Show Great Similarity to Plant–Bacteria Interactions

The effects of PGPR on plant development are not specific to plant–bacteria interactions since similar phenotypes are induced within roots on exposure to fungi. The ectomycorrhizal fungus *Laccaria bicolor* S238N, the truffles *Tuber borchii* strains ATCC 96540 and 43BO and *Tuber melanosporum* strains Bal1 and Rey\_t, *Trichoderma virens* Gv. 29-8, and *Trichoderma atroviride* (IMI 206040) all increase lateral root growth [116–119]. *T. atroviride* also increases root hair length and density [119]. In addition, the truffles inhibit primary root growth [117]. Therefore it is tempting to speculate on the conserved responses to biotic factors.

Like PGPR, fungi affect endogenous plant mechanisms to influence root development. The increased development of lateral roots in response to *T. virens* is accompanied by increased expression of the auxin-inducible marker *DR5:uidA* in the primary root tip and the developing lateral roots and is dependent on functional auxin transport and responses in the plant [116]. Since *T. virens* produces the auxin precursor IAA [116], this suggests that fungal auxin leads to a change in auxin signaling in the plant, ultimately leading to the observed root phenotype. Plants also respond to contact with *L. bicolor* with an increased auxin response. However, although *L. bicolor* produces auxin, auxin produced by the fungus is not the only trigger to induce lateral root formation in *Arabidopsis*. Instead, a volatile compound produced by the fungus induces the phenotype, which potentially leads to enhanced auxin biosynthesis within the plant resulting in the observed root phenotype [118]. This volatile might be ethylene, which is produced by the truffles and, together with fungal auxin, induces the mentioned root phenotype when applied to the roots directly [117]. The possible interplay between auxin and ethylene in response to beneficial fungi is supported by work on *T. atroviride*. *T. atroviride* produces both auxins and ethylene. Ethylene signaling within the plant is important for the fungus-induced root hair phenotype while lateral root induction is ethylene independent [119]. Lateral root induction might instead be dependent on auxin signaling in the plant, which is affected by the bioactive metabolite 6-pentyl-2H-pyran-2-one (6-PP) produced by *T. atroviride* [120]. 6-PP increases auxin responsiveness in the lateral root primordia, as shown by increased *DR5:GFP* expression in the primordia, possibly by changing expression of PIN proteins, which are auxin transporters [120]. The signals from the two hormones might be integrated in a positive feedback loop by MPK6, although the precise interactions have not been uncovered [119].

Together, the research on fungi presents another example of the complex mechanisms underlying the effects of soil microorganisms on root development. In addition, it exemplifies the similarities between the interaction of both bacteria and fungi with plants. Like PGPR, soil

fungi appear to change lateral root and primary root growth primarily by affecting plant endogenous phytohormone signaling. As seen for PGPR-induced effects, while microorganism-produced auxin might induce these effects, there is evidence that other compounds, such as the volatile ethylene, are the causal agents of the effect on root development and that intricate crosstalk mechanisms are involved. These clear similarities between fungal and bacterial effects on root development indicate that results obtained on plant–bacteria interactions are also of general interest in the study of other plant–microbe interactions and might help shed light on general mechanisms of plant–microorganism communication.

### Concluding Remarks and Future Perspectives

Bacteria influence post-embryonic development in many organisms. Examples can be found across the eukaryotic kingdom. Bacteria induce the transition from floating larva to stationary juvenile in a tubeworm [121], stimulate single-celled choanoflagellates to form colonies [122], and regulate the development of epithelial brush border cells in the zebrafish gut [123–125]. It is thus unsurprising that beneficial microbes in the soil affect root development. Here we review recent evidence regarding the effects of PGPR on *Arabidopsis* root development. One clear trend emerges: bacteria manipulate endogenous host mechanisms regulating post-embryonic root development to alter root growth. These findings open exciting new paths for future research (see Outstanding Questions). Research harnessing natural variation in *Arabidopsis* populations [126,127] and novel technologies such as emerging root-imaging techniques [128–131] and high-throughput sequencing technologies [132,133] will be useful for addressing these questions. Ultimately, a more thorough understanding of the interaction between beneficial soil bacteria and plants leading to enhanced plant growth will prove valuable for several research disciplines, including root development, plant–microbe interactions, and bacterial signaling. Moreover, it will nurture the development of sustainable agricultural techniques that use naturally occurring soil microbes to promote plant growth and health while reducing herbicide and synthetic fertilizer use in the field.

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### Outstanding Questions

How do beneficial bacteria evade or suppress the plant immune system?

What is the mechanism for entry of beneficial bacteria into the root? Do the cell wall and Casparian strip play an active or passive role in regulating bacterial (endophytic) colonization?

What bacterially produced compounds affect root development and how does the plant respond to these compounds at the cellular level?

Do bacteria influence the gene oscillations that appear to prime lateral root founder cells and do they thus influence founder cell specification in addition to lateral root development?

What are the differences between volatile-induced and direct contact-induced bacterial effects on root development and how are these differences established?

What is the spatial distribution of bacteria along the root and how are roots colonized in space and time?

Are there universal beneficial microbial partners that contribute to plant health and growth?

Are similar genes and cellular mechanisms involved in PGPR interactions with different plant species?

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