Structure and Functions of the Bacterial Microbiota of Plants

Davide Bulgarelli,[1] Klaus Schlaeppi,[1] Stijn Spaepen,[1,2] Emiel Ver Loren van Themaat,[1] and Paul Schulze-Lefert[1]

[1]Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany; email: schlef@mpipz.mpg.de

[2]Centre of Microbial and Plant Genetics, KU Leuven, B-3001 Heverlee, Belgium

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Abstract

Plants host distinct bacterial communities on and inside various plant organs, of which those associated with roots and the leaf surface are best characterized. The phylogenetic composition of these communities is defined by relatively few bacterial phyla, including Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. A synthesis of available data suggests a two-step selection process by which the bacterial microbiota of roots is differentiated from the surrounding soil biome. Rhizodeposition appears to fuel an initial substrate-driven community shift in the rhizosphere, which converges with host genotype–dependent fine-tuning of microbiota profiles in the selection of root endophyte assemblages. Substrate-driven selection also underlies the establishment of phyllosphere communities but takes place solely at the immediate leaf surface. Both the leaf and root microbiota contain bacteria that provide indirect pathogen protection, but root microbiota members appear to serve additional host functions through the acquisition of nutrients from soil for plant growth. Thus, the plant microbiota emerges as a fundamental trait that includes mutualism enabled through diverse biochemical mechanisms, as revealed by studies on plant growth–promoting and plant health–promoting bacteria.

Contents

INTRODUCTION

In the past 30 years, molecular-genetic analysis of binary plant-pathogen interactions has uncovered the logic of the plant innate immune system, explaining how plants recognize non- and modified-self molecular structures to trigger immune responses and how host-adapted pathogens subvert the immune response to cause disease (74). In parallel work, the cellular signaling pathways underlying binary mutualistic interactions between legumes and nodule-forming rhizobial and mycorrhizal associations were defined and shown to overlap, suggesting that molecular components of these legume-specific networks have nonlegume counterparts in all flowering plants (100). However, these binary parasitic and mutualistic interactions, all of which are linked to the appearance of macroscopically visible disease symptoms or beneficial infection structures such as root nodules, are merely extreme outcomes of a continuum of interorganismal associations. In nature, healthy plants host a remarkable diversity of microbes known as the plant microbiota (22, 30, 78, 85, 89). These microbial assemblies appear to be symptomless at first glance, possibly representing a continuum of symbiosis ranging from commensalistic to mutualistic interactions. The latter provide often-overlooked host services such as indirect pathogen protection and nutrient acquisition from soil for plant growth (see below). Thus, the plant microbiota emerges as a novel trait that extends the capacity of plants to adapt to the environment.

Advances in understanding the molecular basis of fundamental traits are often driven by new technologies. Next-generation sequencing technologies and corresponding bioinformatic tools have begun to transform plant microbiota research (see sidebar, Metagenomics: Sequencing and Computational Methodologies). These DNA sequencing technologies have for the first time enabled systematic culture-independent surveys of the microbiota, revealing its taxonomic structure and the relative abundance of community members.

A habitat is a specific place occupied by a community of organisms for growth and reproduction. Thus, plant organs colonized by microbial communities with a distinctive phylogenetic structure represent different habitats. A niche is defined as the totality of biological

and environmental factors that affect a species in a habitat, and includes how a species uses this environment (132). Thus, the establishment of a population of a particular microbiota member in a community context on a plant organ can be regarded as niche colonization.

Here we present a critical appraisal of plant microbiota research, with a focus on plant-associated bacterial communities. Plants also host fungal and eukaryotic communities, which, although they can be of critical importance, are not the focus here. We discuss common and distinctive features of root and leaf microbiota, present a two-step selection model by which the bacterial root microbiota is recruited from the surrounding soil biome, and show how this model can help to explain derived biological phenomena such as soil suppressiveness. Finally, we discuss a range of biochemical mechanisms underlying rhizobacterial plant growth promotion and pathogen protection and propose that these exemplify traits encoded by the microbiome.

THE HOST AS DRIVER FOR THE ESTABLISHMENT OF RHIZOBACTERIAL ASSEMBLAGES

Soil represents one of the richest microbial ecosystems on Earth (50). However, at high taxonomic rank, a few bacterial phyla—including Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria—recapitulate most of the diversity of contrasting soil biomes (46). **Table 1** compiles published cultivation-independent surveys of the bacterial rhizosphere and endosphere microbiota retrieved from different plant species grown in different soils, and indicates that these two ecological habitats are formed by soil biome community shifts that give rise to a distinctive phylogenetic structure with a few dominating phyla. The underrepresentation of Acidobacteria members and increased proportion of Proteobacteria and Actinobacteria members in the rhizosphere and endosphere suggest that a combination of edaphic and plant host–derived factors shape the bacterial microbiota composition close to and inside plant roots.

METAGENOMICS: SEQUENCING AND COMPUTATIONAL METHODOLOGIES

Questions on community composition ("Who is there?"), function ("What can they do?"), and activity ("What do they do?") can be addressed by sequencing 16S rRNA, DNA, or mRNA from environmental samples. The continual reductions in cost and increases in production speed and read length of sequencing technologies have made high-resolution community profiling a standard laboratory routine (81). Reports on communities close to or inside plants show medium taxonomical complexity (see **Table 2** below), resulting in saturated 16S rRNA profiles: No new phylotypes are found by increasing sequencing depth using a given primer (56). Capturing all functions (genes) in a community is more complicated owing to the high diversity within plant-associated phylotypes (86), contamination with host material, and natural sequencing bias toward the few dominating species or transcripts. The increasing size of generated data sets in comparative functional metagenomics ("How do they differ?") comes, however, with significant costs in computational infrastructure (81) and challenges computational methodology to improve in efficiency, accuracy, and reproducibility (57). Notable efforts of the open-source community enabling cloud compatibility include CloVR (Cloud Virtual Resource) (4), MG-RAST (Metagenomics Rapid Annotation Using Subsystem Technology) (143), and QIIME (Quantitative Insights into Microbial Ecology) (23).

Rhizodeposition Mediating Substrate-Driven Community Shifts of the Soil Biome

One potential molecular mechanism underlying the formation of a distinctive rhizosphere microbiota from soil biomes is rhizodeposition. This process refers to intertwined plant developmental and secretory activities in the root system. Rhizodermis cells secrete a wide range of compounds, including organic acid ions, inorganic ions, phytosiderophores, sugars, vitamins, amino acids, purines, and nucleosides, and the root cap produces polysaccharide mucilage (28). Rhizodeposition also refers to the

Mutualism: a relationship between two organisms that is mutually beneficial

Parasitism: a relationship between two organisms in which one benefits and the other is harmed

Commensalism: a relationship between two organisms in which one benefits without affecting the other

Symbiosis: a close biological relationship among two or more individuals of different species

Table 1 Bacterial phyla dominating rhizosphere and endophyte bacterial assemblages

Host Species	Rhizosphere	Endosphere	Dominating phyla	Reference
Wild oat (*Avena fatua*)[a]	✓		Actinobacteria Firmicutes Proteobacteria	29
Oak (*Quercus* sp.)	✓		Acidobacteria Actinobacteria Proteobacteria	127
Poplar (*Populus deltoides*)	✓		Acidobacteria Proteobacteria	58
		✓	Proteobacteria	
Cultivated potato (*Solanum tuberosum*)[a]	✓		Actinobacteria Bacteroidetes Firmicutes Proteobacteria	138
Cultivated potato (*Solanum tuberosum*)	✓		Actinobacteria Proteobacteria	65
Cultivated potato (*Solanum tuberosum*)		✓	Actinobacteria Bacteroidetes Proteobacteria	90
Sugar beet (*Beta vulgaris*)[a]	✓		Actinobacteria Firmicutes Proteobacteria	95
Cultivated maize (*Zea mays*)[b]	✓		Proteobacteria	19
Cultivated rice (*Oryza sativa*)[c]	✓		Actinobacteria Proteobacteria	78
Cultivated rice (*Oryza sativa*)[d]		✓	Firmicutes Proteobacteria	120
Thale cress (*Arabidopsis thaliana*)	✓		Acidobacteria Planctomycetes Proteobacteria	22
		✓	Actinobacteria Bacteroidetes Proteobacteria	
Thale cress (*Arabidopsis thaliana*)	✓		Acidobacteria Actinobacteria Bacteroidetes Proteobacteria	89
		✓	Actinobacteria Bacteroidetes Firmicutes Proteobacteria	

[a]Data generated with PhyloChip.
[b]Data generated with a custom-designed 16S rRNA gene microarray.
[c]Data generated from whole-metagenome shotgun and 16S rRNA gene clone libraries.
[d]Data generated from Sanger sequencing of endophyte-metagenome shotgun clones.

release of a specialized cell population, called root cap border cells, into the rhizosphere (32). Root cap border cells are particularly attractive candidates for contributors to the rhizosphere effect because this cell population typically remains alive after desquamation from the root corpus into soil (59). For example, root cap border cells of maize remain viable in the rhizosphere for a week or longer (133). As a consequence, rhizosphere soil collected for root microbiota studies is inevitably "contaminated" with live and dead root cap border cells (**Figure 1**). Rhizodeposits account for ~11% of net photosynthetically fixed carbon and 10–16% of total plant nitrogen, although these values vary greatly depending on plant species and plant age (73). The net sequestration of organic carbon and nitrogen by roots is thought to stimulate soil microbial multiplication in the vicinity of root tissues because (*a*) most known soil bacteria are organotrophs, i.e., they derive the energy for growth from organic substrates, and (*b*) the accessibility and availability of organic compounds are limited in most soils (3, 31). In the following we discuss experimental results that have provided the first molecular insights into the presumed role of rhizodeposition in the establishment of a rhizosphere microbiota distinct from that of the surrounding soil.

Arabidopsis roots release border-like cells (BLCs) from the root caps into the exterior environment (134). These cells were named BLCs because their desquamation from the root tip involves the release of organized cell files rather than individual cells, as in many other plant species. Ultrastructural analysis of *Arabidopsis* BLCs revealed numerous Golgi stacks and Golgi vesicles in the cytoplasm, suggesting that these cells have a high secretory activity in the rhizosphere (134). Pharmacological interference of plant cell wall proteoglycan functions by application of 3,4-dehydroproline [which inhibits the O-glycosylation of cell wall proteoglycans, including arabinogalactan proteins (AGPs)] or the β-glucosyl Yariv reagent (which binds and precipitates AGPs) resulted in reduced adhesion of the *Rhizobium* sp. YAS34 strain to *Arabidopsis* BLCs and the rhizodermis in a gnotobiotic test system (134). This points to a potential function of BLC- and rhizoplane-derived cell wall proteoglycans in the attachment of *Rhizobium* to root cells. One caveat in the interpretation of these experiments is that *Rhizobium* sp. YAS34 was originally isolated from the sunflower rhizosphere (134), and it is not known whether this strain is an indigenous member of the *Arabidopsis*

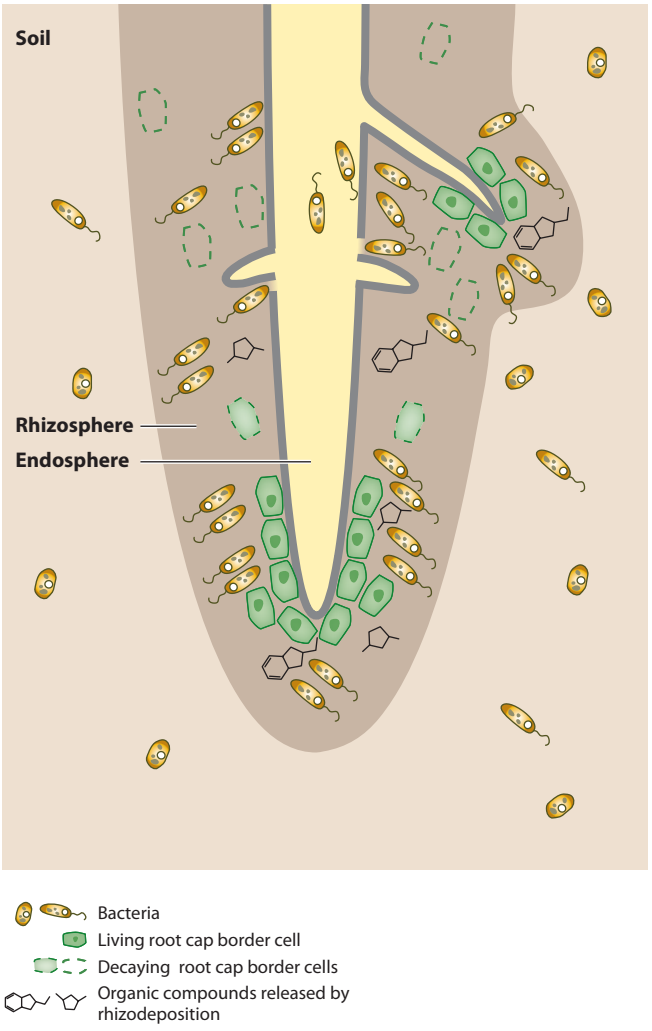

Figure 1

Niche differentiation at the root-soil interface. From outside to inside, the habitats are the soil, rhizosphere, and endosphere. Rhizodeposits generated from root cap border cells and the rhizodermis provoke a shift in the soil biome. Cellular disjunction of the root surface during lateral root emergence provides a potential entry gate for the rhizosphere microbiota into the root interior.

Microbiota: the set of microorganisms of a particular habitat

Soil biome: all soil type–dependent microorganisms in a particular habitat, including nematodes and protists

Microbiome: the set of genomes of the microorganisms in a particular habitat

Rhizosphere: the region of soil surrounding plant roots in which the chemistry and microbiology are influenced by the roots' growth, respiration, and nutrient exchange

Endosphere: the microbial habitat inside plant organs

Edaphic factors: soil properties that influence biological activity

Rhizosphere effect: enhanced bacterial activity in the rhizosphere

Operational taxonomic unit (OTU): a terminal node in a phylogenetic analysis

root microbiota. The identification of several indigenous *Rhizobium* species in the *Arabidopsis* root microbiota (22, 89) should make it possible to examine the proposed function of AGPs in the attachment of host-adapted *Rhizobium* to a nonleguminous root system. The supporting evidence for a link between host-released AGPs and bacterial attachment is the identification of an AGP in a high-molecular-weight fraction from pea root exudates, which is sufficient to induce biofilm formation of *Rhizobium leguminosarum* on an artificial glass surface (146). The biofilm on glass is thought to mimic in vivo *Rhizobium* biofilm formation on roots and root hairs of nonleguminous plants (115, 146).

Genetic support for a function of AGPs in root colonization beyond *Rhizobium* comes from the characterization of an *Arabidopsis* mutant, *rat1*, that is resistant to transformation by *Agrobacterium tumefaciens* (52). *Arabidopsis RAT1* encodes a lysine-rich root-expressed AGP, and *rat1* mutant plants show reduced *Agrobacterium* binding to both the rhizoplane and root hairs, indicating that RAT1 is needed for an initial binding step during *Agrobacterium* root infection. *Agrobacterium* is a definable genus of the family Rhizobiaceae (45), implying a conserved function of root AGPs in the attachment of at least a subset of soil-borne bacteria. Further experimentation is needed to determine how broadly important AGPs are for the indigenous microbiota and whether genetic depletion of *Arabidopsis* root AGPs can be compensated for by particular rhizobacteria during microbiota differentiation.

Evidence for a dynamic root microbiota structure along the longitudinal axis of the root system was obtained by PhyloChip analysis of wild oat (*Avena fatua*) (29). In these experiments, rhizosphere prokaryotic communities (without root tissue) were separately studied at and close to the root tip (0–4 cm from the root tip), in the root hair zone (4–8 cm from the root tip), and at the mature roots (8–16 cm from the root tip). Of the 1,917 taxa detected, ~8% (147 taxa) displayed root zone–dependent enrichment (29). In addition, the highest live bacterial counts were in rhizosphere soil collected from the root tip and root hairs, the next highest were in the rhizosphere of the mature root zone, and the lowest were in bulk soil (29). Thus, if rhizodeposits are causally linked to the formation of a rhizosphere-specific bacterial microbiota, then the observed root zone–dependent enrichment of subsets of this community should reflect local differences in amounts and/or composition of metabolites released along the longitudinal axes of roots. For example, the differentiation and release of root cap border cells only at the root tip (**Figure 1**) is consistent with a dynamic substructure of the microbiota along the longitudinal root axis.

Plant genes encoding membrane-resident ATP-binding cassette (ABC) transporter proteins are plausible candidates for genes mediating the export of small molecules from root cells into the rhizosphere. Mutants of seven *Arabidopsis* ABC transporter–encoding genes that are highly expressed in roots were grown in *Arabidopsis*-accustomed soil over two generations, and the microbiota of their roots with attached soil was compared with that of wild-type plants by automated ribosomal intergenic spacer analysis (ARISA) (6). One ABC transporter mutant, *abcg30*, exhibited differences in ARISA profile compared with the wild type, and this correlated with an altered metabolic profile in *abcg30*-derived root exudates collected from 21-day-old seedlings grown in liquid media. NMR spectroscopy of exudates collected from liquid media–grown *abcg30* plants showed that they contained elevated levels of phenolics and reduced amounts of sugars. Reassessment of the root microbiota profiles of single nonreplicated samples of wild-type and *abcg30* plants by low-pass 16S rRNA gene pyrosequencing suggested an increased abundance of potentially beneficial bacteria in *abcg30* mutant roots. However, this conclusion is based on low-abundance operational taxonomic units (OTUs), and their significance is difficult to assess because the variation of low-abundance OTUs between full factorial replicates in such experiments is generally high (22, 89).

To test a potential driver role of common root exudates in soil biome community shifts, Eilers et al. (41) simulated exudation by adding the low-molecular-weight carbon substrates glucose, glycine, or citric acid to microcosms containing three soils derived from grassland, hardwood forest, and coniferous forest. The addition of each substrate altered the soil community composition in a soil type–dependent and carbon substrate–dependent manner. Across all treatments and all tested soil types, the observed community shifts resulted mainly from an increase in the relative abundance of the subphyla Betaproteobacteria and Gammaproteobacteria and the phylum Actinobacteria, suggesting the existence of specific bacterial taxa that preferentially respond to organic carbon substrate addition. The community shifts in soil are unlikely to be the result of indirect pH shifts because the low-molecular-weight carbon substrate solutions were equally adjusted to pH 7 (41).

Although these experiments ignore the facts that in vivo soil bacteria are exposed to a mixture of exudate molecules and that only a single relatively high test substrate concentration was examined, it is a striking coincidence that in the rhizosphere and/or root endosphere compartments of different plant species, Actinobacteria and Proteobacteria become almost invariably enriched (**Table 1**). Thus, it is possible that the establishment of a distinctive rhizosphere bacterial community is at least partly the result of substrate-driven community shifts fueled by the secretion of photoassimilates from root cells. Supporting evidence for this hypothesis comes from molecular-genetic work on the chemotaxis of the soil-borne pathogenic bacterium *Ralstonia solanacearum*, which invades host plants via roots (148). *R. solanacearum* is specifically attracted by diverse amino acids, organic acids, and root exudates from its host plant, tomato. *R. solanacearum* mutants lacking either *cheA* or *cheW*, two key regulatory components of bacterial chemotaxis, were found to be fully nonchemotactic but retained otherwise normal swimming behavior. The nonchemotactic mutants reached the same population size as the wild type in a soil soak assay but exhibited reduced virulence. Importantly, the chemotaxis mutants were as virulent as the wild-type strain when inoculated directly into the plant stem (148), which is consistent with the idea that bacterial chemotaxis makes a contribution to the early phase of host colonization through the perception of root exudates in the rhizosphere. Similarly, a nonchemotactic *cheA* mutant of the plant growth–promoting bacterium *Pseudomonas fluorescens* resulted in strongly reduced competitive tomato root colonization ability and identified malic acid and citric acid as major chemoattractants for this microbe in the tomato rhizosphere (34). By applying distinct approaches such as in vitro chemotactic assays, transcriptome studies on bacterial gene expression, and colonization assays with wild-type and benzoxazinoid-deficient corn mutant plants, a recent study demonstrated that another plant growth–promoting bacterium, *Pseudomonas putida*, is recruited to plant roots by chemotaxis toward the benzoxazinoid secondary metabolites (99). Although these studies provide compelling evidence for a role of root exudates in attracting individual rhizobacteria, it remains to be shown whether this function is retained in a community context and whether a mixture of exudate molecules is sufficient to enforce the characteristic taxonomic community differentiation seen in the root microbiota (**Table 1**).

Direct evidence for a net carbon flux from plants to rhizobacterial communities can be obtained by stable-isotope probing (SIP) techniques. SIP in combination with microbiota DNA profiling (DNA-SIP) offers unprecedented opportunities to obtain deeper insights into interorganismal carbon flow at the plant-soil interface (24). The rhizosphere represents an ideal experimental system for SIP-based community profiling because the labeled substrate, typically $^{13}CO_2$, can be added to create a defined atmosphere in phytotrons in which atmospheric $^{13}CO_2$ is converted into organic carbon by the Calvin-Benson cycle in green leaves. Long-distance transport of organic ^{13}C from shoot to root, its release, and its subsequent

capture by the root microbiota can be measured by purifying labeled ("heavy") ^{13}C rhizobacterial chromosomal DNA from the nonlabeled ("light") DNA fraction using CsCl density gradient centrifugation. A key advantage of DNA-SIP is that the label serves as a selective tag for active bacteria within the root microbiota whose growth is stimulated by root exudation.

In combination with low-resolution microbial ribotyping, SIP has been utilized to explore the potential effect of altered glucosinolate metabolism on bacterial and fungal communities in the *Arabidopsis* rhizosphere (20). Glucosinolates are a class of Capparales-specific phytochemicals previously shown to have antimicrobial activity in plant-microbe and plant-insect interactions (11). Microbial DNA samples of the rhizosphere and roots of a transgenic *Arabidopsis* line expressing the sorghum cytochrome P450–encoding gene *CYP79A1*, which is known to produce high levels of the exogenous tyrosine-derived *p*-hydroxybenzyl glucosinolate (7), were inspected by low-resolution denaturing gradient gel electrophoresis (DGGE) fingerprinting following $^{13}CO_2$ labeling and compared with wild-type *Arabidopsis* plants (20). Although glucosinolate products were undetectable in rhizosphere soil, Alphaproteobacteria (mainly Rhizobiales) and fungal communities discriminated both the active rhizosphere and root endophyte communities of the *CYP79A1*-expressing line from those of the wild type. However, these findings do not provide clues about whether endogenous *Arabidopsis* glucosinolates contribute to the establishment of the *Arabidopsis* root microbiota. Despite these limitations, DNA-SIP and advances of techniques that are able to trace the fate of minerals and organic compounds in situ, such as nanometer-scale secondary ion mass spectroscopy (69), promise to generate high-resolution quantitative maps of nutrient flow at the root-soil interface (25).

Host Genotype–Dependent Fine-Tuning of the Root Microbiota

Using high-resolution 16S rRNA gene pyrosequencing of bulk soil, rhizosphere, and root compartments collected from eight *Arabidopsis* ecotypes grown in two soil types, Lundberg et al. (89) identified among 778 measurable OTUs a total of 12 OTUs exhibiting host genotype–dependent quantitative enrichment in the root endophyte compartment. Utilizing a similar sequence-based 16S ribotyping platform but two other natural soils, Bulgarelli et al. (22) identified only one OTU of the bacterial root endophyte community that showed significantly different quantitative enrichment between the two *Arabidopsis* ecotypes tested. In both studies, soil type and the respective soil bacterial biomes had a greater influence than the host genotype on the composition of root endophyte communities. Thus, a significant but weak host genotype–dependent effect acts in the selection of *Arabidopsis* root-inhabiting bacterial communities. Micallef et al. (96) reported differences in both the composition and relative abundance of rhizosphere community members among eight tested *Arabidopsis* ecotypes by applying terminal restriction fragment length polymorphism (T-RFLP) and ARISA. However, it remains unclear how many differential T-RFLP peaks result from sampling inaccuracies (the rhizosphere was collected using scalpel blades) and how many genotype-specific signals are reproducible in replicate experiments, i.e., using independent soil samples collected from the same field plot.

Other than the *Arabidopsis* reports (22, 89, 96), few studies have explored the magnitude of host genotype–dependent variation on bacterial root microbiota profiles. Utilizing PhyloChip, a high-density 16S rRNA gene probe array that can detect up to 8,741 known OTUs, Weinert et al. (138) examined the root microbiota of three cultivars of field-grown potato plants (rhizosphere plus root-inhabiting bacterial communities) in two different soils. Of the 2,432 OTUs detected, 9% showed a quantitative cultivar dependence in one of the soils tested and 4% showed a dependence in both. The host genotype–dependent OTUs belong mainly to the phyla Actinobacteria, Chloroflexi, Firmicutes, and Proteobacteria (138). Consistent with a previous DGGE analysis of the same

biological material (137), a greater number of OTUs (28%) differentiated the root microbiota of these potato plants grown in different soils (138). Thus, similar to the *Arabidopsis* studies (22, 89), soil type influences the potato root microbiota profiles to a greater extent than host genotype does. Although these data were obtained from a single growing season and are limited by the preselected PhyloChip probe set, the conclusions are well supported by statistical analysis. Unfortunately, the lack of data on the biomes of the corresponding unplanted soils precludes numerical information on the expected potato root rhizosphere effect.

Two other reports of field experiments with potato plants illustrate the difficulties in inferring general conclusions on the potato microbiota when different sampling methods are employed (65, 66). These experiments involved a comparison of bulk soil and rhizosphere compartments of six potato cultivars grown in two soil types. Bacterial 16S rRNA gene pyrosequencing revealed members of the phyla Actinobacteria and Alphaproteobacteria as dominant taxa in both the unplanted soil biome and rhizosphere communities (65). Based on the relative abundance of 16S rRNA gene sequences assigned to bacterial genera, the structure of the rhizosphere assemblages was different from that of the unplanted bulk soil biomes at each of three tested developmental stages (young leaf development, florescence, and senescence) (65). However, a significant host genotype–dependent rhizosphere effect was detected only in young potato plants. This differentiation occurred mainly on the axis that explained ~5% of the observed variation in a principal components analysis and is therefore weak. In addition, hierarchical clustering of the relative abundance of the major bacterial classes and phyla from the same samples did not reveal a clear host development–dependent or host genotype–dependent effect on rhizosphere microbial profiles.

Although currently available bacterial root microbiota studies suggest a weak host genotype–dependent effect, it is important to mention that the resolution power of sequence-based 16S rRNA ribotyping is inherently limited to the species level or higher taxonomic ranks. However, subspecific genetic variation in pathogenic microorganisms, including bacteria, has a key role in host genotype–dependent colonization (119). Thus, if subspecies genetic variation of microbiota members contributes to host colonization success, the actual host genotype–dependent effect cannot be determined with available community fingerprinting technologies.

When wooden splinters from two tree species were incubated in the same natural soils used to define the *Arabidopsis* root-inhabiting bacterial microbiota, approximately 40% of the root-inhabiting OTUs also colonized this dead plant material (22). This microbiota subcommunity consists largely of Proteobacteria and is not specific to *Arabidopsis*, and probably represents saprophytic bacteria that populate the roots of any plant species, including decaying plant litter. Notably, the other 60% of the *Arabidopsis* root microbiota is dominated by Actinobacteria, followed by Proteobacteria and Bacteroidetes (22). For a deeper interpretation of these findings, it is relevant that upon termination of root primary growth (i.e., when roots reach their maximal length), secondary growth ensues, characterized by root thickening and the appearance of secondary phloem and secondary xylem. The latter tissue is typically responsible for the woody appearance of mature root systems and results from the apoptotic death of cell files, which leaves behind large amounts of lignified cell wall cellulose microfibrils (97). Within a few weeks after germination of *Arabidopsis* seeds, part of the primary root (rhizodermis, cortex, and endodermis) is replaced by new cells during secondary thickening (38). Thus, woody material is an integral part of mature root systems utilized for most root microbiota studies. In this wider context it is possible that Actinobacteria, Proteobacteria, and Bacteroidetes represent early root endophytes and that the Actinobacteria members are outcompeted during root secondary growth. Alternatively, there is no dynamic succession

of root microbiota members during root development, but woody parts and metabolically active root cells are instantly populated by distinct bacterial subcommunities. Irrespective of these alternative niche-filling mechanisms of live and dead root cells, the detection of potentially saprobic bacteria in the roots of live *Arabidopsis* plants could point to their activity in the decomposition of organic matter after plant death.

A Two-Step Selection Model for Root Microbiota Differentiation

A comparison of the bacterial and fungal root microbiota of mature poplar (*Populus deltoides*) trees growing at two natural sites revealed strikingly different endophyte community compositions compared with the surrounding rhizosphere (58). 16S rRNA gene pyrosequencing of the bacterial endophyte communities displayed an order-of-magnitude reduction in richness (number of OTUs identified by rarefaction analysis) and were dominated by members of Proteobacteria (>80% of retrieved OTUs). In contrast, Acidobacteria dominated rhizosphere assemblages and was underrepresented in the root-inhabiting communities (58). Fungal rhizosphere and endophyte samples had similar amounts of Pezizomycotina, whereas Agaricomycotina was more abundant in the root endosphere. It is possible that undersampling of the endophyte compartment partly contributed to the marked differences in the community structures of the rhizosphere and endosphere. It is also possible that complex interactions between the fungal and bacterial microbiota contributed to the rhizosphere and endosphere community differentiation, because poplar is unusual among higher plants in that it engages in symbiotic interactions with both endomycorrhizal Glomeromycota fungi and ectomycorrhizal Ascomycotina and Basidiomycotina fungi. With these limitations and the peculiar poplar biology aspect in mind, this shows that the root interior represents a microbial habitat on its own and is not fortuitously filled by rhizosphere members.

Qualitatively similar observations were reported in two independent studies on the bacterial root microbiota of *Arabidopsis* (22, 89). In both studies, the microbiota inhabiting root tissues is markedly differentiated from the one that populates the rhizosphere or unplanted soil. This was evident from both a reduced richness (estimated through rarefaction curves) of the root-inhabiting communities and concomitant increases in the abundance of Actinobacteria, Bacteroidetes, and Proteobacteria. In contrast, Acidobacteria members that dominate both unplanted soil and the rhizosphere were virtually excluded from the root endosphere (22, 89). An important finding of these two studies is that the endosphere taxonomic profiles are remarkably similar (mainly Actinobacteria, Bacteroidetes, and Proteobacteria), although the plants were grown in four different soils on two continents. In addition, the rhizosphere community differentiation in comparison with bulk soil was weak in all four tested soils (22, 89). These results argue strongly against fortuitous niche filling of the endosphere from rhizosphere assemblages and predict the existence of different host-controlled mechanisms underlying the differentiation of rhizosphere and endosphere communities.

A collective synthesis of the available literature suggests a two-step selection process, gradually differentiating the root microbiota from the surrounding soil biome. In this model, rhizodeposition fuels an initial substrate-driven community shift in the rhizosphere, which converges with host genotype–dependent fine-tuning of microbiota profiles during endophyte microbiota differentiation (**Figure 2**). Accordingly, substrate-driven selection in the rhizosphere is expected to persist in the endosphere. Note that the magnitude of selection in the rhizosphere compared with that of the endosphere can vary greatly in different plant species and as a function of the host genotype (**Table 1**). Likewise, the magnitude of rhizosphere community differences relative to that of the soil biome can vary in different soil types. One prediction of this model is the existence of genetic adaptation of the host to different soil types;

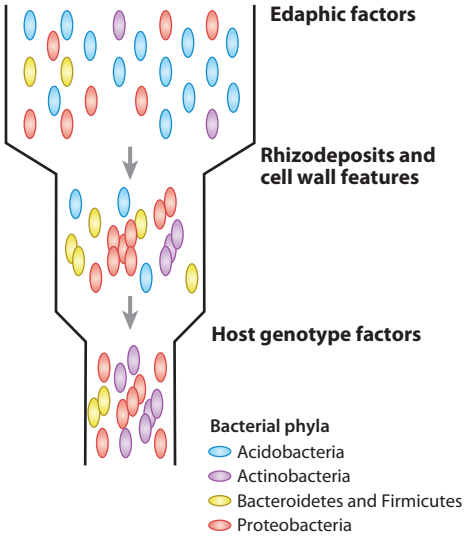

Figure 2

A two-step selection model for root microbiota differentiation. Edaphic factors determine the structure of bacterial communities in soil biomes. In the first differentiation step, rhizodeposits and host cell wall features promote the growth of organotrophic bacteria, thereby initiating a soil biome community shift. In the second step, convergent host genotype–dependent selection close to and within the root corpus fine-tunes community profiles thriving on the rhizoplane and within plant roots.

i.e., optimal plant growth depends on specific combinations of host genotype–dependent and soil type–dependent bacterial start inoculum.

Among numerous factors that could explain the observation of distinctive rhizosphere and endophyte communities within the root microbiota, the innate immune system is a prime candidate for the selection of a distinctive root endophyte microbiota. Plants have evolved an elaborate innate immune system consisting of two classes of immune receptors that detect the presence of nonself molecules both inside and on the surface of host cells (74). Nonself recognition activates powerful immune responses to terminate microbial multiplication of pathogens. The identification of an increasing number of pattern recognition receptors on the plant cell surface during the past decade is intuitively difficult to reconcile with the colonization of the root interior by soil-derived endophytic microbial communities because this class of immune receptors detects a wide array of microbe-associated molecular patterns (MAMPs) (17). Characterized MAMPs recognized by cognate cell surface receptors are epitopes derived from bacterial flagellin, the translation elongation factor Tu, bacterial lipopolysaccharide, or fungal chitin (13, 17). The discovery of root endophyte communities with a defined taxonomic structure could be reconciled with the current framework of plant innate immunity if these microbes are capable of immune response interception, as has been demonstrated for pathogenic microbes (18). It is also conceivable that endophytes evolved effective MAMP camouflage mechanisms to escape immune receptor detection. Finally, it is possible that the innate immune system is activated upon endophyte colonization but this limits endophyte multiplication at microbial titers that are well below those of pathogenic bacteria causing disease symptoms. Clearly, future experimentation is needed to discriminate between these three models, which are not necessarily mutually exclusive.

DISEASE-SUPPRESSIVE SOILS: A DERIVED BIOLOGICAL PHENOMENON OF THE ROOT MICROBIOTA

Disease-suppressive soils are those in which little or no disease occurs under conditions that are favorable for disease development (77). Disease suppressiveness can be a natural property of certain soils and persist in the absence of cultivation or can be induced after a monoculture of the same crop species for several years, followed by a severe disease outbreak (14). In both cases, the bacterial soil biome plays a critical role: Pathogen inoculation of pasteurized suppressive soils invariably leads to reestablishment of the host disease (95). Whereas natural disease suppression is not limited to a particular plant species and affects a broad range of pathogens, induced disease suppression arises from specific

host-pathogen combinations, suggesting a primary role for the root microbiota in controlling the disease (14). Host plants protected against soil-borne pathogens through soil disease suppressiveness do not appear to harbor cryptic disease-resistance genes against the respective pathogens (77), supporting the idea that this trait is delegated to the root microbiota.

PhyloChip analysis of the rhizosphere microbiota of sugar beet plants grown on soils either suppressive of or conducive to *Rhizoctonia solani*, a major fungal pathogen of sugar beet, revealed that the two conditions did not significantly alter the detected number of bacterial taxa and that both soil types were dominated by members of Actinobacteria, Firmicutes, and Proteobacteria (95). Likewise, heat treatments, amendment of conducive soil with a small amount (10%) of suppressive soil, and suppressive soil inoculation with *R. solani* did not provoke a significant effect in the diversity of the analyzed rhizosphere bacterial microbiota (95). These data suggest that disease suppression might arise from factors other than the mere presence or absence of certain bacteria. Consistent with this, Bray-Curtis dissimilarity matrix analysis calculated on the relative abundance of the identified bacterial and archaeal OTUs discriminated the rhizosphere microbiota of the suppressive soils from that of the conducive soils (95). Burkholderiaceae, Lactobacillaceae, Pseudomonadaceae, and Xanthomonadales were identified as the most dynamic in the data set—i.e., these taxa were responsive to all soil conditions tested. In contrast, Actinobacteria members largely accounted for the observed differentiation among the tested suppressive soil, suppressive soil inoculated with *R. solani*, and conducive soil (95).

Interestingly, the enrichment of Actinobacteria in the *Arabidopsis* root microbiota depends on metabolically active root cells (22), and the wide range of antimicrobial compounds secreted by members of this bacterial phylum (10) suggests a possible role of such compounds in indirectly protecting sugar beet against the soil-borne fungal pathogen. In a parallel experimental attempt, a culture-dependent isolation of bacteria from the suppressive soil was conducted and yielded a disproportionate amount of pseudomonads (95). Several *Pseudomonas* strains were identified that are significantly enriched in the disease-suppressive soil compared with conducive soils. This is notable because the relative abundance of *Pseudomonas* producing the antibiotic compound 2,4-diacetylphloroglucinol (2,4-DAPG) correlates with the control of another soil-borne fungal pathogen, *Gaeumannomyces graminis*, in soil suppressive of the "take-all disease" caused by this fungus in cereals (107). Only one of the isolated *Pseudomonas* strains from the *R. solani* disease-suppressive soil conferred protection against the pathogenic fungus in a plant bioassay, indicating that more bacteria than the above-mentioned Actinobacteria might contribute to the observed soil suppressiveness. However, random transposon mutagenesis of this *Pseudomonas* strain revealed that some mutants have the competence to colonize the rhizosphere at similar levels compared with the wild-type strain but fail to confer disease protection (95). This suggests that *Pseudomonas* rhizosphere colonization competence can be uncoupled from disease suppression (95). Although the exact molecular mechanisms triggering the establishment of disease-suppressive soils remain largely obscure, the ensuing soil biome community shift(s) initiated by severe disease outbreak likely contribute to the phenomenon of soil suppressiveness.

MICROBIOTA AND PLANT DOMESTICATION

Since its inception ~10,000 years ago, plant domestication has produced a large number of cultivated plants from wild ancestors through continuous anthropogenic selection to meet the food and feed demand of human societies (106). Domestication has progressively homogenized plant genotypes, thereby eroding natural genetic variability present in nondomesticated ancestors (37). Because host genotype–dependent selection is an essential

component of the two-step selection model for root microbiota (**Figure 2**), the hypothesis that domestication has inadvertently affected microbiota profiles seems reasonable. It has been postulated that old cultivated forms and their wild ancestors were generally exposed to more marginal soils before the invention of synthetic fertilizer–driven agricultural production, and their gene pools might have a different adaptive capacity to engage in probiotic associations with rhizosphere microbes compared with the gene pools of present-day cultivars (144). The root microbiota might therefore represent an untapped trait for future rational plant breeding through the selection of host genotypes that capture an optimal microbiota from a given soil type to reduce synthetic fertilizer inputs. Experimental data testing this idea, although unfortunately sparse, are discussed below.

To test the hypothesis that crop evolutionary history shaped how the five main genetic groups of maize interact with soil bacteria in the rhizosphere, Bouffaud et al. (19) examined their rhizobacterial community composition using a 16S rRNA taxonomic microarray that targets 19 bacterial phyla. Differences in the community composition of 21-day-old seedlings grown in the same European soil were found in the abundance of certain Betaproteobacteria and *Burkholderia* members and were subsequently validated by quantitative polymerase chain reaction (PCR). However, most of the community structures were common to the five genetic groups, which is reminiscent of a weak host genotype–dependent activity in the composition of the *Arabidopsis* root bacterial assemblages (89). Notably, the few differences in community profiles did not correlate with genetic distances between the five tested maize groups or individual lines. These data suggest that the genetic structure of maize that arose during crop diversification, but not the extent of maize diversification in itself, influences the selection of rhizobacterial communities in maize seedlings. It remains to be seen whether this also applies to the maize root endosphere and can be replicated using 16S rRNA gene sequencing technology that targets all known bacterial phyla.

T-RFLP analysis of the microbiota retrieved from surface-sterilized seeds and stems of 14 genotypes of corn cultivars, landraces, and ancestors (teosinte) revealed distinct seed endosphere profiles of plants grown in distinct pedoclimatic regions (72). Once these plants were grown for one generation in the same soil and under the same climatic conditions, endophyte diversity disappeared, suggesting the existence of a seed-heritable core endosphere microbiota across the genus *Zea*. However, because the applied seed surface sterilization method does not destroy microbial DNA detectable by PCR, it remains possible that the observed T-RFLP profiles represent surface-attached bacteria rather than *Zea* seed endophytes.

Teosinte: the wild ancestor of cultivated maize

Phyllosphere: the microbial habitat defined by the surface of aboveground plant organs

BACTERIAL COMMUNITIES OF THE PHYLLOSPHERE

Microbial communities dwell on and in aerial plant organs. The term phyllosphere refers to aboveground plant surfaces as a habitat for microbes. The bulk of this surface is provided by green leaves, and it is thought to represent one of the largest microbial habitats on Earth. Compared with fungi and archaea, bacteria are the most prevalent phyllosphere-colonizing microbes, with bacterial titers averaging approximately 10^6–10^7 microbial cells per square centimeter of leaf area (85). Phyllosphere microorganisms are exposed to acute fluctuations in temperature, humidity, and UV light irradiation and face limited access to nutrients (62). This differs from the comparatively weak and buffered fluctuations of abiotic conditions prevailing in the rhizosphere. Phyllosphere microbial communities impact global carbon and nitrogen cycles and provide microbial services to the host, e.g., indirect pathogen protection (85, 141).

In comparison with diverse microbial environments such as coastal seawater habitats and farm soil, the phyllosphere represents an environment of reduced bacterial complexity (30). This is similar to other host-associated

Table 2 Bacterial phyla dominating phyllosphere bacterial assemblages

Host species	Dominating phyla	Reference
Soybean (*Glycine max*)[a]	Actinobacteria Bacteroidetes Proteobacteria	30
56 tree species[b]	Actinobacteria Bacteroidetes Firmicutes Proteobacteria	107
Cultivated rice (*Oryza sativa*)[a]	Actinobacteria Proteobacteria	78
Cultivated lettuce (*Lactuca sativa*)[b]	Bacteroidetes Firmicutes Proteobacteria	108
Cultivated spinach (*Spinacia oleracea*)[b]	Actinobacteria Proteobacteria	87
Salt cedar (*Tamarix* sp.)[b]	Actinobacteria Firmicutes Proteobacteria	47
Several plant species[c]	Firmicutes Proteobacteria	141

[a]Data generated with shotgun metagenomics and 16S rRNA clone libraries.
[b]Data generated with pyrosequencing of 16S rRNA gene amplicons.
[c]Data generated with 16S rRNA gene clone libraries.

habitats, including the rhizosphere and the vertebrate gut microbiota. Thus, relatively few bacterial phyla define the phylogenetic structure of phyllosphere communities (**Table 2**): Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, of which the last often dominates the phyllosphere community. A few bacterial genera, including *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, *Bacillus*, *Massilia*, *Arthrobacter*, and *Pantoea*, appear to compose the core of phyllosphere communities. DNA and protein samples collected from leaf surfaces of field-grown soybean and clover as well as from a wild population of *Arabidopsis thaliana* identified *Sphingomonas* spp. and *Methylobacterium* spp. as the most common community members, both belonging to the class of Alphaproteobacteria (30).

Members of the genus *Sphingomonas* may contribute to plant health, as evidenced by the suppression of disease symptoms and reduced growth of the foliar pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 on *A. thaliana* under laboratory conditions (68). Interestingly, this beneficial service to the host was not observed in sphingomonads isolated from air, dust, or water, indicating that the capacity for biocontrol is unique to plant-adapted strains. Using a forward-genetic in planta screen, 10 mutants of *Sphingomonas* sp. Fr1 were identified that have intermediate disease-suppressive capabilities (but retain leaf colonization competence) and map to seven genomic regions (135). This points to the existence of several parallel molecular mechanisms, each contributing partially to the disease-suppression trait of *Sphingomonas* sp. Fr1.

Factors Explaining Community Composition

Phyllosphere communities for 10 tree species, all located within the same 35-hectare area, have been surveyed using a pyrosequencing-based approach (110). The variation found between individual trees of the same species (intraspecific variation) and

between distinct species (interspecific variation) was investigated. In addition, bacterial phyllosphere variation was tested between samples collected from the same individual (intraindividual variation). The bacterial community variation was largest between samples from different tree species and lower at the intraspecific and intraindividual levels. However, interspecific variation, measured in UniFrac distances, was only slightly higher (~0.70) compared with those found among interspecific and interindividual samples (~0.66 and ~0.64, respectively). This indicates that the phyllosphere bacterial microbiota displays considerable variability in community composition even in replicate samples from the same host plant. When intraspecies community variability was tested as a function of geographic distance by comparing phyllosphere communities from *Pinus ponderosa* sampled from several locations around the globe, minimal geographic differentiation was observed (110). These observations support the notion that the host plant species is a determinant for the structure of the phyllosphere community. Root-associated bacterial assemblies, in contrast, are defined largely by soil type (i.e., the bacterial start inoculum present in the surrounding soil biome), and the host genotype is responsible for the fine-tuning of community structure during the establishment of the root endophyte microbiota (see the two-step selection model shown in **Figure 2**).

In this context, it is relevant that the characterization of the leaf microbiota is still fragmentary; there have been no studies directly comparing epiphytic and endophytic leaf microbiota profiles by culture-independent methods. Similarly, only a handful of studies have systematically compared leaf- with root-associated communities collected from the same plant individuals (78). Fundamental physiological differences need to be taken into account when comparing rhizosphere and phyllosphere communities. Unlike root exudation, in which significant amounts of photoassimilates are released into the rhizosphere space, there is no evidence for an equivalent mechanism that releases large quantities of soluble organic compounds to the leaf surface. Thus, instead of a gradual substrate-driven community shift of the soil biome initiated at a distance from the root corpus in the rhizosphere, the selection of phyllosphere communities appears to take place solely at the immediate leaf surface. Although the leaf cuticle and plant cell wall molecules in principle provide ample organic matter for bacterial growth, soluble organic compounds are scarce on the leaf surface (85). These differences in the abundance of organic substrates on leaf and root surfaces might at least partly enable the differentiation of distinctive phyllosphere and rhizosphere communities through a common principle, substrate-driven selection (see below evidence for distinctive molecular adaptation strategies of phyllosphere bacteria).

Source of Inoculum

The defined phylogenetic structure of the low-complexity phyllosphere communities prompts questions regarding the source of its start inoculum. Intuitively, one might think of air and its aerosols, which flow around leaves and are known to transport bacteria. However, the typical bacterial titer in air as determined by different methods ranges from 10^1 to 10^5 cells per cubic meter (43), which is orders of magnitude lower than the typical titer in soil, which ranges from 10^6 to 10^9 cells per gram (142). In addition, aerosol-associated bacteria typically have a mixed and variable origin, ranging from marine and soil to plant and animal sources, and must survive in an extremely nutrient-poor environment exposed to UV light. Notably, abundant sequences assigned to *Sphingomonas* and *Pseudomonas* were identified in clone libraries of several aerosol samples, indicating that air presents one route of transmission for these genera (43). Neighboring plants and plant debris constitute another important immigration source, as these bacteria have already adapted to the phyllosphere. For a Mediterranean site, Vokou et al. (136) determined the relatedness between airborne

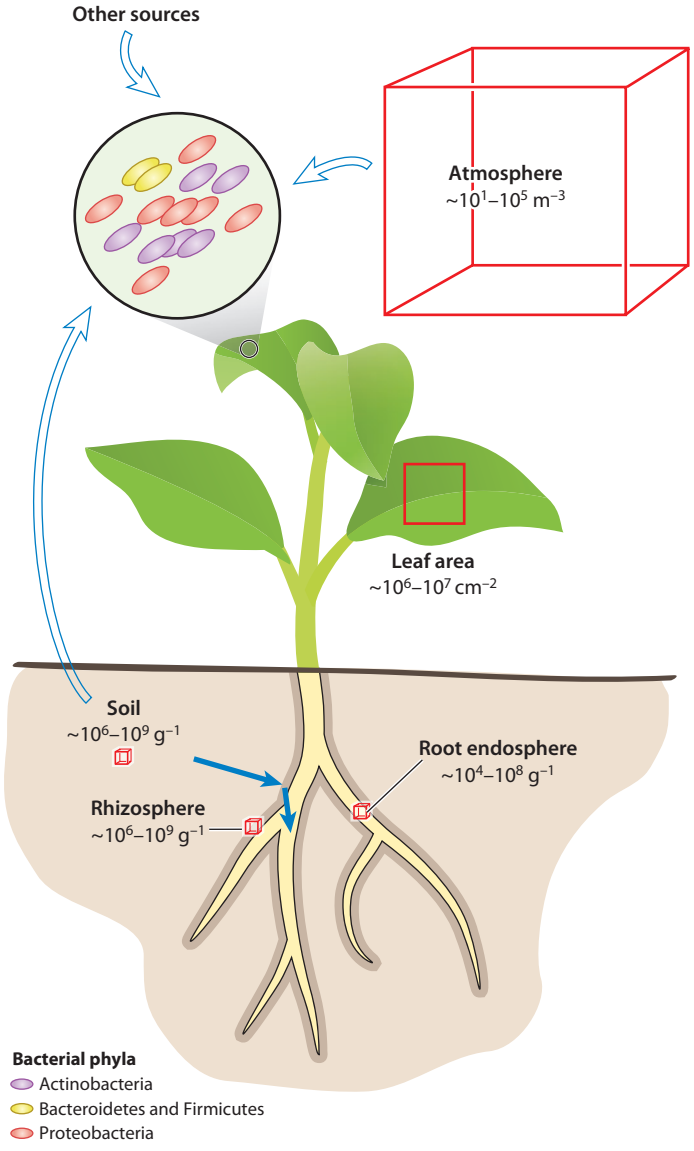

Figure 3

Bacterial titers and phyllosphere community composition. Numbers of bacterial cells in phyllosphere, atmosphere, rhizosphere, and root and soil bacterial communities were taken from References 43, 85, 123, and 142, respectively. An exact comparative enumeration of bacteria in these microhabitats based on a literature search is not possible, as the experimental setups differ in many variables, such as plant species, cultivation media, sampling unit (e.g., fresh or dry root weight), and bacterial diversity (colony-forming units based on reisolation of a single strain versus community colony-forming units). The taxonomic structure of phyllosphere communities is dominated by Actinobacteria (*purple*), Bacteroidetes and Firmicutes (*yellow*), and Proteobacteria (*red*) (see **Table 2**). Open and solid arrows represent inoculation routes for the phyllosphere and root microbiota, respectively.

bacterial populations and the phyllosphere bacterial communities of nine perennial plant species using DGGE followed by cloning and sequencing of dominant bands. Only 2 of 28 taxa were present in both the air and the phyllosphere, whereas 8 and 18 unique bands were detected, respectively, documenting the high degree of dissimilarity between these two microbial habitats. Another case study used a metaproteogenomics approach to compare the phyllosphere communities of paddy-field-grown rice (*Oryza sativa*) plants with the corresponding rhizosphere communities in relation to the flooding water of the paddy field (78). Hierarchical cluster analysis of the proteome composition indicated a closer relatedness between phyllosphere and water communities than between phyllosphere and rhizosphere communities, possibly pointing to a water-based start inoculum for the phyllosphere of paddy rice. In comparison with the root microbiota, the origin of the bacterial phyllosphere microbiota appears to be much more variable and remains ill defined (**Figure 3**).

Microbial Traits for Adaptation to the Phyllosphere Environment

Remarkable insights into phyllospheric lifestyles of bacteria have been obtained by combining metagenomic and metaproteomic approaches. Delmotte et al. (30) identified microbial proteins, which appear to reflect differential adaptation strategies to the leaf environment of two abundant phyllosphere colonizers, *Sphingomonas* spp. and *Methylobacterium* spp. For example, *Methylobacterium* expresses proteins allowing the use of methanol, a by-product of plant cell wall metabolism, as its carbon and energy source (126), indicating that these bacteria adapt via a specific methylotrophic one-carbon metabolism to the phyllosphere. In contrast, multiple transport proteins, including TonB-dependent receptors, were found from *Sphingomonas* spp., suggesting the exploitation of a large substrate spectrum, which could serve as an alternative adaptation strategy to scavenge diverse plant

metabolites present in low amounts on the leaf surface for bacterial growth.

Microbial Assemblies of Other Plant Organs

In comparison with rhizosphere and green leaf habitats, little is known about microbial environments in other plant organs. Limited information is available on bacterial communities thriving on flowers, fruits, and seeds. Highly abundant Enterobacteriaceae species were isolated on synthetic media from petal-associated bacterial communities of *Saponaria officinalis* and *Lotus corniculatus*, and the composition of these communities was clearly different from those found on green leaves (75). Similarly, based on cultivation-dependent methods, bacteria are the most abundant colonizers of flowers, fruits, and seeds. For example, bacteria of Styrian oil pumpkin flowers and fruits reach densities of 10^7 and 10^4 cells per gram of tissue, respectively, whereas seed-associated bacteria reach at most 10^2 colony-forming units (CFU) (49). In grapevine, the lowest bacterial titers were observed in seeds compared with the titers in flowers and berries, but all three microenvironments harbor at least three orders of magnitude less bacteria than the rhizosphere (27). It has been speculated that bacterial communities of flowers and seeds serve as reservoir for biocontrol bacteria with antagonistic functions against microbial pumpkin diseases.

PLANT GROWTH–PROMOTING MICROORGANISMS

Plant growth–promoting microorganisms are mainly soil- and rhizosphere-derived organisms that are able to colonize plant roots in significant numbers (10^5–10^7 CFU per gram of fresh root) and influence plant growth in a positive manner under certain environmental and soil conditions (123). Most molecular research has focused on rhizobacteria, also classified as plant growth–promoting rhizobacteria (PGPRs). The best-studied examples belong to diverse genera, such as *Azospirillum*, *Gluconacetobacter*, *Pseudomonas*, and *Rhizobium*, although some gram-positive genera are also well studied (e.g., *Bacillus* and *Paenibacillus*). There has always been a bias in the isolation of PGPRs toward diazotrophic bacteria owing to the historical assumption that biological nitrogen fixation is an important mechanism for plant growth promotion (see discussion below). Other mechanisms for direct plant growth promotion were later described and elucidated, such as phytohormone production, nutrient solubilization, and nitrogen metabolism. Indirect mechanisms of plant growth promotion are related mainly to the suppression of (soil-borne) pathogenic and deleterious microorganisms by exclusion and antagonism, and these mechanisms are attributed more to general plant health than to plant growth promotion.

PGPRs: plant growth–promoting rhizobacteria

Although thousands of plant growth–promoting bacterial strains have been isolated during the past few decades, the exact mode of action from inoculation of a potentially beneficial microorganism until the final outcome (yield increase) is still very much a black box. This likely reflects our sparse knowledge of molecular processes that control yield in an agricultural context. First of all, PGPR research has been lacking model organisms, which would allow better comparisons of data between different laboratories. Second, molecular and systems approaches are underused, although in recent years a catch-up operation to implement state-of-the-art technologies (mainly for *Pseudomonas* and *Azospirillum*) has been employed. In addition, mechanistic insights were mostly inferred from artificial laboratory setups, hampering extrapolation to agricultural settings. Although field trials have shown that PGPRs have the potential to increase plant yield under certain environmental and soil conditions, most results are not reproducible under other conditions, raising questions about the wide-scale application of these microorganisms. A prominent factor affecting field trials is the influence of the indigenous field biome and how it can influence the outcome of inoculation experiments. Thus, in the context

of an indigenous soil biome and distinctive rhizosphere and endosphere microbiota, seed coating with individual PGPRs for subsequent rhizosphere colonization is essentially a niche competition experiment in which resource partitioning, competitive exclusion, or coexistence determines the effectiveness of a given PGPR. In this respect, the term rhizosphere competence is commonly used to refer to the survival and colonization potential of PGPRs, although ultimately this phenomenon needs to be formalized in the wider context of niche theory (132).

Bacterial traits such as motility, chemotaxis, attachment, growth, and stress resistance contribute to the overall competence of a bacterium to survive in the rhizosphere and successfully colonize plant tissues. Rhizosphere competence is mostly overlooked when identifying PGPRs owing to the quest for mechanistic insights in the growth-promoting effect. However, effective rhizosphere competence can be a key factor for the successful application of PGPRs. One such important trait is flagellar motility, because it is fundamental for a directed movement toward the plant root and the initial adhesion phase. *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Azospirillum brasilense* mutants lacking (polar) flagella are severely affected in (or even incapable of) plant root colonization (35, 130). Also, at the community level, an overrepresentation of clusters of orthologous groups of proteins related to flagellar biosynthesis is observed in the rhizosphere compared with bulk soil (8), confirming the importance of this trait in rhizosphere competence. Another important competence trait is chemotaxis (the movement of cells toward or away from certain chemicals), and clusters of orthologous groups of proteins related to chemotaxis are overrepresented but less diverse in the rhizosphere compared with bulk soil, indicating the selection of a subgroup by plant roots (8, 21). It will be interesting to see whether future whole-genome information on entire rhizosphere communities allows us to define a core set of physiological functions needed for rhizosphere colonization (competence).

Plant Growth Promotion

Once established on or in the plant (rhizosphere, rhizoplane, or endosphere), rhizobacteria can influence plant growth via different molecular mechanisms. We first discuss biochemical mechanisms employed by rhizobacteria for the mobilization and provision of plant nutrients and then review mechanisms of rhizobacterial interference with plant hormone levels for plant growth promotion. Finally, we describe how bacterium-derived volatile organic chemicals and signal molecules that regulate population-dependent bacterial behavior impact plant growth.

Biological nitrogen fixation. Biological nitrogen fixation is the process to reduce gaseous dinitrogen to ammonia by the nitrogenase enzyme complex ($N_2 + 8H^+ + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$) and is well known in rhizobia-legume symbiosis (accounting for up to 460 kg fixed N hectare^{-1} year^{-1}). This process is not restricted to symbiotic microorganisms; biological nitrogen-fixing capacity in vitro has also been demonstrated for rhizosphere and endophyte bacteria. However, in most plant-bacteria systems the nitrogen attributed to the plant is less than 10 kg fixed N hectare^{-1} year^{-1}. Significant nitrogen fixation under field conditions has been shown in sugarcane and rice, mostly using the ^{15}N natural abundance technique, with Brazilian sugarcane varieties having at least 40 kg fixed N hectare^{-1} year^{-1} (128).

The use of nitrogen-fixing-defective mutant strains (Nif$^-$, defective in an essential nitrogen-fixation gene) has in a few cases provided direct evidence that biological nitrogen fixation is responsible for growth promotion, such as in the interaction of *Azoarcus* sp. BH72 with Kallar grass and the interaction of *Klebsiella pneumoniae* 342 with wheat. The mode of transfer of fixed nitrogen, transferred directly from atmospheric dinitrogen or indirectly via death and mineralization, is unknown (64, 67). It must be noted that both bacteria are validated root endophytic bacteria, retrieved in high numbers from

interior plant tissues upon inoculation [up to 7×10^7 CFU per gram of dry root for *Azoarcus* (111) and 6×10^8 CFU per gram of fresh root for wheat (67)]. In less intimate interactions, such as rhizosphere- and rhizoplane-colonizing bacteria, nitrogen fixation does not contribute significantly to plant growth promotion and can be seen as a survival strategy of bacteria under low nitrogen levels.

Nitrogen metabolism. Nitrogen is generally considered one of the major limiting nutrients in plant growth. Available genome sequences of rhizosphere and endophyte bacteria reveal in most cases a versatile carbon and nitrogen metabolism. One specific conversion in the nitrogen cycle has been studied intensively: dissimilatory nitrate reduction or denitrification, by which nitrate (NO_3^-) is reduced to nitrite (NO_2^-) as an alternative respiratory pathway. Nitrite can further be converted to nitrogen oxides (N_2O and NO) or ammonia. Its role is plant growth promotion is related mainly to the latter compounds. NO is a potent signaling molecule in plants, altering root growth and proliferation in an auxin-dependent manner (79). In *A. brasilense*, neither inoculation of a mutant strain producing only 5% of the wild-type NO level (by a mutation in a key gene for periplasmic nitrate reductase) nor inoculation of the wild-type strain in combination with an NO scavenger was able to induce (lateral) root formation, as was observed for inoculation with the wild-type strain alone (98).

Another example of how nitrogen cycling can contribute to plant growth is the tritrophic interaction between certain endophytic fungi, insects, and plants. Behie et al. (12) recently showed that the fungus *Metarhizium* is able to transfer insect-derived nitrogen to the plant via hyphae by parasitizing and killing soil-borne insects. Using ^{15}N-injected larvae, they showed that in this interaction, 12–48% of the plant nitrogen content is insect derived. The association between plant and fungus is probably mutualistic, because a plant carbon transporter, allowing the exchange of carbon, is required for successful colonization (44).

Phosphorus solubilization. Strategies to improve phosphorus availability/uptake can contribute significantly to plant growth, because less than 5% of the phosphorus content of soils is bioavailable to plants. Microorganisms with the capacity to solubilize mineral phosphorus are abundant in most soils (up to 40% of the culturable population) and can be easily determined by plating on a solidified medium with incorporation of an insoluble phosphorus form (e.g., hydroxyapatite). Halo formation around colonies indicates the phosphorus solubilization capacity of these strains. Well-known isolates belong to *Bacillus*, *Pseudomonas*, or *Penicillium* genera. Mineralization/solubilization is achieved by the production of organic acids (such as acetate, succinate, citrate, and gluconate) or phosphatases, liberating orthophosphate from inorganic and organic phosphorus pools. Several genes involved in phosphorus solubilization have been found and characterized (112). However, owing to the lack of in-depth studies, it is difficult to differentiate between direct microbial solubilization and indirect plant root stimulation by microbes allowing better nutrient uptake.

In this context, arbuscular mycorrhizal symbioses have an important role in phosphorus nutrition under phosphorus-deficient conditions. Many studies have demonstrated that arbuscular mycorrhizae can be seen as extensions of the plant root system, exploiting the soil for phosphorus. In this sense they can be compared with root hairs, but they forage a broader space (in the centimeter range) around the roots. A subset of plant phosphate transporters are specifically expressed in root cortical cells containing arbuscular mycorrhizal feeding structures (arbuscules) and are needed for efficient transport of fungus-derived phosphate across a symbiotic membrane (periarbuscular membrane) into the host cytoplasm (70, 109, 147).

Siderophore production. Similar to phosphorus, iron is abundant in soil, but it is not very available to plants owing to the low solubility of Fe^{3+} oxides. Plants have developed different strategies to counteract this low availability. In

the first strategy (the reduction strategy, found mainly in dicots and nongraminaceous monocots), protons and organic acids are released to decrease the soil pH, thereby increasing iron availability. In the second strategy (the chelation strategy, found mainly in grasses), plant roots release low-molecular-weight iron-chelating molecules (e.g., mugineic acid). These siderophores can efficiently bind iron and are then taken up by root cells (71).

Like plants, microorganisms can release organic acids and a broad range of siderophores under iron-limiting conditions. In this way, a complex competition for iron occurs in the rhizosphere between different microorganisms and between microorganisms and plants. Because bacterial siderophores generally have a higher affinity for iron than phytosiderophores do, the outcome of this competition can be unfavorable for plants. In addition, microbes can even degrade several phytosiderophores. Therefore, more in-depth studies are needed to estimate the importance of siderophore production in plant growth promotion (83, 91).

In one specific, well-documented case, bacterial siderophores can indirectly contribute to plant growth promotion. Most soil-derived fluorescent pseudomonads can efficiently scavenge iron via siderophore production (e.g., pyoverdine). In this way, they antagonize some fungal plant pathogens (e.g., *Fusarium oxysporum*) and restrict their growth in the rhizosphere, thereby enhancing plant health indirectly (40).

Phytohormone biosynthesis and interference. Phytohormones are chemical compounds that promote and influence plant growth and development. Phytohormones are often divided into five major classes—auxins, cytokinins, gibberellins, abscisic acid, and ethylene—although more recently other compounds with hormonal activity have been identified, such as strigolactones and brassinosteroids (116). In the growth medium of many soil- and plant-associated bacteria, phytohormonal production is frequently observed, in multiple cases even including different compounds produced by a single strain (104). However, the extent to which these contribute to plant growth promotion has not been proven for all compounds. Therefore, we restrict this part to two well-documented cases: auxin production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity.

Auxin production. The family of molecules with auxin activity is involved in many aspects of plant growth and development. The most abundant member is indole-3-acetic acid (IAA). Although this molecule was isolated and identified decades ago (for a historical perspective, see 1), the major pathway for IAA biosynthesis was just recently discovered (92, 125, 145). The main precursor for biosynthesis is tryptophan, but multiple biosynthesis pathways have been described in plant-associated microorganisms, including pathogens. Auxin production is a common feature of many soil- and plant-associated bacteria. The best-characterized auxin biosynthesis routes in bacteria are designated the indole-3-acetamide (IAM) and indole-3-pyruvate (IPyA) pathways. In the first pathway, well known from pathogenic bacteria, tryptophan is converted to IAM by a tryptophan monooxygenase; in a second step, IAA is formed by conversion of IAM by an IAM hydrolase. In the second pathway, found mainly in beneficial bacteria, tryptophan is transaminated to IPyA; in a second, rate-limiting step, IPyA is decarboxylated by an IPyA decarboxylase or phenylpyruvate decarboxylase to indole-3-acetaldehyde, which is finally oxidized to IAA (spontaneously or by an aldehyde oxidase/dehydrogenase). The observation that many PGPRs could produce IAA, in combination with inoculation experiments with mutant strains altered in auxin production, has led to the conclusion that auxin production is a major plant growth–promoting trait (124). For *A. brasilense*, a direct link between IAA production and altered root morphology was demonstrated in wheat inoculation experiments: A mutant strain defective in the IPyA decarboxylase could not induce the same morphological changes (36). In greenhouse experiments with wheat under suboptimal nitrogen fertilization, plants

inoculated with the wild-type strain had a higher yield than control plants or plants inoculated with the mutant strain (122). It was hypothesized that bacterial auxin production leads to root proliferation, resulting in a higher total root surface, which allows the plant to absorb more nutrients and water from the soil (80).

In some fungal species, such as *Trichoderma*, *Piriformospora*, and nonpathogenic *Fusarium* species, auxin plays a role in plant growth stimulation. In most examples, fungal auxin biosynthesis is involved in this growth promotion (60). However, this is not the case for *Piriformospora indica*: In that species, auxin biosynthesis is necessary only for root colonization in the biotrophic phase (61). However, *P. indica* induces a higher auxin concentration inside Chinese cabbage via an exuded compound of the fungus (82).

The regulation of auxin biosynthesis has been extensively studied for many bacteria. The main regulatory factors are environmental factors (such as carbon limitation, pH, and matrix potential) and plant factors (such as specific compounds or surfaces) (for a review, see 124). An interesting and rather unusual regulation has been observed for *A. brasilense*: The expression of the key gene (encoding IPyA decarboxylase) is induced by IAA itself (positive-feedback regulation) (129). Important genetic factors regulating auxin biosynthesis in gammaproteobacteria include RpoS (a general regulator in response to stress and starvation) and the two-component system GacS/GacA (involved in competitiveness). The expression of key genes therefore shows a typical stationary-phase-dependent expression (76, 102).

Plant growth is not stimulated only by IAA production. In particular cases, IAA degradation by microorganisms can also stimulate root elongation, as illustrated by the interaction of *P. putida* 1290 with radish plants. The source of IAA could be the plants themselves or other IAA-producing microorganisms. Thus, IAA degraders can have a function in the rhizosphere in auxin homeostasis by elevating or reducing local auxin concentrations (84).

ACC deaminase activity. The phytohormone ethylene was first described as a fruit-ripening hormone but is now known to have a much broader role in other processes, such as senescence, abscission, and pathogen-defense signaling. Under diverse stresses, ethylene biosynthesis is induced, thereby inhibiting root growth and plant growth (2). Some microorganisms can interfere with ethylene biosynthesis by expression of the enzyme ACC deaminase, encoded by the *acdS* gene. This enzyme converts the ethylene precursor ACC to α-ketobutyrate and ammonia. These microorganisms can enhance plant growth by metabolizing ACC exuded by plant roots. Because the ACC concentration outside the roots decreases, ACC exudation increases and ethylene biosynthesis inside the plant stalls owing to the lack of precursor. This attenuates ethylene-dependent inhibitory responses and therefore increases plant growth, especially under stress conditions (54, 55). In addition, ACC deaminase activity can be enhanced by microbial auxin production because the auxin induces the biosynthesis of ACC synthase in the plant, thereby increasing the biosynthesis of ACC (5). It will be interesting to examine whether rhizobacteria producing AcdS and auxin frequently co-occur in indigenous root microbiota and whether their co-occurrence enhances plant growth promotion. The importance of ACC deaminase activity in plant growth promotion has been extensively studied not only by using mutants in *acdS* but also by overexpressing *acdS* in plants. *acdS*-expressing microorganisms and plants are able to alleviate the growth inhibition induced by ethylene synthesis under stress conditions, such as flooding, drought, toxic compounds, and pathogen attack (for reviews, see 53, 55).

Interference with quorum sensing. QS is a key mechanism to regulate gene expression in a population-dependent manner by the accumulation of signal molecules. At a certain threshold concentration (quorum), a regulator is triggered allowing downstream regulation of gene expression. The *N*-acylhomoserine lactone (AHL)–based system is well documented

in gram-negative bacteria and allows them to coordinate their behavior at a population level. Hence, QS is involved in several important processes, such as virulence, biofilm maturation, symbiosis, and survival (48). QS is not restricted to prokaryotes; it can also be an interkingdom signal, a well-developed concept in bacteria-vertebrate interactions (63). A few reports also mention a role in bacteria-plant interactions. In *Medicago truncatula*, AHL can alter protein expression, especially proteins involved in plant defense. However, the plant response depends on the AHL structure and the specific tissue (93). In tomato, AHL-producing bacteria induce systemic resistance against *Alternaria* in a salicylic-acid- and ethylene-dependent manner, pointing toward a role for AHL in the biocontrol of pathogens (118). Schikora et al. (117) recently showed that the mitogen-activated protein kinase AtMPK6 is required for AHL-dependent defense responses in *A. thaliana*. Plants can actively interfere with QS sensing by producing QS-mimicking compounds. Although the exact role of these compounds is not known, they have been proposed to depress the virulence of pathogenic bacteria or improve symbiosis (51, 105). In terms of plant growth promotion, one report demonstrated the capacity of AHLs to modify the root architecture of *Arabidopsis*, similar to a classical auxin response; however, auxin signaling pathways are not involved in the AHL response (101).

Volatile compounds. Direct contact between microorganisms and the plant is not always necessary for growth promotion. Some microbes release volatile organic compounds (VOCs) with a growth-promoting capacity. The best-documented case is *Bacillus subtilis*, which produces the active compounds 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol. A knockout mutant in the biosynthesis pathway for both compounds demonstrated the direct involvement of VOCs in growth promotion (114). Later, the production of VOCs with growth-promoting activity was shown for other bacterial species/genera, such as *Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, *Burkholderia*, and *Serratia*, and the spectrum of compounds was broadened toward 1-hexanol, indole, and pentadecane (16, 114). In *B. subtilis*, VOC production also induces systemic resistance (113). VOC-mediated signaling in plants is highly complex because almost all hormonal pathways have been shown to be involved in the signaling. A clear role for auxin homeostasis and signaling has been demonstrated using a microarray approach and reporter lines (113, 150).

VOC production by fungi is known to be involved in self and interspecies recognition (140). The role of VOCs in plant interactions is less well documented, although in a few examples fungal ethylene emission can alter the root architecture of the host plant.

Biological Control

Biological control, or biocontrol, is the process of suppressing deleterious/pathogenic living organisms by using other living organisms. In this review, we restrict the discussion to microorganisms that can suppress pathogenic microorganisms directly or indirectly, thereby conferring plant protection. Biocontrol has been extensively studied not only under laboratory conditions but also in field situations, leading to several commercial products. Most products are based on *Bacillus* and *Trichoderma* strains owing to (seed) formulation issues, although *Pseudomonas*-based products have also been commercialized in recent years (15). The success of *Bacillus* strains in commercialization is based on the extensive knowledge of the modes of action and applicabilities of these strains both in laboratory settings and in greenhouse and field experiments. In addition, many antimicrobial molecules involved in pathogen suppression have been isolated and characterized (15, 42, 103).

Biosynthesis of antimicrobial compounds. Microorganisms can synthesize a wide range of compounds with antimicrobial activity. These compounds can be derived from the secondary metabolism or are (modified) proteinaceous molecules derived from ribosomal synthesis or nonribosomal peptide synthesis. The

production of antimicrobial compounds has been extensively studied in pseudomonads, bacilli, and *Trichoderma* species, including the identification of biosynthesis pathways and their regulation. Most commercial biocontrol products contain strains belonging to these groups. Well-known and characterized compounds are phenazines, 2,4-DAPG, pyoluteorin, pyrrolnitrin, cyclic lipopeptide surfactants, zwittermycin A, and bacteriocins (15, 42, 103, 139). The heterocyclic nitrogen-containing phenazines have a broad antimicrobial spectrum and have been identified in *Pseudomonas*, *Burkholderia*, *Streptomyces*, and *Brevibacterium* species. The biosynthesis starts from the branch-point molecule chorismic acid and involves the conserved *phz* gene operon. As for most antimicrobial compounds, biosynthesis is regulated by two-component regulatory systems and environmental conditions (39, 94). Although biocontrol strains do not directly promote plant growth, they can influence PGPRs that directly stimulate plant growth, as illustrated for a 2,4-DAPG-producing *P. fluorescens* strain that enhances the phytostimulatory effect of *A. brasilense* by altering the expression of genes involved in plant growth promotion. The authors of this study speculated that 2,4-DAPG is a signal molecule that coevolved in complex plant-microbe interactions (26).

Induced systemic resistance. Inoculation of plants with nonpathogenic bacteria can induce resistance against a broad spectrum of pathogenic organisms in both below- and aboveground parts. This induced systemic resistance (ISR) depends mainly on jasmonate and ethylene signaling. In this way, plants are primed to react more quickly and strongly to a pathogen attack. ISR has been observed for many microorganisms and their cellular derivative determinants (so-called MAMPs, such as flagella, cell envelope components, and siderophores) (33, 149). Well-characterized ISR-inducing microbes include several *Pseudomonas*, *Bacillus*, and *Serratia* species and *Trichoderma harzianum*. Most plant responses have been studied in *A. thaliana*, but ISR has also been observed in bean, radish, rice, tobacco, and tomato (33, 88, 121).

Plant Growth–Promoting Rhizobacteria in a Community Context

Above, we discussed the role of PGPRs in their interactions with plants and which mechanisms can be responsible for the observed (positive) plant responses (**Figure 4**). Many studies applying strains impaired in a particular mechanism

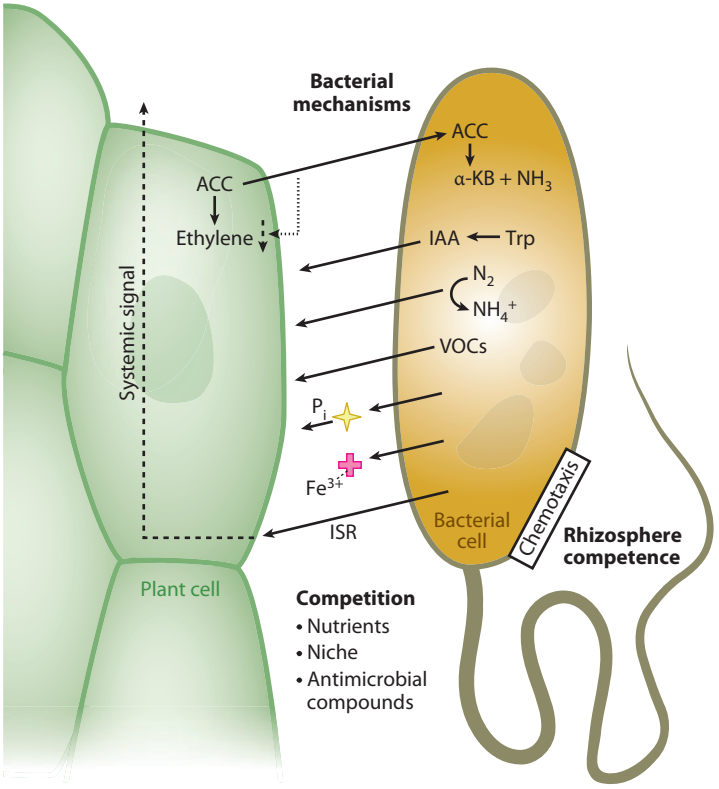

Figure 4

Biochemical mechanisms by which rhizobacteria mediate plant growth promotion and plant health. Bacterial rhizosphere competence is illustrated by the polar flagellum and chemotaxis. Several plant growth–promoting traits discussed in this review are depicted: 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (lowering plant ethylene levels), auxin [indole-3-acetic acid (IAA)] biosynthesis, biological nitrogen fixation, volatile organic compound (VOC) production, phosphorus solubilization (by the secretion of organic acids or phosphatases, represented by the *star*) and siderophore production (represented by the *cross*). Additional abbreviations: ISR, induced systemic resistance; α-KB, α-ketobutyrate; P_i, inorganic phosphate.

Syntrophic interactions: interactions in which one species lives off the products of another species

have shown that a partial plant growth promotion can be observed upon inoculation. This has led to the proposition that multiple mechanisms encoded in a single organism work together, also known as the additive hypothesis (9). However, direct proof of this has not been provided. Another important challenge for further research is the identification of syntrophic interactions. Combined strains have already been applied, but an extensive quest for mutualistic combinations can strengthen the plant growth–promoting effect and thus the outcome and reproducibility of field experiments/applications. In this context, it is also worth mentioning that most PGPR research does not take into account the community level that is already present in the rhizosphere. This complex environment is also a major factor in the outcomes of experiments. Therefore, syntrophic combinations may allow a more stable plant growth–promoting effect in a community context.

The Rhizosphere: A Future Model for Molecular Principles Underlying Niche Formation

The available amount of nutrients in the soil is limiting. Although the rhizosphere is nutrient rich in comparison with bulk soil owing to rhizodeposition and root exudates, competition between microorganisms determines the outcome of the interaction with the plant. As indicated above, microorganisms are attracted to the roots by chemotaxis toward root exudates (mainly sugar, amino acids, and organic acids). Once in contact with the root, the microorganisms can (firmly) attach to the root and occupy potential binding sites for other organisms, including pathogens (i.e., niche competition). Several studies have shown that most organisms are preferentially attached at nutrient-rich niches on the roots, such as places where lateral roots emerge, root hair zones, and junctions between epidermal cells (88, 131).

Owing to nutrient limitation, strains that are able to efficiently scavenge available nutrients and/or possess a versatile metabolism (allowing them to use a broad spectrum of carbon and nitrogen sources) have a competitive advantage over other environmental inhabitants. With the increasing availability of genome sequences and expression data for rhizosphere and plant-associated microorganisms, insights into the metabolic fluxes and conversions are expected to increase in the coming years. Some specific cases of nutrient competition for phosphorus and iron acquisition have already been discussed above.

SUMMARY POINTS

1. Members of the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria dominate root rhizosphere and endosphere bacterial assemblages.
2. Root-derived rhizodeposits provide organic substrates that drive the differentiation of the soil biome in the rhizosphere to give rise to host genotype–individualized endosphere bacterial communities.
3. Phyllosphere communities are dominated by the same few bacterial phyla as rhizosphere communities. However, the source of phyllosphere inocula and assembly cues remains essentially unknown.
4. The promotion of plant growth and plant health by microorganisms has been described for many decades. Multiple (direct or indirect) molecular mechanisms are responsible for this growth promotion, although a general framework for PGPRs is still missing.

FUTURE ISSUES

1. In-depth functional analysis of plant microbiota requires the development of reference plants and the definition of minimal experimental standards to maximize the comparison and integration of data generated by different laboratories. Likewise, the PGPR field requires model organisms to further explore the diversity of plant growth–promoting strategies, especially as the community context has been overlooked as a factor modulating the experimental outcome.
2. The development of open-access and indexed bacterial culture collections representing all microbiota members as defined by deep culture-independent 16S rRNA ribotype sequences will provide essential future tools. This will enable systematic analysis of synthetic communities for plant growth and plant health functions under defined nutrient conditions in the laboratory. In parallel, whole-genome sequencing of such collections will allow functional insights into host-microbiota interactions at a much deeper resolution than 16S rRNA-based ribotyping approaches provide.
3. The development of model systems and functional assays with synthetic communities will deconvolute ecosystem complexity, permit the definition of molecular principles underlying niche filling and niche competition, and aid in the identification of syntrophic community interactions.
4. Comparisons of the rhizosphere, root endosphere, and phyllosphere on the same plant material for a systematic assessment of community members, especially by applying metagenomic approaches, will reveal bacterial traits for adaptation to these habitats.
5. Host-microbiota biology offers the possibility to test niche adaptation theory and presents a framework to test evolutionary transitions from commensalistic to mutualistic or pathogenic lifestyles of community members.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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