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Rhizosphere chemical dialogues: plant–microbe interactions

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Every organism on earth relies on associations with its neighbors to sustain life. For example, plants form associations with neighboring plants, microflora, and microfauna, while humans maintain symbiotic associations with intestinal microbial flora, which is indispensable for nutrient assimilation and development of the innate immune system. Most of these associations are facilitated by chemical cues exchanged between the host and the symbionts. In the rhizosphere, which includes plant roots and the surrounding area of soil influenced by the roots, plants exude chemicals to effectively communicate with their neighboring soil organisms. Here we review the current literature pertaining to the chemical communication that exists between plants and microorganisms and the biological processes they sustain.

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Introduction

Prokaryotes and eukaryotes have coexisted for millions of years on earth. It is estimated that humans have 10^{13} human cells and 10^{14} bacterial cells including the endogenous bacterial flora [1]. As a result of this long association, prokaryotes have developed both beneficial and detrimental relationships with eukaryotes. As autotrophic organisms, plants play a central role in sustaining all other life forms. Unlike mammals, plants are sessile, thus releasing an array of chemical signals to interact with other organisms. The root system, which was traditionally thought to provide anchorage and uptake of nutrients and water, is a chemical factory that mediates numerous underground interactions. These include mutualistic associations with beneficial microbes, such as rhizobia, mycorrhizae, endophytes and plant-growth promoting rhizobacteria (PGPR)

and parasitic interactions with other plants, pathogenic microbes and invertebrate herbivores. Plants release enormous amounts of chemicals through their roots, at a significant carbon cost, to combat pathogenic microorganisms and attract beneficial ones. Rhizosphere interactions are affected by many different regulatory signals, of which only a few have been identified, recalling a quote by Leonardo da Vinci that ‘*We know better the mechanics of celestial bodies than the functioning of the soil below our feet*’ [2]. Rhizosphere interactions are not solely driven by roots but are highly integrated with and influenced by residing organisms and local edaphic factors. Soil-inhabiting mutualists and parasites, both prokaryotic and eukaryotic, are actively involved in signaling with a host (Figure 1). Therefore, rhizosphere interactions are very dynamic and can be altered by addition or loss of any of the players.

A large body of literature exists about rhizosphere interactions [3–5]. In this review, we summarize the current knowledge of rhizosphere chemical communication between plant roots and their associated microorganisms. Central to this discussion is the recent progress made in understanding rhizosphere chemical dialogues between plants and different components of the microbial community. We end with a discussion of how these chemical dialogues may improve plant fitness at the community level and discuss the new challenges faced by researchers.

Chemical signaling between plants and mutualists

Plant roots release a wide range of compounds that are involved in attracting beneficial organisms and forming mutualistic associations in the rhizosphere. These compounds include sugars, polysaccharides, amino acids, aromatic acids, aliphatic acids, fatty acids, sterols, phenolics, enzymes, proteins, plant growth regulators and secondary metabolites. The most important rhizosphere mutualisms described are between plants and mycorrhizae or rhizobacteria.

Mycorrhizal associations are present in almost all land plants and are essential biological constituents of the rhizosphere. Mycorrhizae are grouped into two categories: endomycorrhizae (arbuscular, AM) and ectomycorrhizae. The AM symbiosis represents the most widespread and ancient plant symbioses, originating about 450 million years ago [6]. About 6000 species in the Glomeromycotina, Ascomycotina and Basidiomycotina families have been recorded as mycorrhizal and with more sensitive molecular techniques this number is increasing [7]. Similarly, more

than 200 000 plant species host mycorrhizal fungi, but a relatively small number of mycorrhizal types are known [8]. The biotrophic interfaces that are formed between plant roots and the fungus result from recognition of exchanged cues. There is an extensive list of plant genes that are predicted to play a role in facilitating AM interactions [9*,10*], but comparatively few identified in the fungus [11*,12]. Thus little knowledge exists about signaling processes between symbionts, the pathways related to symbiosis-specific development of AM fungi in root tissues, or mechanisms of nutrient exchange between them [12,13,14,15*].

The establishment of AM symbioses begins with the colonization of a compatible root by hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots. This is followed by appressorium formation and entrance into the cortex to form specialized structures called arbuscles. Before colonization, it is assumed that a continuous dialogue of signals is exchanged between the symbionts to establish colonization. Since this symbiosis lacks host specificity it has been suggested that either the plant-derived signals are conserved throughout the plant kingdom or that a broad range of related compounds are involved. Plant-released compounds like sugars and amino acids are potential fungal stimuli but phenolic compounds, particularly flavonoids, are known as key signaling components in many plant–microbe interactions [16*]. There are vast quantities of data on the effect of flavonoids on AM hyphal growth, differentiation, and root colonization [16*], and specific effects depend on the chemical structure of the compound [17]. It was recently found that flavonoids exhibit a genus-specific and species-specific effect on AM fungi [18]. In addition, strigolactones, a group of sesquiterpene lactones exuded by *Lotus japonicus* roots, were shown to induce hyphal branching in AM fungi, a pre-requisite for successful root colonization fungi [19**]. Strigolactones present in the root exudates of a wide range of plants act display specificity as signals for AM fungi but did not affect other fungal species such as *Trichoderma*, *Piriformospora*, *Botrytis cinerea* and *Cladosporium* sp. [19**]. A further hypothesis is that strigolactones are not only involved in inducing AM hyphal branching factor but also act to attract AM fungi to roots [20]. However, more studies are needed to clarify both the specificity and roles of strigolactones in establishing mycorrhizal associations. The production and exudation of strigolactones are dependent on nutrient availability. Recently, Yoneyama *et al.* [21] reported that nitrogen and phosphorus deficiency enhanced the secretion of a strigolactone, 5-deoxysatrigenin in sorghum plants. Besides strigolactones, some studies demonstrate that calcium ions are an intracellular messenger during mycorrhizal signaling, at least in a pre-contact stage [22**].

Even less understood than the signaling between plants and mycorrhizae is the interaction of mycorrhizae with

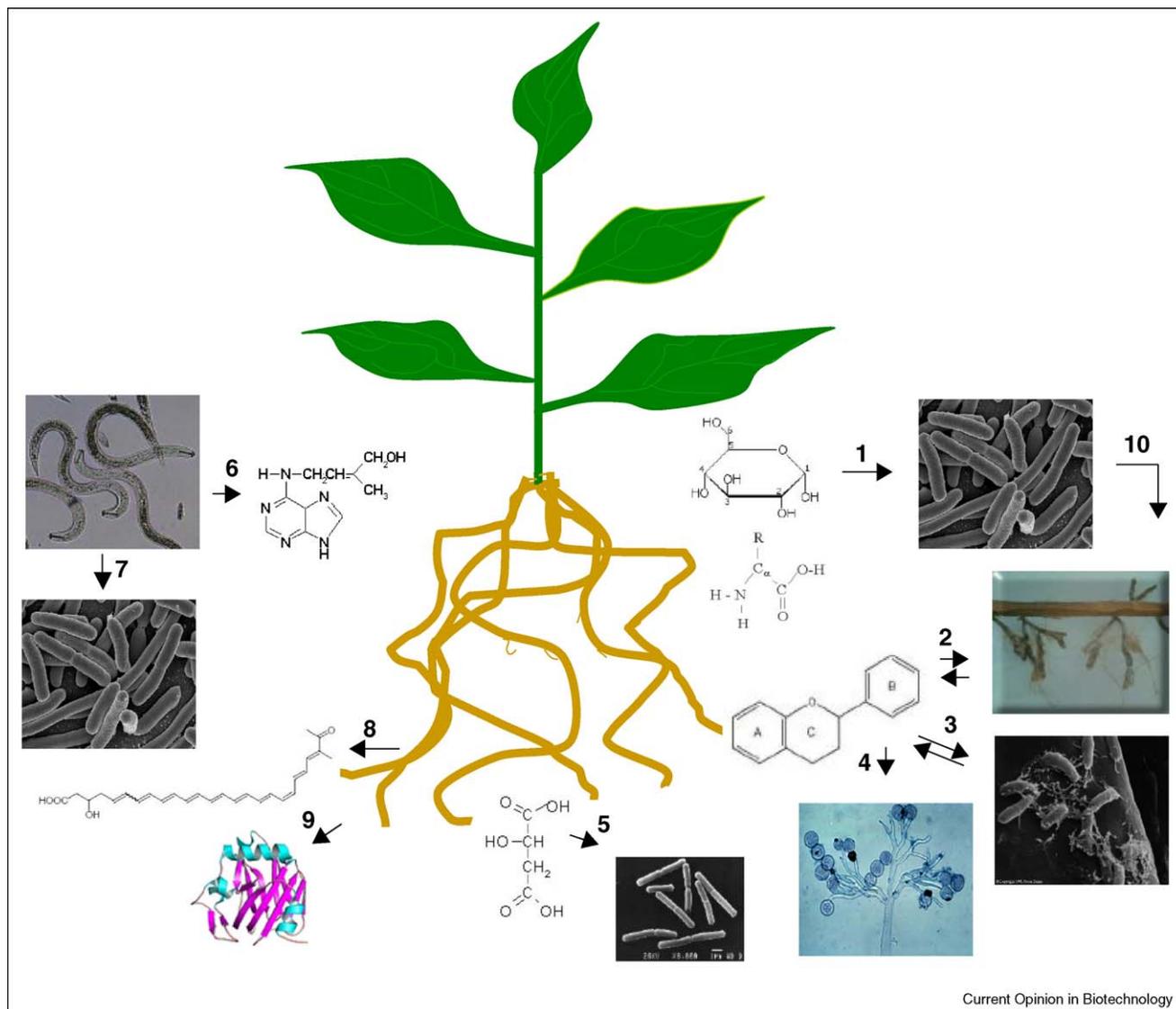
other soil microbes. It has been demonstrated that AM fungal exudates directly impact soil bacterial community composition [23], and some bacteria associated with AM can improve colonization, root branching and antifungal properties [7,24]. Future goals should include identifying all players of these signaling networks, particularly the signals and receptors that open the door to symbiosis formation. Other major challenges include unraveling the signaling events in tri-partite interactions (plant–AM–bacteria) to better understand how soil bacteria and AM fungi associate. Although, some structural properties that regulate interspecies interactions are known [25*] the bacterial–mycorrhizal network still remains to be elucidated.

As mentioned, flavonoids play a key role in the early signaling events of legume–rhizobia interactions [26]. The legume rhizosphere chemically attracts rhizobia by secreting flavonoids and related compounds [27]. Subsequently, the NodD protein of rhizobia perceives specific flavonoids through one or two-component regulatory systems, initiating transcription of nod genes that encode the biosynthetic machinery for a bacterial signal, the Nod factor. Nod factors are lipochitooligosaccharides consisting of β -1, 4-linked *N*-acetyl-glucosamine backbones with four or five residues with an acyl chain at C2 in the non-reducing end and decorated with acetyl, sulfonyl, carbamoyl, fucosyl or arabinosyl moieties at defined positions depending on the rhizobial strain [28]. Perception of the Nod factors by the plant induces multiple signaling pathways that initiate root hair infection and nodule formation. There are other nonflavonoid related compounds like xanthenes, vanillin and isovanillin that induce NodD gene expression, but they are required at much higher concentrations than flavonoids [29*], and thus their importance in natural environments is questionable. Recently, Cai *et al.* [30**] reported that canavanine, a compound present in the seed coat and root exudates of various legume plants, is toxic to many soil bacteria but not to rhizobial strains that possess specific transporter to transport (detoxify) this compound. They also suggested that host legumes secrete canavanine into the rhizosphere to select beneficial rhizobia. Further studies are warranted to identify factors determining host–rhizobium specificity.

Molecular communication between host and pathogens

There are four main groups of plant pathogens [31] but only two of them are major players in the soil; fungi and nematodes. Comparatively fewer bacteria are considered to be soilborne plant pathogens; however, some well-studied exceptions include *Ralstonia solanacearum* (bacterial wilt of tomato) and *Agrobacterium tumefaciens*, the casual agent of crown gall disease [32,33]. Fungi and oomycetes, physiologically and morphologically similar but phylogenetically distinct groups of organisms, are the

Figure 1



Pictorial illustration of the chemical communication that exists between plant roots and other organisms in the complex rhizosphere. Plant roots secrete a wide range of compounds, among those sugars and amino acids are engaged in attracting (chemotaxis) microbes (1), flavonoids act as signaling molecules to initiate interactions with mycorrhiza (AM fungi) (2), rhizobium (3) and pathogenic fungi (oomycetes) (4), aliphatic acids (e.g. malic acid) are involved in recruiting specific plant growth promoting rhizobacteria (*Bacillus subtilis*) (5), nematodes secrete growth regulators (cytokinins) that are involved in establishing feeding sites in plant roots (6) and nematodes secrete other compounds (organic acids, amino acids and sugars) involved in attracting bacteria and in bacterial quorum sensing (7). Knowledge of the roles of other types of compounds, such as fatty acids (8) and proteins (9), secreted by roots in the rhizosphere and other multi-partite interactions (10) remains unknown.

most predominant soilborne pathogens. Like plant-mutualist associations, pathogens also utilize chemical signals in early steps of host recognition and infection. Before the establishment of infection, *Phytophthora sojae* zoospores are chemically attracted by daidzein and genistein secreted by soybean [34]; however, the nature of the isoflavone receptor on the zoospores remains unknown. Most plants produce antimicrobial secondary metabolites, either as part of their normal program of growth and development or in response to pathogen

attack and those antimicrobial compounds protect plants from a wide range of pathogens [35]. Preformed anti-fungal compounds, called phytoanticipins, occur constitutively in healthy plants and act as chemical barriers for fungal pathogens. By contrast, phytoalexins are antimicrobial compounds induced in response to pathogen attack but not normally present in healthy plants. These two groups of compounds have proven very effective for a wide range of fungal pathogens. However, most studies pertaining to these compounds were conducted in leaves,

not in the roots or in the rhizosphere. Recently, Bednarek *et al.* [36] reported that *Arabidopsis* roots and leaves differ greatly in the accumulation of indolic and phenylpropanoid compounds upon infection with *Pythium sylvaticum*. On the basis of this report, one can hypothesize that the literature pertaining to the phytoalexin and phytoanticipin responses after leaf infections will differ from root infection studies. Following this observation, Badri *et al.* [37] reported that differential genome-wide expression profiles in roots upon the independent addition of three important plant signaling molecules (SA, MeJA and NO) to the roots and that these profiles were different than those from leaves treated with the same signaling molecules described in the literature. There is a need to better understand how phytoalexins, phytoanticipins and other secondary metabolites act to inhibit root fungal pathogens. The development of a rice–*Magnaporthe grisea* (causal agent of blast disease) pathosystem would be particularly useful as this fungal pathogen is capable of infecting both leaves and roots of rice plants [38**]. In addition, this system would allow us to identify if the role of phytoalexins or phytoanticipins involved in plant defense against this fungal pathogen on the leaves is the same as in roots. Further research is warranted on soilborne fungal pathogens because they cause a considerable yield loss to crops compared with foliar pathogens [39*].

Nematodes are complex, worm-like eukaryotic invertebrates that rank among the most numerous animals on the planet [40]. Most nematodes in soil are free living, and consume bacteria, fungi and other nematodes, but some can parasitize plants. The majority of crop damage is caused by both root-knot nematodes (RKN) and cyst nematodes [41]. It is generally thought that nematodes perceive their environment through chemosensory perception. Typically, RKN must locate and penetrate a root, migrate into the vascular cylinder and establish a permanent feeding site. These events are accompanied by extensive signaling between the nematode and the host, and are well described at the level of identifying proteins that are secreted by nematodes during the migratory phase [41,42]. However, the identification of initial signaling molecules (non-protein signaling compounds) released from the host to attract nematodes is still at a primitive stage. Nematodes with a wide host range respond to root-released compounds/diffusates from a wide range of hosts, whereas species with a restricted host range hatch only when presented with signals from that host [43]. Recently, Horiuchi *et al.* [44*] reported that *Medicago* roots released a volatile (dimethyl sulfide) that attracted nematodes (*C. elegans*), which acted as vectors for rhizobia and effectively enhanced nodulation. However, detailed information about the reciprocal initial signal exchange between nematodes and host is lacking with the exception of the role of cytokinins in host–nematode relationships [45*]. The nematodes secrete

cytokinins that play a role in cell cycle activation and in establishing the feeding site as a nutrient sink in the host roots. Another recent study demonstrated that how nematode secretions (non-proteinaceous compounds) interact with soil-inhabiting bacteria [46*] by using the model nematode *Caenorhabditis elegans*. This study will open a new avenue of research to study the chemical interactions of other parasitic nematodes with their hosts.

Quorum sensing (QS) and rhizosphere communication

The exquisitely coordinated gene expression that resulted in production of bioluminescent proteins by the marine bacteria *Vibrio fischerii* was once considered an interesting novelty, and it was several years before the scientific importance of this discovery was realized. It has now been determined that coordinated activity among microbial cells using diffusible chemical signals is a widespread phenomenon, called ‘quorum sensing’ or ‘cell-to-cell communication’. Although the chemical signals and mechanisms of QS systems vary, the most prevalent form of QS signals used by plant-associated bacteria are acyl homoserine lactones (AHLs), which vary in the length, oxidation state, and degree of saturation of their acyl side chains to provide a degree of species specificity. At threshold concentrations, these AHLs form complexes with their cognate receptors, which bind to DNA and act to regulate expression of specific genes, effectively allowing populations of individual cells to act as a collective unit. This is a simplified explanation as increasing evidence suggests that signal concentration alone does not dictate the activation or repression of QS-controlled genes, but that local environment and spatial distribution of cells are also important contributing factors [47,48*].

The behaviors that are influenced by QS are extremely varied but from a broader ecological perspective they facilitate nutrient or niche acquisition, modulate collective defense against competitors, and permit community escape in the face of population destruction [49]. In plant-associated bacteria, QS is often involved in establishing successful associations, whether they are symbiotic or pathogenic. The role of QS in the pathogenesis of *Erwinia carotovora* and *Agrobacterium tumefaciens* on their respective plant hosts are well characterized. The enteric phytopathogen *E. carotovora* produces a number of QS-regulated virulence factors, such as pectinases, cellulases and proteases and some strains produce a β -lactam antibiotic that is thought to provide niche protection to *E. carotovora* once it has established an infection [50]. However, whether plant factors are involved in establishing or inhibiting QS to determine host specificity of *E. carotovora* is unknown.

Conversely, it has been established that chemicals from the plant host contribute to infection by the tumor-inducing bacteria *A. tumefaciens*. The signal-receptor pair

(TraI/TraR) responsible for regulation of QS in *A. tumefaciens* occurs on the Ti (or tumor-inducing) plasmid, which is required for gall formation in host plants. An infection occurs when a segment of this plasmid is integrated into the nucleus of host plant cells, resulting in the production of opines that can then be utilized as a novel source of nitrogen and carbon [51]. The presence of opines, which are only found in the plant tumor, then upregulate expression of the bacterial TraR gene. Thus, the QS system, which allows for conjugation and replication of the Ti plasmid, is only effectively activated after infection, resulting in a questionable role for QS in *A. tumefaciens* pathogenicity [52]. To complicate matters, *A. tumefaciens* also produces a protein BlcC (formerly AttM) that has lactonase activity, which it was suggested may negatively regulate QS through signal degradation [53,54], a phenomenon called 'quorum quenching'. Another study showed that the presence of the plant defense metabolite salicylic acid resulted in increased expression of this lactonase and inhibition of virulence (*vir*) genes carried on the Ti plasmid [55]. However, the biological significance of this plant-induced lactonase to act as a quorum quencher was not substantiated by *in planta* data and appears to have only a transient effect [56].

Quorum sensing has also been implicated as an important factor in the symbiotic association between legumes and rhizobia, although many details of its involvement are still emerging. Many rhizobia displaying mutations of their QS systems have reduced ability to infect root hairs and/or form nodules [57–59]. Additionally, several legumes have been shown to secrete compounds that can interfere with bacterial QS [60,61,62], and *Medicago truncatula* responded differentially with regards to root exudation and protein expression to AHLs produced by its symbiont *Sinorhizobium meliloti* and an opportunistic pathogen *Pseudomonas aeruginosa* [63]. However, among the plant-produced QS agonists and antagonists that may play a role in legume/rhizobia interactions, the only one that has chemically identified is L-Canavanine, an arginine analog [62]; thus, it has been predicted that the observed QS inhibition may be an indirect effect potentially caused by protein misfolding of transcription regulators [59].

Endophytes as chemical factories

In addition to interacting with microorganisms in the rhizosphere, plants are internally colonized by endophytic bacteria and fungi. Endophytic bacteria can be defined as bacteria that reside within living plant tissue without causing substantive harm to their host. Diverse arrays of bacterial genera have been reported to be endophytic [64,65]. The community structure of endophytic bacteria was shown to be strongly affected by the plant species, up to the level of the cultivar [66], pointing to species-specific associations between endophytes and their plant host [67]. On the contrary, some endophytic bacteria

seem to be quite promiscuous when it comes to host plant colonization and plant beneficial effects, such as the *Burkholderia cepacia* Bu72, which was isolated from yellow lupine [68] but also significantly improved biomass production of poplar DN-34 [67,69]. Therefore, before applying plant growth promoting endophytic bacteria preliminary studies to confirm the plant growth promoting synergy of the selected endophytes and the plant species are required.

A close relationship exists between endophytic and rhizosphere bacteria and many facultative endophytic bacteria can also survive in the rhizosphere, where they can enter their host plant via the roots. Root colonization by rhizosphere bacteria involves several stages [70] and endophytic bacteria are hypothesized to follow a similar process. In the initial stage, bacteria move to the plant roots either passively via soil water fluxes or actively via specific induction of flagellar activity by plant-released compounds. In a second step, non-specific adsorption of bacteria to the roots takes place, followed by anchoring (third step), and resulting in the firm attachment of bacteria to the root surface. Specific or complex interactions between the bacterium and the host plant, including the secretion of root exudates, may arise that can result in the induction of bacterial gene expression (fourth step). Endophytic bacteria can subsequently (fifth step) enter their host plant at sites of tissue damage, which naturally arise as the result of plant growth (lateral root formation), or through root hairs and at epidermal junctions [71]. In addition, plant exudates leaking through these wounds provide a nutrient source for the colonizing bacteria.

Endophytic bacteria can improve plant growth and development in a direct or indirect way. Direct plant growth promoting mechanisms of endophytic bacteria may involve nitrogen fixation [65,72], the production of plant growth regulators such as auxins, cytokinins and gibberellins [73–75], suppression of the production of stress ethylene by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [76,77], and alteration of sugar sensing mechanisms in plants [78]. For instance, alteration of biosynthesis and/or metabolism of trehalose *in planta* have been shown to increase tolerance to drought, salt and cold [79]. It is therefore noteworthy that several endophytic bacteria from, for example, poplar were able to efficiently metabolize trehalose [67]. Endophytic bacteria can also indirectly benefit plant growth by preventing the growth or activity of plant pathogens through competition for space and nutrients [80], antibiosis [81], production of hydrolytic enzymes [82], inhibition of pathogen-produced enzymes or toxins [83] and through induction of plant defense mechanisms [84].

A systems biology approach to better understand the synergistic interactions between plants and their beneficial

endophytic bacteria represents an important field of research, which is facilitated by the recent sequencing of the genomes of several plant species and several endophytic bacteria. For instance, the genome comparison between the poplar endophyte *S. maltophilia* R551-3 and the opportunistic pathogen K279a pointed to the existence of insertion hotspots in the core genome of this species [85]. The mechanisms responsible for colonizing plants and for antagonistic activity of *S. maltophilia* strains against plant pathogens seem similar to those responsible for colonization of human tissues and for pathogenicity. Furthermore, antibiotic resistance and synthesis was found to be part of the core genome. Therefore, the application of rhizospheric and endophytic bacteria, such as *S. maltophilia* or *B. cepacia*, to control plant pathogens or promote plant health should be very carefully considered, as they may have potential as opportunistic pathogens.

Rhizoremediation

Plant-assisted bioremediation or phytoremediation holds promise for *in situ* treatment of polluted soils. The general subject of phytoremediation has been reviewed by numerous journal articles and book chapters, and aspects specific to the rhizosphere are included. A recent review article by Wenzel [86] comprehensively covers the rhizosphere processes and management in plant-assisted bioremediation of soils; therefore, an extensive discussion is not warranted here. However, it is very clear that the understanding of the plant–microbial consortia in the rhizosphere will enhance our ability to engineer plants for phytoremediation purposes very effectively as described by Dzantor [87] and Ryan *et al.* [88]. Further emphasis should be put on evaluating results obtained from simplified lab experiments to heterogeneous natural conditions under such as complex rhizosphere environments (multiple plants–multiple microbes).

PGPRs interaction with plant roots

The rhizosphere is the playground and infection court for soilborne pathogens and also a battlefield, where both microflora and microfauna interact with soilborne pathogens and influence the outcome of pathogen infection [39]. However, several beneficial microorganisms that reside in the rhizosphere can inhibit the growth and activity of soilborne pathogens. The activity and effects of beneficial rhizosphere microorganisms on plant growth and health are well documented for bacteria like *Pseudomonas*, *Burkholderia* and fungi like *Trichoderma* and *Gliocladium*. Similar to the involvement of flavonoids in legume–rhizobia signaling, root-secreted compounds (both flavonoids and other secreted compounds) modulate the interaction between plants and PGPRs and these interactions are reviewed by several articles [3,5,89,90]. But, the specific root-released signal in recruiting specific bacteria species is poorly understood. Recent evidence [91] demonstrated that L-malic acid secreted from plant roots is involved in specifically recruiting *Bacillus subtilis*

FB17 but not other *Bacillus* sp. This suggests that each beneficial rhizobacteria needs a specific signal to colonize the host. All PGPRs have indirect positive effects on plant health by inhibiting soilborne pathogens by means of competition and antibiosis [39]. PGPRs also have direct positive effects on plant health by inducing systemic resistance (ISR) in order to prepare the plants from pathogen attack or by exposing the plants to PGPR-released compounds such as 2,3-butanediol, pyoverdine, and lipopeptide surfactants [39*]. However, most experiments examining the mechanisms of PGPRs deal with only a single host–single PGPR interaction. In nature, the rhizosphere contains millions of microbes including PGPR, pathogens and microfauna. Further studies are needed to unravel these multiplex interactions at a molecular level to enhance their utilization for agricultural benefits.

Novel tritrophic interactions

Root secreted compounds are also being studied for their involvement in tritrophic interactions (plant–microbe–nematode) in the rhizosphere. Only a few examples are well documented in this line of research [3], such as plant–AMF–parasitic weed interactions [92], legume–nematode–rhizobium associations [44], and the attraction of entomopathogenic nematodes to insect damaged roots [93]. All these interactions are studied at laboratory levels by using simplified model systems but the knowledge about how these interactions might occur effectively in the complex rhizosphere under natural conditions still remains scarce.

Can proteins in the exudates be chemical signals?

While there is abundant information on the role of root secreted secondary metabolites in rhizosphere plant–microbe interactions, the role of exuded proteins is poorly studied. Recent evidence demonstrates that microbes can modulate root exudation of proteins and that plants can do the same in soil bacteria [94]. These reports confirm that the composition of proteins exuded by plant roots is dynamically effected by the organisms in their surroundings. A recent review by Mathesius [95*] discussed the use of proteomics to study root–microbe interactions. Most of the conclusions reached to date are based on results obtained from simple laboratory experimental models. Some of the secreted proteins are starting to be identified but nothing (almost) is known about how these secreted proteins from different organisms interact at interspecies/inter-generic levels or what effect they have on other organisms in the rhizosphere. Most importantly, research needs to be conducted to determine if the proteins retain their enzymatic activities in the rhizosphere.

Future challenges

Rhizosphere chemical dialogues are the language of communication between plant roots and microbes in

the area where the soil and roots are in close proximity. Several lines of evidence implicate root-secreted exudates as signaling agents that play a key role in these interactions. Researchers have already identified some of the factors initiating the dialogues in the rhizosphere that drive these interactions. However, there are still numerous other factors/determinants yet to be identified to better understand these interactions at an ecological level. The rhizosphere is considered to be common ground for ecologists, molecular biologists and plant biologists to further explore these novel interactions occurring in this complex zone. Recent technology development in the areas of 'omics' such as proteomics, metabolomics, transcriptomics and secretomics allow us to further underpin these interactions efficiently for agricultural benefit. A combination of data analyses obtained from these 'omics' studies will further strengthen our capability to visualize a complete picture of these complex multi-species interactions.

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