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Rhizospheric plant-microbe interactions: microRNAs, a key mediator

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Abstract:

The importance of microorganisms in plant development, nutrition and stress resistance is consensus and has led to a more holistic approach of plant-microbe interactions, under the holobiont concept. The structure of plant microbiota is often described as host-driven, especially in the rhizosphere, where microbial communities are shaped by diverse rhizodeposits. Gradually, this anthropogenic vision is fading and being replaced by the idea that plants and microorganisms co-shape the plant microbiota. Through co-evolution, plants and microbes have developed cross-kingdom communication channels. We propose that microRNAs are crucial mediators of plant-microbe interactions and microbiota shaping in the rhizosphere. Moreover, we suggest, as an alternative to generally unsuccessful strategies based on microbial inoculants, microRNAs as a promising tool for novel holobiont engineering.

Keywords: miRNA; extracellular vesicles (EVs); rhizospheric microbiota; inter-kingdom communication; holobiont engineering

Highlights

- Plants interact with a diversity of microbes, especially in the rhizosphere. The plant and its associated microbes form a co-evolved unit called “holobiont”, implying the emergence of bi-directional interkingdom communication channels.
- MicroRNAs may be key mediators of plant-microbe interactions, as they are well-conserved throughout the living, can be conveyed by extracellular vesicles on long-distances and are part of interkingdom interactions.
- Plant microRNAs are uptaken by gut microbiota and phytopathogens, resulting in a modulation of microbial gene expression, hence of microbial community activity and composition. In return, microbial microRNA-like RNAs target plant genes and act on their fitness.

Cross-kingdom communication in the rhizosphere *via* microRNAs

Plant-associated microorganisms are essential for the well-being of plants: they play a crucial role in development, nutrition and resistance to various stresses [1]. Notably, the **rhizosphere** (see [Glossary](#)) provides a rich environment in which diverse microbial communities, including plant beneficial microorganisms, live abundantly, in close relation with the plant. Together, a plant and its associated microbes form a coordinated unit, referred to as a **holobiont** [2]. In order for this association to remain coordinated, it is necessary to have some form of regulation within the microbial communities and with the plant. Currently, there is still a gap in our knowledge with regards to the assembly and composition dynamics of the rhizospheric **microbiota**. Generally, it is thought that the plant performs a selection of its microbial partners through the influence of its **rhizodeposits**, and especially its **root exudates** [3]. However, this uni-directional

selection is starting to be questioned [4]. Indeed, the **co-evolution** of the plant-microbe holobiont suggests a bi-directional communication, notably regarding the shaping of the rhizospheric microbiota. The composition and function of these communities are modulated by a variety of molecules and signals [5]. These past years, a growing interest in small RNAs (sRNAs, [Box 1](#)) and more specifically microRNAs as mediators of cross-kingdom communication has emerged [6]. Conveyed by **extracellular vesicles** (EVs), mammal host microRNAs and dietary plant microRNAs have been shown to be implicated in regulating the intestinal microbiota, by modulating both bacterial gene expression and species abundance [7–9]. Regarding the commonalities between the gut microbiota in animals and the rhizospheric microbiota in plants [10,11], combined with their key role in holobiont nutrition and health, and the universality of the RNA “language”, the implication of microRNAs in plant-rhizospheric microbiota **cross-kingdom** interactions is very plausible, but completely understudied.

In this Opinion, we suggest that microRNAs, conveyed by EVs, constitute a cross-kingdom bi-directional communication channel between the plant and its associated rhizospheric microorganisms ([Figure 1, Key Figure](#)). Supported by experimental evidence of the connection between plant microRNAs and the intestinal microbiota [9] and between plant microRNAs and pathogens [12–15], we propose that microRNAs are implicated in shaping the abundance, composition, functions and activities of the rhizospheric microbiota.

74

75 **Who shapes the rhizospheric microbiota?**

76 From the very start of their lives, as a seed and then through all stages of development,
77 from root to shoot, plants are surrounded and populated inside and outside by
78 microorganisms. The significant role of these microorganisms in the survival and growth
79 of the plant is fully accepted and has been thoroughly reviewed [1]. Moreover, it has
80 been stated that plant-associated microbes allow the plant to better adapt to their
81 environment [16]. The plant microbiota can be acquired from its environment, through
82 horizontal transmission, but also directly in the seed from the parent, through vertical
83 transmission [17]. The environmental acquisition of microbes and the laws governing
84 their assembly in the plant holobiont are poorly understood. It is generally thought that
85 during root penetration in the bulk soil, the soil microbiota gradually differentiates into the
86 rhizospheric microbiota through contact with rhizodeposits, which greatly impact the
87 composition of the microbiota by having antimicrobial properties or by serving as a
88 **substrate**, for example [18]. After this first community shift, the microbiota composition
89 will be fine-tuned in a compartment-specific manner (i.e. endosphere, rhizoplane or
90 rhizosphere) [16], but also depending on host genotype. Indeed, characteristics
91 determined by plant genetic factors, such as root morphology and root exudate quality
92 and quantity were shown to shape the rhizospheric microbiota [3]. In addition, other
93 factors such as the plant immune system, plant developmental stage and season, were
94 shown to be greatly involved in shaping the rhizospheric microbiota [19]. The existence
95 of a core microbiota in certain plants, regardless of soil origin and fertilization regimes,

further suggests that the assembly and selection of these communities is at least partly plant-driven [19].

Even though the assembly of rhizospheric microbial communities seems to be mainly the result of **selection**, it is important to consider the other processes of community assembly: **dispersal**, **drift** and **speciation** [20]. It is suggested that these processes modulate, at varying degrees through plant development, the establishment of microbial communities in the rhizosphere [20]. Changes in the microbiota and its functional diversity are not the sole result of the acquisition of novel microbial strains, selected by the host plant. Such variations in the holobiont are also the consequence of specific **microbial amplification/reduction** and **horizontal gene transfer** (HGT) [21]. The combination of these three processes allow rapid adaptation of the plant holobiont to challenging and changing environments. These shifts from originally present microbial communities, to communities specifically tailored to the situation, have been observed in many studies. An example of microbial abundance modulation by the plant was described in rice: under drought stress, the shift in composition of root microbial communities was characterized by a specific enrichment of certain bacterial taxa, known as plant growth promoting rhizobacteria (PGPR) and/or resisting drought stress [22]. The authors of this study suggested that the change in root exudation, due to drought stress, could be the origin of the selective enrichment of certain microbes. In addition to a change in microbial composition, stressful conditions can also impact microbial activity in the rhizosphere. In hydrocarbon-contaminated soils, both microbial composition and activity are modulated: for example, *via* a specialization of the microbiota, through a selective enrichment of hydrocarbon degraders and PGPRs [23] or *via* a stimulation of

specific microbial activities like hydrocarbon degradation, in the microbes already present [24].

The regulation of rhizospheric microbiota is not solely driven by plants, but also by the microbes themselves. Indeed, they can modulate the plant's environment and even reprogram the plant to their advantage *via* exudation of phytohormones, volatile organic compounds, quorum-sensing molecules and antimicrobials [5]. Shaping of the rhizospheric microbiota is also based on microbial metabolite substrate preference [25] and interactions between microbial members. Microbial interactions can be based on cooperative mechanisms, such as quorum-sensing and metabolite exchange under nutrient-poor conditions, or competitive mechanisms, such as secretion of antimicrobial compounds [19], but it remains unclear which type of interaction dominates communities. Overall, microbial interactions can induce an alteration in gene expression within the communicating microbes [3]: hence, microbial interactions modulate both shape and function of the microbiota. Based on their role in regulating plant-associated microbial communities, some argue that symbionts are ecological engineers of the holobiont [4]. Thus, on top of plant regulation of the rhizospheric microbiota, microbial interactions in the rhizosphere also play an essential role in structuring the plant microbiota.

The role of microRNAs in bi-directional cross-kingdom communication

Between the host plant and the associated microorganisms, there is a constant two-way dialogue, adapted to cross-kingdom communication (Box 2). A well-known example is during the establishment of the rhizobium-legume symbiosis, in which the plant

141 produces flavonoids that induce bacterial *nod* genes, resulting in the secretion of Nod
142 factors, which in turn initiates nodule formation, that house the rhizobacteria that will
143 there fix atmospheric nitrogen and transfer it to the plant [26,27].

144 As discussed previously, a plethora of signalling molecules are exchanged in the
145 rhizosphere. Interestingly, a majority of microbial signalling molecules used to
146 communicate with the host plant are also part of inter-microbial communication [5].
147 Knowing that these compounds affect plants, in their development or immune system,
148 these signals may allow the microbiota to control the plant, or plants have simply
149 evolved to perceive them. With regards to the holobiont concept, these two possibilities
150 could co-exist, as there is constant bi-directional communication between host and
151 microbes.

152 In the past years, interkingdom communication studies have bloomed, especially
153 regarding host-microbiota interactions and their implications for human health. Amongst
154 the different possible mediators of such conversations, sRNAs, and more specifically
155 microRNAs, have sparked a growing interest [28] (Box 1).

156 MicroRNAs are a class of small non-coding RNAs (~21 nucleotides), implicated in
157 regulating gene expression: either by repressing the translation or by directly cleaving
158 the target mRNA. MicroRNAs were first discovered in nematodes [29,30] which then led
159 to their identification in fishes to flies and mammals, highlighting their conservation
160 across the animal kingdom [31]. Shortly after, plant microRNAs were described, for the
161 first time, in *Arabidopsis thaliana* [32] and then, microRNA-like RNAs (miRNAs) were
162 characterized in fungi [33] (Box 1).

163 It was previously thought that microRNAs had appeared with pluricellular organisms
164 and that their function was limited to intercellular communication within a single
165 organism. Since then, microRNAs or microRNA-like RNAs have also been found in
166 unicellular organisms: first, in *Chlamydomonas reinhardtii* [34,35], then in oomycetes
167 [36] and in bacteria [37], the latter being referred to as microRNA-size small RNA
168 (msRNA) [38]. The discovery of microRNA-like RNAs in unicellular organisms suggests
169 that microRNA-based gene regulation is an ancient mechanism and could have
170 appeared prior to pluricellular life [34]. MicroRNA, or at least very similar molecules, are
171 indeed evolutionarily well conserved and produced throughout all branches of life.

172
173 Moreover, microRNAs are not restricted to intercellular communication within a tissue,
174 an organ or an organism: they can be transported on long distances, between
175 individuals and from one species to another. With regards to other signalling
176 compounds, the ancient and widespread RNA “language” is particularly suited for cross-
177 kingdom communication, as it is employed by all organisms which use RNA-based
178 regulation: RNA provides a universally conserved, stable, communication channel [39].
179 MicroRNAs, as mediators of such conversations, are conveyed by nanovesicles,
180 generally named EVs, although Outer Membrane Vesicles (OMVs) is sometimes used
181 for Gram-negative bacteria [40]. In eukaryotic cells, EVs originate from the fusion of
182 multivesicular bodies with the plasma membrane, whereas bacterial EVs or OMVs are
183 produced through external budding of the bacterial outer membrane [40]. These vesicles
184 function as a protective transportation structure for RNA, but also proteins and lipids.
185 Before encapsulation, these molecules are selectively sorted into EVs or OMVs [40],

suggesting a functional targeting. Cross-kingdom microRNA exchanges have been described for a multitude of species and interactions (Table 1). Many studies have focused on host-pathogen interactions, often emphasizing on sRNA-mediated **RNA interference** (RNAi) mechanisms which are conserved virulence mechanisms shared by many pathogens to suppress host immunity [13], whereas the host may target virulence-related genes in the pathogen [41]. Evidence of such host-pathogen interactions were demonstrated between mammals and bacteria [42,43]; nematodes and mammals [44]; plants and fungi [12,14,15,41,45]; plants and insects [46] and between parasitic plants and host plants [47]. Actual bi-directional cross-kingdom RNAi, through sRNA trafficking, was first described between *Arabidopsis thaliana* and a fungal pathogen, *Botrytis cinerea* [13].

However, more and more studies are now highlighting the role of microRNA exchanges in non-pathogenic relationships, with many focusing on host-microbiota interactions in the gut. Indeed, host microRNAs from the intestinal epithelium cells have been shown to impact the composition of the gut microbiota by specifically entering certain bacteria and regulating the transcription of a wide range of housekeeping and sugar degradation genes, thereby impacting bacterial growth [7,8]. Interestingly, ingested plants release EVs in the gut, where they are preferentially uptaken, along with their content in plant microRNA, by certain target bacteria, inducing a modification of their gene expression [9,48]. In turn, their production and secretion of metabolites may be altered, leading to differential development of bacterial species interacting with these target bacteria [9]. Hence, ingested plants are capable of shaping the gut microbiota of mammals through

208 microRNA-based communication, partly explaining the effect of diet on the gut
209 microbiota.

210 Given these recent advances on the impact of host microRNAs and plant microRNAs on
211 the gut microbiota, we propose that plants and their rhizospheric microbiota also
212 communicate *via* microRNAs, regulating microbial composition and activity in the
213 rhizosphere. Besides, it has recently been shown that bacterial vesicles can carry their
214 DNA cargo into other bacteria [49], a mechanism called “vesiduction” [50], but also into
215 host eukaryotic cells [51]. Hence, it is easily conceivable that msRNA are also conveyed
216 in such a manner and may mediate cross-kingdom communication. Plant microRNAs
217 are tissue specific, and many hundreds are found in the root tissues [52], and it is thus
218 likely that a subset of these, selected through co-evolution with surrounding organisms,
219 would be excreted via EVs in the rhizosphere.

220 **Holobiont engineering and current RNAi applications**

221 Under the holobiont era, the role of microorganisms in shaping their own communities,
222 concurrently to plant influence, is becoming a subject of interest. In many fields, such as
223 ecological engineering, scientists have aimed to successfully engineer the plant's
224 microbiota, in order to optimize the ecosystemic services provided by the plant
225 holobiont. Many biotech companies, worldwide, have made large efforts to create
226 specific **inocula** containing carefully selected microorganisms, in order to improve crop
227 production. However, many of these methods do not translate efficiently from lab to field:
228 inoculated microbes can be in competition with native communities or simply washed
229 away before being able to establish themselves stably. Inoculation of non-native species

also raises questions about potential invasive species. Hence, microbiome engineering is slowly evolving into “holobiont engineering” [53], which is already applied in agricultural practices such as grafting, organic or low-farming and plant breeding strategies which include microbiota [54]. Holistic biotechnologies are also being developed such as approaches based on soil memory [55] or synthetic microbiomes [56]. We suggest that these holobiont engineering techniques could also be based on cross-kingdom communication mediators such as microRNAs.

Currently, cross-kingdom RNAi technology for crop protection already exists and is largely investigated, notably Host-Induced Gene Silencing (HIGS) which uses genetically modified plants able to express specific sRNA or double-stranded RNAs (ds-RNA) which target specific genes in pathogens [57]. However, HIGS requires the use of genetically modified plants, which is controversial, and even banned in certain countries. To counter these issues, environmental RNAi quickly emerged: sRNA or ds-RNA are directly applied on the host plant, resulting in the silencing of specific genes in pathogens and disease control [58]. These environmental RNAi methods are named Spray-Induced Gene Silencing (SIGS). However, SIGS technologies are still in their infancy and encounter many problems, such as penetration, application effects and production costs: currently, there are no available SIGS-based products on the market [59,60].

Alternatively, the use of microRNA-encoded peptides (miPEPs) [61,62], as a tool to amplify the expression of specific plant microRNAs, could be an interesting perspective. miPEPs are small regulatory peptides, encoded in plant pri-miRNAs (Box 1), capable of enhancing the transcription of their corresponding microRNA, resulting in a stronger

repression of the target mRNA [61]. When synthetic miPEP171b was applied on *Medicago truncatula* the expression of the microRNA171b was increased and led to enhanced mycorrhization by *Rhizophagus irregularis* [63]. If our hypothesis, that microRNAs are exchanged between the plant and its rhizospheric microbiota, is confirmed, then the use of miPEPs to stimulate the synthesis and release of certain beneficial plant microRNAs could be a promising tool for many fields in ecological engineering. Indeed, miPEPs could indirectly modulate the rhizospheric microbiota, without adding exogenous inocula. Moreover, the application of miPEPs is straightforward and does not require genetic transformation [62], which makes miPEP technology an exciting pathway for holobiont engineering.

Concluding remarks and future perspectives

Regarding our current knowledge about the role of host and plant microRNA on the composition and activity of the gut microbiota, the universality of the RNA “language”, combined with the potentiality of RNAi technology, our opinion is that microRNAs at least partly shape the rhizospheric microbiota. Such a point of view implies that microRNA-based microbial community manipulation has an enormous potential for agriculture, forestry and environmental remediation. Evidently, more fundamental research is needed to decrypt the implication of microRNAs in plant-microbe communication and especially the impact of plant microRNAs on rhizospheric microbial communities. If our hypotheses reveal to be correct, then a new field of perspectives regarding our knowledge of plant-microbe interactions and holobiont engineering possibilities will arise, with a plethora of Outstanding Questions.

276

277 [Glossary](#)

278 **Co-evolution:** process during which two or more species, sharing a close ecological
279 relationship, evolve simultaneously

280 **Cross-kingdom:** involves species from distinct taxonomic high ranks, i.e. animals,
281 plants, fungi, protists bacteria or archaea

282 **Dispersal:** refers to the movement of species between communities, from one site to
283 another

284 **Drift:** random change in population size due to births and deaths, which can happen in
285 small, low-diversity communities

286 **Extracellular Vesicles (EVs):** spherical structures with a lipid-membrane, comprising
287 microvesicles to exosomes, ranging from 30nm to 1000nm, secreted by a wide diversity
288 of organisms. Plant EVs range mostly from 20nm to 500nm. EVs are greatly involved in
289 intercellular communication as they contain and transport proteins, lipids, nucleic acids
290 and other molecules.

291 **Holobiont:** originally termed by Lynn Margulis as an organism, animal or plant, and its
292 symbiotic associations, “holobiont” now refers to a host macrobe and, more generally, its
293 numerous associated microbes

294 **Horizontal Gene Transfer (HGT):** a mechanism of gene exchange between bacterial
295 cells

296 **Inoculum** (pl. inocula): microbial sample destined to be added to a plant or soil, for
297 example

298 **Microbial amplification/reduction**: respectively, an increase/decrease in numbers of a
299 certain group of associated microbial species, due to environmental conditions, also
300 known as “enrichment/depletion”

301 **Microbiota**: community of microorganisms living in a specific region (e.g. human
302 intestine, skin or the rhizosphere)

303 **Rhizodeposits**: variety of carbon- and nitrogen-rich compounds released by roots into
304 their environment (root-cells, mucilage, root exudates, soluble lysates and volatile
305 compounds), responsible for shaping the rhizosphere microbiota [18]

306 **Rhizosphere**: portion of soil surrounding the root system, greatly influenced by the plant
307 notably *via* its rhizodeposits. Microorganisms living in this habitat vary from those living
308 on the rhizoplane (root surface) or in the endosphere (inside plant tissues).

309 **RNA interference (RNAi)**: Eukaryotic process of gene silencing, at the post-
310 transcriptional level, by small non-coding RNAs (siRNA, miRNA and piRNA).
311 Prokaryotes have similar mechanisms such as microRNA-like regulatory systems and
312 CRISPR-Associated System [64]

313 **Root exudates**: a class of rhizodeposits secreted by plants in the meristematic region of
314 root tips, such as sugars and amino acids, but also organic acids, nucleotides, peptides,
315 fatty acids and secondary metabolites. The composition of root exudates depends on
316 plant genotype, its developmental stage and environmental conditions [3].

Selection: result of biotic and abiotic influence on the reproductive success (i.e. fitness) of an individual or species. This is the most studied process regarding microbial communities composition differences, due to plant exudates or environment, whereas the study of drift, dispersal and speciation in the microbial field is more complex to approach, if even possible [20].

Speciation: evolutionary process through which novel species form

Substrate: nutritious substance used by microorganisms to grow and feed on to meet their nutritional requirements

Box 1. An overview of plant and microbial small RNAs

Plants produce a wide variety of small RNAs (sRNAs), each capable of regulating gene expression through different silencing pathways. These 21 to 24-nucleotide RNAs originate from hairpin precursors or double-stranded RNA (dsRNA) which are processed by an RNase III Dicer-like protein (DCL) forming sRNA duplexes, with 2'-O-methylation at 3' ends, which in turn are loaded into Argonaute proteins (AGO) in order to function and repress expression of target RNAs of complementary sequence [65]. Plant sRNAs encompass small-interfering RNAs (siRNAs) and microRNAs (miRNAs).

SiRNAs transcripts are converted by an RNA-Dependent RNA Polymerase (RDRP) into long dsRNAs, which are then processed by DCL proteins into a siRNA duplex. Plant siRNAs are categorized into secondary siRNAs and heterochromatic siRNAs (hetsiRNAs). They are involved in post-transcriptional gene silencing (PTGS), but mostly in RNA-directed DNA methylation (RdDM) and transcriptional gene silencing (TGS) [65].

339 MicroRNAs encoded by MIR genes are transcribed by RNA polymerase II (Pol II) into a
340 hairpin structure, the primary-miRNA (pri-miRNA). A DCL protein then processes pri-
341 miRNA into a smaller stem-loop structure, precursor-miRNA (pre-miRNA), which is
342 processed again by DCL protein forming a mature miRNA duplex (Figure 1). Once
343 loaded into AGO proteins, forming the multi-protein RNA-Induced Silencing Complex
344 (RISC), miRNAs can then repress target gene expression by mRNA cleavage or
345 translation inhibition [65].

346 Together, plant small RNAs, especially microRNAs, are involved in many regulatory
347 processes and play essential roles in plant development and resilience to stresses. The
348 conservation of microRNA or microRNA-like RNAs and their mechanisms across the
349 branches of life suggests their importance for many organisms and their potential role as
350 mediator of cross-kingdom communication.

351 For instance, in the rhizosphere, the plant interacts with diverse microorganisms,
352 capable of producing microRNA-like RNAs. Similarly to eukaryotic microRNA, bacterial
353 ~22nt RNA fragments, namely msRNA, obtained from hairpin structures have been
354 discovered [37,38,66] (Figure 1), but their function remains unknown, even though their
355 abundance suggests an important functional role. Aside msRNAs, bacteria possess a
356 variety of small RNAs, mainly characterized as trans-acting sRNA and cis-acting sRNA,
357 but many new bacterial small regulatory RNAs are to be defined [67].

358 Fungi are eukaryotic organisms and possess diverse small RNAs, however it is still
359 under debate if they produce microRNAs. Most studies have demonstrated the presence
360 of microRNA-like RNA (miRNA) in fungi, which are not produced by the same

biogenesis pathway as real microRNAs (Figure I) [33,68]. Interestingly, the evolutionarily ancient lineage of oomycetes also possesses microRNAs [36,69].

Box 2. Plant-microbe interactions under the hologenome theory

The hologenome theory states that the combined genomes of the holobiont (e.g. the plant genome and the associated microbial genomes), is one of the biological units subjected to natural selection [21]. This concept is mainly based on: (i) all animals and plants are associated with a diversity of microbes, (ii) a proportion of these microbes are transmitted between holobiont generations, (iii) host-microbiota interactions impact the holobiont fitness [70]. This theory could explain the speed at which genetic variation happens in holobionts: the microbiota evolves at a fast rate due to short generation times, horizontal gene transfer and arrival of new microbes in the community [21]. The strong bonds established between a host and its microbiota, combined with the power of microbial evolution, must have great consequences on co-evolution and macroevolution mechanisms in the holobiont. In the case of the plant holobiont, co-evolution between plant and microbes suggests the existence of cross-kingdom bi-directional communication. Regarding the diversity and dynamics of the rhizospheric microbiota, it is probable that the host plant co-evolves with only a portion of its microbiota, namely the core microbiota, which is often characterized as stable and transmittable to offspring. However, these two-way interactions allow regulation of the hologenome, by comprising the essential roles of both plant and microbes in holobiont fitness.

Host	Interacting organism	Small RNA mobility	Small RNA exchanged	Small RNA vehicle	Targeted genes	Effect	Reference
Human & Mice	Gut microbiota (<i>F. nucleatum</i> , <i>E.coli</i> ...)	From mammal host to bacteria	microRNA	EVs	dnaK operon, 16S rRNA and rpoB (in <i>Fn</i>) & RNase P,	Regulation of bacterial growth	[7]

384

385 **Table 1. Examples of cross-kingdom communication through small RNAs**

					rutA, yegH and fucO (in <i>E. coli</i>)		
Human & Mice	Gut microbiota	From mammal host to bacteria	microRNA	EVs	β -galactosidase AMUC_RS06985	Upregulation of target gene, enrichment of <i>Akkermansia muciniphila</i> , increasing regulatory T cells & ameliorating multiple sclerosis	[8]
Ginger (<i>Zingiber officinale</i>)	Gut microbiota (<i>Lactobacillaceae</i>)	From plant to bacteria	microRNA	Exosome-Like Nanoparticles	Monooxygenase ycnE	Regulation of bacterial growth & increase in indole-3- carboxaldehyde (I3A)	[9]
Ginger, grapefruit (<i>Citrus paradisii</i>), carrot (<i>Daucus carota</i>), grape (<i>Vitis vinifera</i>)	Mice gut cells	From plant to mice	microRNA	Exosome-Like Nanoparticles	?	Induction of anti- inflammation genes expression	[48]
Soybean (<i>Glycine max</i>)	Rhizobia bacteria	From bacteria to plant	tRNA-derived small RNA fragments (tRFs)	?	Orthologs of <i>Arabidopsis</i> genes involved in root hair/plant development	Positive regulation of rhizobial infection and nodulation formation in soybean	[71]
<i>Arabidopsis thaliana</i>	<i>Botrytis cinerea</i>	From plant to fungal pathogen	sRNA	EVs	Vesicles-trafficking pathway, vacuolar protein sorting	Decrease in fungal virulence	[72]
Cotton plant (<i>Gossypium sp.</i>)	<i>Verticillium dahliae</i>	From plant to fungal pathogen	microRNA	?	Virulence genes: Ca ²⁺ -dependent cysteine protease & an isotrichodermin C-15 hydroxylase	Silencing fungal virulence genes	[12]
Wheat (<i>Triticum aestivum</i>)	<i>Puccinia striiformis f. sp. tritici (Pst)</i>	From fungal pathogen to plant	microRNA-like RNA (milRNA)	?	Pathogenesis- related 2 (PR2)	Suppression of plant defenses	[14]
<i>Arabidopsis thaliana</i> & <i>Nicotiana benthamiana</i>	Parasitic plant <i>Cuscuta campestris</i>	From parasitic plant to host plant	microRNA	?	TIR1, AFB2 and AFB3 (auxin receptors), BIK1 (kinase receptor), SEOR1 (phloem protein) and HSFB4 (transcriptional repressor)	mRNA cleavage & secondary siRNA production	[47]

386

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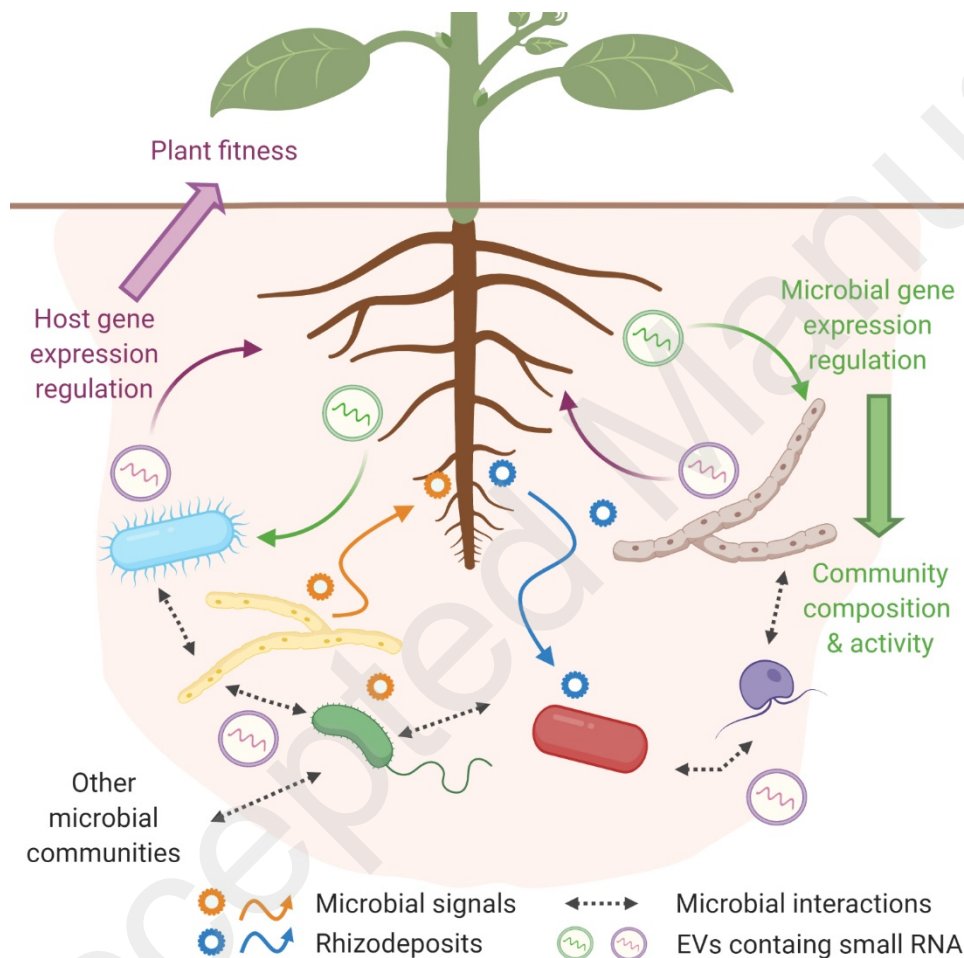
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Key figure

Model of cross-kingdom interactions in the rhizosphere through exchanges of extracellular vesicles, containing small RNA

Figure 1. A suggested representation of plant-microbe interactions in the rhizosphere: the selective power of the host plant is demonstrated through the rhizodeposits (in blue) which play a great part in shaping the microbiota. Rhizodeposits are diverse in form and function, they allow signalling with other organisms and their environment. Microbe development in the rhizosphere depends on these compounds and is somewhat regulated by them, as microbes feed preferentially on certain rhizodeposits as substrates. In turn, microbes produce a variety of signalling molecules (in orange) such as volatile organic compounds, phytohormones, quorum-sensing molecules, etc.... which are perceived by the plant. We suggest that plants and microbes also communicate *via* extracellular vesicles (EVs) which contain small RNAs, and notably microRNAs or microRNA-like RNAs. Host plant EVs containing microRNA (in green) can modulate microbial gene expression to its advantage by regulating community composition and activity (bold green arrow, representing the effect of microRNA-mediated regulation). In return, microbes can also regulate host plant gene expression through their EVs containing microRNA (in purple), thus controlling their close environment and plant fitness (bold purple arrow). Additionally, the rhizospheric microbiota members also interact between themselves and with other microbial communities (black dashed arrows), potentially through EVs containing small RNAs. Not to scale.

Outstanding questions

- Are rhizospheric bacteria and fungi able to incorporate plant microRNAs, conveyed or not by extracellular vesicles? If so, can microbial genes be modulated by plant microRNAs? What genes are targeted? To what effect?

- Are members of the rhizospheric microbiota capable of producing extracellular vesicles? If so, do they contain small RNAs? microRNAs? microRNA-like RNAs? To who are destined these vesicles: the host plant? other members of the community?
- As microRNA-size small RNAs exist in bacteria, what are the bacterial mechanisms of RNA interference, aside from the CRISPR-mediated system?
- To which extent can plant/microbial microRNAs play a role in shaping the rhizospheric microbiota and its associated functions? What role do plant microRNA play in microbial transcriptomics? Can they affect community activity and/or composition?
- Do the secreted microRNAs vary depending on environmental conditions, such as abiotic stresses, in order to modulate accordingly the surrounding organisms? What are the selective mechanisms involved in microRNAs sorting in extracellular vesicles?
- How can we use this potential novel interkingdom communication channel to develop holobiont engineering tools, in the future, such as miPEPs? How can these modifications be durable and what are the implications for long-term use?

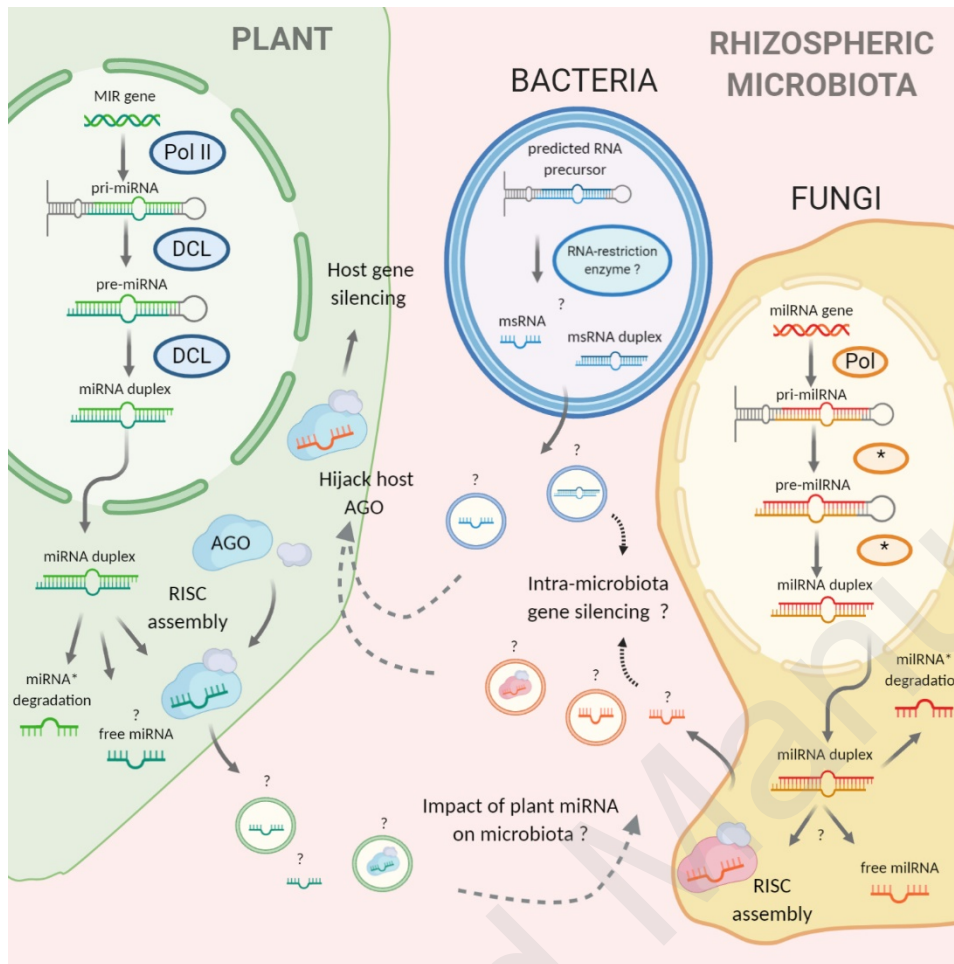


Figure from Box 1

Figure I. A comparison of microRNA and microRNA-like RNA biogenesis in plants and microbes. In this figure, microRNA production and interspecies exchanges (dashed arrows), in the rhizosphere, are compared. The biosynthetic pathway of plant microRNAs has been extensively studied and is mainly composed of DCL and AGO proteins. Fungi produce milRNA through at least four different mechanisms [33]: after transcription of milRNA gene, the processing of pri-milRNA into mature milRNA involves a combination of enzymes (represented as *) such as Dicer, QDE-2 (an Argonaute protein), MRPL3 (an RNase III) and the exonuclease QIP. Interestingly, there is a Dicer-independent microRNA biogenesis pathway, which opens up an opportunity to consider

microRNA production in bacteria and archaea which lack Dicer enzymes, but do have Argonaute-like proteins [33]. Details on bacterial msRNAs biogenesis are still to be discovered, however multiple studies have demonstrated the presence of msRNAs in different bacterial strains and have predicted similar hairpin RNA precursors, validated by both msRNA and msRNA*. It is suggested that an RNA-restriction enzyme, such as MazF found in *E. coli*, may be involved in msRNA processing [38]. Once the mature microRNA is synthesized, many questions remain unanswered as to their movement and function between species: are they transported in EVs? in OMVs? Do they travel bound to an AGO protein or freely? What is the role of plant microRNAs on the microbiota? What microbial genes are targeted and for what purpose? How does this regulation shape the microbiota? In return, fungal small RNAs have been shown to use plant AGO proteins in order to silence host genes, however it is unknown if bacteria use this mechanism.