CellPress

Opinion

Let the Core Microbiota Be Functional

Philippe Lemanceau,[1,*,@] Manuel Blouin,[1] Daniel Muller,[2] and Yvan Moënne-Loccoz[2]

The microbial community that is systematically associated with a given host plant is called the core microbiota. The definition of the core microbiota was so far based on its taxonomic composition, but we argue that it should also be based on its functions. This so-called functional core microbiota encompasses microbial vehicles carrying replicators (genes) with essential functions for holobiont (i.e., plant plus microbiota) fitness. It builds up from enhanced horizontal transfers of replicators as well as from ecological enrichment of their vehicles. The transmission pathways of this functional core microbiota vary over plant generations according to environmental constraints and its added value for holobiont fitness.

Trends

Recent advances in next-generation sequencing technology have boosted the field of plant–microorganism interactions, especially in rhizosphere ecology.

The principle of a core microbiota has been proposed to describe the microbial community that is systematically associated with a given plant genotype.

So far, this core microbiota was mostly defined on the basis of DNA sequences with taxonomic value, and not on their functional relevance.

However, biogeography studies suggest that microbial reservoirs may differ depending upon soil types, thus questioning the universal distribution of the taxonomic core microbiota under various environmental conditions.

The microbiota recruited by a given plant genotype in different environments seems to share greater functional similarity than taxonomic similarity.

Plants–Microorganisms: A Joined Success Story

Land colonization by plants has been a formidable undertaking that was made possible with the help of friendly soil mutualistic microbes. Plants sustain their microbial partners by providing them with organic carbon that is accessible directly inside plants or released by the roots in the form of rhizodeposits [1,2]. This leads to the stimulation of a range of microbial functions that modulate hormone balance, interfere with plant and microbe communication, enhance plant mineral nutrient and water uptake, promote plant growth, and suppress soilborne diseases (Table 1). These microbial functions represent a key asset to the plant because individual plants cannot migrate when environmental conditions shift away from the optimum. Therefore it came as no surprise that plant roots have developed strategies aimed at selecting useful microbial communities whose features depend on the genetic and physiological traits of the host plant but also on the local soil microbial reservoir. This implies that the microbial communities that roots recruit from a given soil microbial reservoir or **microbiome** (see Glossary) can vary among plant genotypes, and indeed each plant genotype teams up with a core range of microbial partners. So far, this core **microbiota** has been defined on the basis of taxonomic markers. However, as stressed by Violle *et al.* [3], when considering ecological traits, functional issues need to receive prime consideration if we are to understand the ecology of the core microbiota and to exploit its potential for plant growth and health. In this paper we argue that the **functional core microbiota** is in fact a basic component of plant functioning and success, and therefore should be given first priority.

The Taxonomic Core Microbiota

Plants are colonized by and interact with a high diversity of microorganisms. This microbiota influences the physiology, growth, and health of the host plant to such an extent that plants may form with their associated microbiota single entities, termed **holobionts**, in which plants and their microbial partners contribute positively or negatively to the overall stability and **fitness** of the system [4,5]. Recent studies have defined a core range of species (or proxies such as operational taxonomic units, OTUs) of the microbiota associated with a particular plant species or variety. In their seminal papers, Ludenberg *et al.* [6] and Bulgarelli *et al.* [7] concluded from

[1]Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, 21000 Dijon, France
[2]UMR Ecologie Microbienne, CNRS, INRA, VetAgro Sup, UCBL, Université de Lyon, 69622 Villeurbanne, France
[@]Twitter: @PLEMANCEAU

*Correspondence: philippe.lemanceau@dijon.inra.fr (P. Lemanceau).

deep-sequencing analyses of 16S rRNA gene diversity in *Arabidopsis thaliana* roots that there is a typical core 'microbiome' recruited on the basis of the ability of community members to metabolize root exudates. In the present paper the above referred microbiome defined on the basis of taxonomic markers is defined as the **taxonomic core microbiota**. This focus stemmed from methodological advances in the characterization of microbial diversity, whose newly available tools mainly relied on large-scale analyses of the polymorphism of target taxonomical sequences. Nevertheless, the level of resolution achieved for taxonomic microbiota analysis has led to the identification of classes that are commonly associated with plants (Proteobacteria, Actinobacteria, Firmicutes, and Bacteriodes [8]), whereas a taxonomic classification at the lower (genus or species) hierarchy levels might reveal distinct microbiota for a given plant genotype in different environments. This represented a major progress for microbial ecology studies but did not address the integration of functional issues and their implications in terms of partner evolution, metabolic investment in the interactions, and fitness. Moreover, in contrast to Beijerinck [9], biogeographic studies point out that everything is not everywhere [10]. Thus the microbial reservoir in which plants recruit their partners may differ, resulting in variations in their taxonomic core microbiota according to the soil. This implies that a new strategy should be implemented for the molecular characterization of the core microbiota, and which should not be solely based on taxonomic markers but should also be based on functional traits recruited by plants among microbial taxa, even though the latter may differ between soils.

The Functional Core Microbiota

It can be argued that plant–microorganism evolution favors the recruitment of microbial populations, possibly belonging to different taxa according to the soil type, but sharing the ability to ensure favorable functions for the host plant. In this case the focus is no longer on the microbial taxa recruited by the host plant, but rather on the microorganisms that ensure key functions for holobiont fitness, especially by promoting the nutrition (mineral nutrients for plants, exudates for microorganisms) and health of the components of the holobiont. These functions result from activities coded by genes (**replicators**) within organisms considered as **vehicles**, after Dawkins' famous assumption [11]. We therefore argue for the key importance of a functional core microbiota – which can be defined as a whole set of microbial vehicles, including replicators coding for essential functions for holobiont fitness. More specifically, a given replicator may be distributed across different microbial genetic backgrounds according to soil microbial reservoirs. Within a given reservoir, this replicator may be represented across a range of vehicles. The resulting functional redundancy [12] ensures that functions are maintained despite environmental fluctuations [13]. The delineation of the functional core microbiota will depend on the physiology and ecology of the plant associated with the core microbiota. Thus, closely related plant genotypes would recruit complementary functions of microbial origin to complement their own functions, and indeed targeted genetic modification of a plant variety modified the range of microbial partners (and the corresponding metabolic functions) that were recruited [14]. Consequently, the functional core microbiota is expected to exhibit a greater degree of similarity when plants are phylogenetically close. However, this remains to be investigated. First results on this issue have not confirmed the hypothesis, but only one microbial function was considered [15]. We further argue that the functional core microbiota will be associated to these plants irrespective of the soil provided that its replicators are present within the rhizosphere microbial community, even when the vehicles harboring them differ among soils. Such decoupling between phylogeny and function, which at the scale of a given taxa is likely to concern pangenomic genes that mostly do not belong to the core genome, has been evidenced for leaf-associated microbiota [16].

The functional core microbiota could ensue from various evolutionary scenarios (Figure 1A–D) in which the evolutionary pathways of plants and microorganisms are more or less tightly connected. The greatest degree of connection (Figure 1D) corresponds to the holobiont [4]

Glossary

Fitness: survival and reproductive success of an individual in a given environment.
Functional core microbiota: a subset of the microbiota associated with a given host irrespective of the macroenvironment (e.g., soil type) and that encompasses microbial vehicles carrying replicators with essential functions for holobiont fitness (Table 1).
Holobiont: an entity encompassing an individual host (e.g., a plant) and its associated microbial community (i.e., the microbiota). On the basis of its properties, the holobiont may be assimilated into a superorganism.
Microbiome: an adaptation to microorganisms of the ecological concept of biome, the latter being defined as a set of biotic communities typical of a biogeographical area and named on the basis of plant (and animal) taxa emblematic of this area. On this basis, a microbiome encompasses the microbiota present in a given environment (e.g., in the rhizosphere).
Microbiota: the ecological community of commensal, symbiotic, and pathogenic microorganisms as characterized by their abundance, taxonomic diversity, and functional diversity.
Replicator: an entity (e.g., a DNA sequence) able to reproduce itself exactly [8], thus representing a fundamental unit of natural selection, the basic unit that survives or fails to survive.
Superorganism: a multispecies community that functions as an organizational unit [17,18]. This unit of functional organization [17] corresponds to a unit whose subsets have stronger and more numerous interactions with one another than with the environment of the system.
Taxonomic core microbiota: a subset of the microbiota associated with a given host irrespective of the macroenvironment (e.g., soil type), as characterized by taxonomic markers (e.g., 16S rRNA sequences).
Vehicle: an entity (i.e., an individual organism, or group of organisms) that carries replicators. In contrast to replicators, vehicles do not reproduce themselves exactly, but they represent the phenotypic level at which selection occurs. Vehicles that are more successful than others in ensuring the survival of their replicators are selected preferentially by the plant.

Table 1. Microbial Functional Traits Promoting Plant Fitness, and the Corresponding Genes (Replicators) and Microorganisms (Vehicles)

Functional traits		Replicators (corresponding gene(s) and/or operons)	Vehicles (microorganisms carrying the corresponding replicators)	Refs
Plant hormone balance				
Auxins	Indole-3-acetic acid	*ppdC/ipdC/iaa*	*Agrobacterium, Rhizobium, Bradyrhizobium, Azospirillum, Pseudomonas, Pantoea, Enterobacter cloacae, Bacillus, Paenibacillus*, etc.	[49]
	Auxin catabolism	*iac* cluster	*Pseudomonas putida*	[49]
	Phenylacetic acid catabolism	*paaCYBDFGHIJKWLN*	Various Proteobacteria, Actinobacteria, Firmicutes, some Bacteroidetes, Chlorobi and *Deinococcus–Thermus*	[50,51]
Gibberrelin synthesis		*cyp*	Various bacteria (*Azospirillum, Rhizobium, Acetobacter diazotrophicus, Herbaspirillum*) and fungi (*Fusarium, Gibberella*)	[52,53]
Cytokinin synthesis		Not characterized	*Bacillus*	[54,55]
Coronatine synthesis (jasmonate mimic)		*cfa*	*Pseudomonas synringae, Streptomyces scabies*	[56]
Ethylene synthesis		*efe*	Various bacteria (*Azospirillum, Pseudomonas*, etc.), and fungi (*Penicillium*, etc.)	[57]
1-Aminocyclopropane-1-carboxylate (ACC) deaminase (regulation of ethylene synthesis)		*acdS*	Various bacteria and fungi	[58,59]
Nitric oxide synthesis		*nirK or nirS*	Various bacteria and Archeae	[60]
Acetoine/2,3-butanediol synthesis		*budABC*	*Bacillus, Paenibacillus, Rhizobium*, Enterobacteriaceae	[32,61]
Salicylic acid synthesis		*ics* and *ipl*	*Pseudomonas, Azospirillum, Mycobacterium, Paracoccus, Vibrio*, etc.	[62]
γ-Aminobutyric acid (GABA) catabolism		*gabT* and *gabD*	Various bacteria and fungi	[63]
Abscisic acid (ABA) synthesis		*bcaba*	Various bacteria and fungi	[64]
Molecular communication				
Type-3 secretion systems promoting mycorrhizal symbiosis		*hrc* or *rsc*	Several fluorescent *Pseudomonas* spp.	[65,66]
Pathogen inhibition		*hrc*	*Pseudomonas fluorescens*	[67]

Table 1. (continued)

Functional traits		Replicators (corresponding gene(s) and/or operons)	Vehicles (microorganisms carrying the corresponding replicators)	Refs
Lactonase mediating quorum-quenching leading to disease suppression		*aiiA*	*Pectobacterium carotovorum*	[68]
Plant nutrition				
Phosphate solubilization	Pyrroloquinoline quinone	*pqqBCDEFG*	Various Proteobacteria	[32,69]
	Gluconate (or citrate) synthesis	*gcd*	γ-Proteobacteria	[69]
	Phytase	*phyB*	Various bacteria and fungi	[70]
Nitrogen fixation		*nifDHK*	Various bacteria and Archeae	[15]
Nitrification		*amoCAB*	Some Proteobacteria, Thaumarchaeota, Nitrospira, and *Nitrospira*	[71]
Iron nutrition	Pyoverdines	*pvd*	Fluorescent *Pseudomonas* spp.	[72,73]
	Other siderophores	Non-ribosomal peptide synthetase genes	Various bacteria and fungi	[74]
Adaptation of plants to drought				
Trehalose synthesis		*ostAB*	*Rhizobium etli*	[75]
Polysaccharide synthesis		EPS	*Rhizobium* sp.	[76]
Disease suppression/pathogen inhibition				
2,4-Diacetylphloroglucinol synthesis		*phl*	Several fluorescent *Pseudomonas* spp.	[77]
Hydrogen cyanide synthesis		*hcnABC*	Various Proteobacteria	[32]
Phenazine synthesis		*phz*	*Pseudomonas chlororaphis*	[78,79]
Pyrrolnitrin synthesis		*prn*	*Pseudomonas chlororaphis*	[78,79]
Pyoluteorin synthesis		*plt*	*Pseudomonas protegens*	[78,79]
2-Hexyl-5-propyl-alkylresorcinol synthesis		*dar*	*Pseudomonas fluorescens*	[80]
Pyoverdine-mediated iron competition		*pvd*	Several fluorescent *Pseudomonas* spp.	[81]
Iturins		*itu*	*Bacillus* species	[82]
Fengycins		*fen*	*Bacillus* species	[83]
Surfactins		*srf*	*Bacillus* species	[84]
Orfamide		*orf*	*Pseudomonas* species	[85]
Viscosin		*vis*	*Pseudomonas* species	[86]
Massetolide		*massABC*	*Pseudomonas* species	[84]

Table 1. (continued)

Functional traits		Replicators (corresponding gene(s) and/or operons)	Vehicles (microorganisms carrying the corresponding replicators)	Refs
Disease suppression/plant defense				
Lipopolysaccharides		LPS	Various bacteria (pathogens and PGPRs)	[86]
Flagelline		*flg*	Various bacteria (pathogens and PGPRs)	[87,88]
peroxidase and α-dioxygenase		*epl1* and *sm1*	*Trichoderma*	[89]
2,4-Diacetylphloroglucinol (DAPG)		*phl*	*Pseudomonas protegens*	[90]
Siderophores	Pyoverdines	*psb, pvd*	*Pseudomonas putida*	[90,91]
		pvd	*Pseudomonas protegens*	[92]
	Pyochelin and pyocyanin	*pch-phz*	*Pseudomonas aeruginosa*	[93]

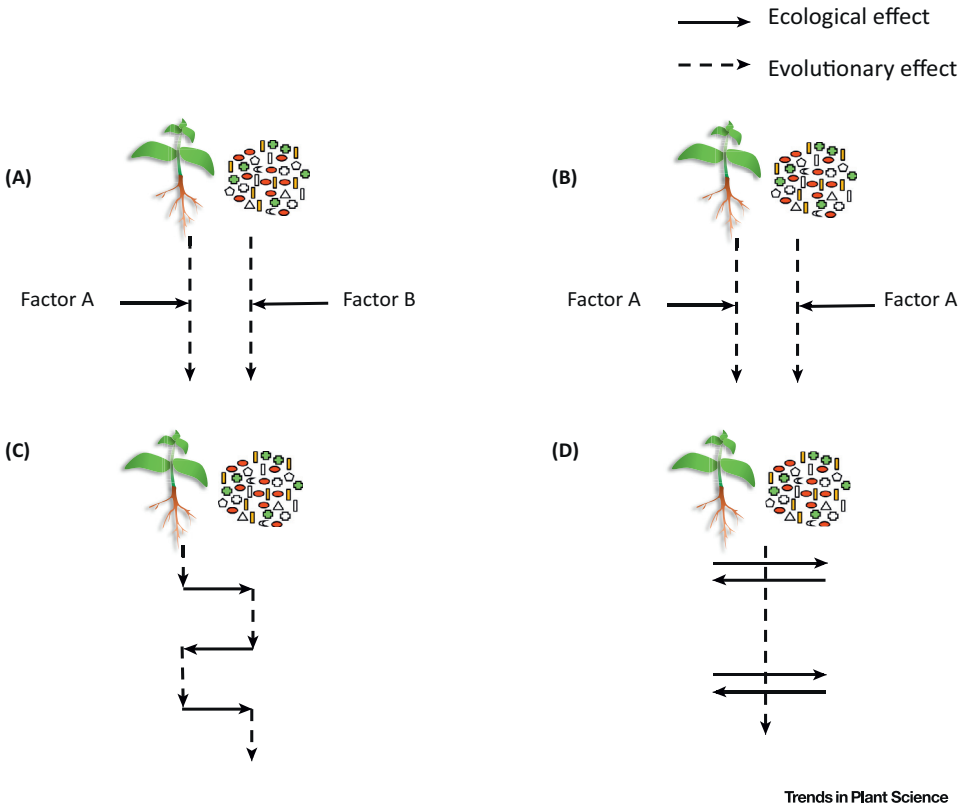

Figure 1. Different Scenarios of Evolutionary Trajectories of Plants and Microbiota. (A) Independent evolution of the two components in response to distinct environmental factors. (B) Parallel evolution of the two components in response to the same environmental factor. (C) Evolution of each component as a function of the response of the other component (coevolution *sensu stricto*). (D) Evolution of the interactions between the two components (i.e., evolution of the holobiont). Scenarios (C) and (D) lead to covariation of the two components (coevolution *sensu lato*), resulting in the emergence of a functional core microbiota. Different scenarios may occur simultaneously. The microbiota is represented schematically by different items corresponding to individuals; different shapes correspond to different populations, different colors correspond to different replicators, and colored items are vehicles carrying the corresponding replicators. Dashed lines represent evolutionary effects; plain lines represent ecological effects.

described as a **superorganism** [17], 'the superorganism being a multispecies community that functions as an organizational unit' [18]. However, evolutionary pathways may have run in parallel in response to different environmental selective constraints (Figure 1A).

These evolutionary processes may not be favorable and may lead to deleterious interactions (Figure 2). Nevertheless, we hypothesize that positive feedback loops increase the survival and reproduction (i.e., the fitness) of the plant and its associated microorganisms (Figure 2B) under selection pressure. Natural selection leads not only to the dissemination of holobionts that benefit from positive feedback loops but also to the regression of holobionts that suffer from negative feedback loops (Figure 2B,C). Based on these evolutionary processes, functional core microbiota are believed to be beneficial to plants overall.

The vital functions of plants evolve together with their phenological stages, therefore the functional microbiota is bound to change over time too. This hypothesis is supported by variations in rhizodeposit composition over time (according to plant phenology) and space (according to root zones) [1], as indicated by changes in the taxonomic microbiota [19,20].

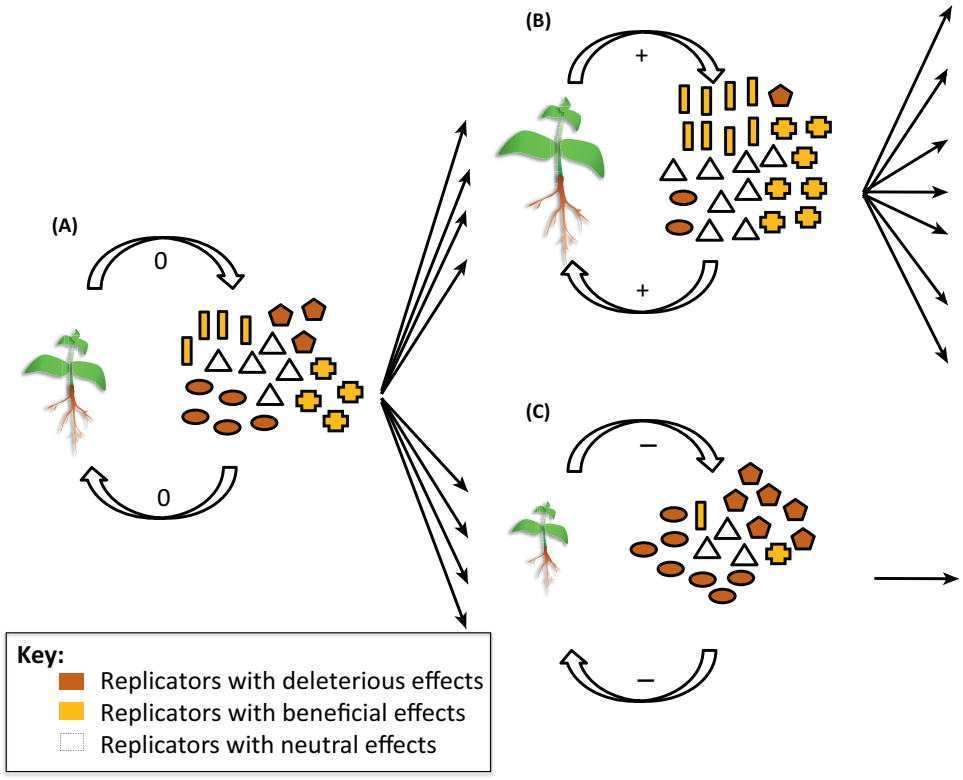

Figure 2. Schematic Representation of Natural Selection of Positive Feedback Loops Between Plants and Functional Core Microbiota. The functional core microbiota encompasses genes (replicators) with positive, neutral, or negative effects on plant growth and health. (A) When replicators with positive and negative effects are present in the same proportions, their net effects on the plant and on the plant feedback are null. Depending on possible mutations in the microbiota, new feedback loops may develop as follows: (B) when positive feedback loops develop, plant growth and health are promoted, resulting in an increased microbiota-carrying capacity of the rhizosphere; holobiont fitness increases, leading to its progressive spread; (C) when negative feedback loops develop, plant growth and health are depressed, resulting in a reduced microbiota-carrying capacity of the rhizosphere; holobiont fitness decreases, leading to its progressive elimination. The microbiota is represented schematically by different items corresponding to individuals; different shapes correspond to different populations, different colors correspond to different replicators, and colored items are vehicles carrying the corresponding replicators.

Rationale for a Functional Core Microbiota

The presence of these host-associated microbial replicators coding for shared functions while being distributed among different genetic backgrounds (i.e., vehicles) has been illustrated recently. Bulgarelli *et al.* [21] showed that the host genotype (*Hordeum vulgare*) had little impact on microbial taxonomical sequences, defining here the taxonomic core microbiota, but had a great impact on sequences with functional value, defined here as the replicators of the functional core microbiota. Similarly, the microbial populations associated with different samples of the alga *Ulva australis* only displayed 15% taxonomic similarity versus 70% functional similarity [22]. Ofek-Lalzar *et al.* [23] reported the presence of a core set of functional genes associated with microbial colonization of the roots of two plant species (*Triticum turgidum* and *Cucumis sativus*) grown in the same soil. The identified replicator sequences coded for functions mainly related to microorganism physiology that were likely implicated in interactions with plants such as host colonization or regulation of virulence [22,23]. In addition, Louca *et al.* [16] reported that, among 22 bromeliads, a remarkably similar functional community structure was observed, whereas the taxonomic composition within individual functional groups (i.e., fermenters, aerobic chemoheterotrophs) was highly variable. The variability of the taxonomic

composition within a given functional group is not specific to plant microbiota, and is also found in human microbiota [24]. The natural selection of a functional community through enrichment in populations carrying replicators beneficial for the host plant is nicely illustrated by the well-known decline of take-all disease of wheat, caused by the soilborne fungus *Gaeumannomyces graminis tritici* (Ggt). Take-all decline is observed following repeated cropping of the host plant in the presence of Ggt. Successive crops allow the build-up of a functional microbial community composed of fluorescent pseudomonads belonging to different species. These bacteria carry genes (replicators) coding for a class of antimicrobials (2,4-diacetylphloroglucinol) that reduce the saprophytic growth of Ggt and thereby the severity of the disease [25,26]. It should be noted that this phenomenon has been described in very different soils because replicators can be carried by different vehicles depending on the taxonomic diversity of the resident *Pseudomonas* populations that may vary depending upon the soil [27]. Table 1 lists other examples of functional traits implicated in plant hormonal balance, molecular communication, plant nutrition, drought tolerance and phytoprotection, together with the corresponding replicators and the microbial vehicles carrying them. This table illustrates how a given replicator may be present in different genetic backgrounds.

The principle that natural selection operating on a functional microbial community impacts on the plant is also supported by the results of artificial selection experiments during which microbial communities with special functions were transferred to successive generations of plants. The principle of the selection of microbial communities over successive generations was validated in the absence of plants [28]. In the presence of plants, host traits such as plant shoot biomass [29] and flowering time [30] were also altered via artificial selection of rhizosphere microbial communities.

In addition to this gradual promotion of microbial populations bearing specific functions within the rhizosphere or root community (Figure 2A), other pathways may be hypothesized. Within a given microbial population, plants may favor the frequency of specific alleles that are the most beneficial (Figure 3B) [15] through natural selection over successive microbial generations. Lastly, microbial replicators could be disseminated in various vehicles via horizontal gene transfer (HGT) between different species [31]. A recent analysis of genes contributing to phytobeneficial functions indeed suggests that HGT may have played an important role in the emergence of plant growth-promoting rhizobacteria (PGPR) [32], which are key members of the plant microbiota. For instance, *Azospirillum* PGPR evolved from an aquatic niche to a rhizosphere lifestyle by horizontal acquisition of genes from diverse soils and plant-associated bacteria [33], pointing to HGT of microbial functions within and even between holobionts. Plants may promote HGT of conjugative plasmids [34] and symbiosis islands among bacterial taxa [35], which mediate rhizosphere adaptation [36] via their rhizodeposits (Figure 2C).

By analogy to superorganisms, such as bee colonies, whose components are coordinated by molecular signaling intended for the well-being of the whole colony, we propose that this type of regulation similarly coordinates the components of the holobiont, in other words plants plus functional core microbiota. Thus, molecular mediators may impact on both the host physiology (e.g., nutrition, development, immunity) and the associated microbiota [37]. It is interesting to note that the hormone salicylic acid implicated in plant defense mechanisms has recently been shown to have an additional effect – a modification of the structure of the rhizosphere microbiota – which might also be significant in terms of plant health [38]. A better knowledge of the complex signaling that regulates the selection of microbial populations bearing replicators deserves particular attention. This raises questions about the regulation that leads to enrichment for populations of antimicrobial-producing *Pseudomonas* in Ggt-infected necrotized roots but not in healthy roots; perhaps this entails signaling phenomena in addition to trophic effects from metabolites released by damaged roots.

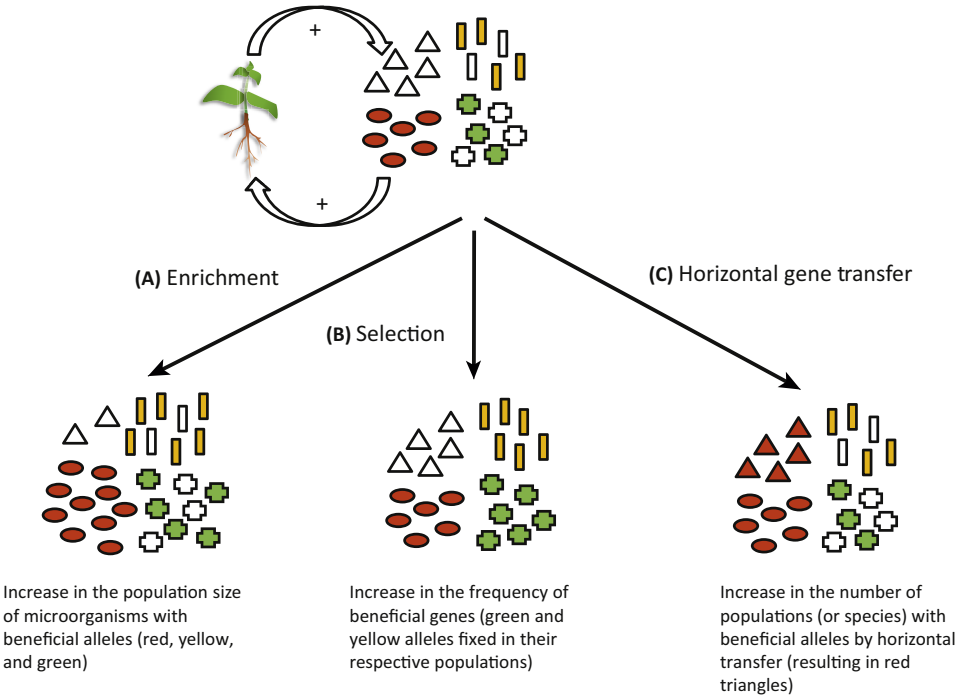

Figure 3. Schematic Representation of the Mechanisms Involved in the Recruitment by Plants of Beneficial Replicators for Holobiont Fitness. Plants may promote beneficial replicators within the functional core microbiota through: (A) enrichment, by increasing the size of the microbial populations (vehicles) carrying beneficial replicators, (B) selection, by increasing the frequency of beneficial alleles within a given population, and (C) horizontal gene transfer, by promoting the transfer of beneficial replicators among populations. The microbiota is represented schematically by different items corresponding to individuals, with different shapes corresponding to different populations; the different colors (green, red, and yellow) correspond to replicators that are carried by different vehicles (colored shapes).

Transmission and Stability of the Functional Core Microbiota

The fact that similar microbial functional groups are faithfully associated with individuals in various taxa (*Ulva* [22], bromeliads [16], humans [24]) suggests that the functional core microbiota recruited by a given host is somehow stable over time. This stability ensues from the genetic transmission of replicators but also from non-genetic environmental transmission. This hypothesis is in line with the 'extended synthesis of evolution' theory based on the concept of inclusive inheritance [39] which integrates genetic and non-genetic heredity [40]. Non-genetic heredity includes transgenerational epigenetic inheritance, somatic inheritance, and ecological inheritance [39–41]. Danchin *et al.* [39] proposed the existence of a link between environmental stability and the mode of transmission of information, whether genetic or not. Thus, a piece of information that changes rapidly relative to the generation time of a given organism will not be passed on, but if the same piece of information varies more slowly, it could be passed on via the environment, parental effects, epigenetics, or the genes involved in the most stable pieces of information. In that case, these pieces of information will be passed on in a quasi-irreversible manner (Figure 3).

The functional core microbiota represents an environmental component for plants, and vice versa. It can be inherited vertically through successive generations via seeds [42], or horizontally via the environment (i.e., via the soil microbial reservoir shared by parents and their descendants), as in the case of take-all decline. Vertical transmission corresponds to 'parental-effect' heredity, while horizontal transmission corresponds to 'ecological inheritance' transmission. If endophytes turned out to be transmissible via the cytoplasm of plant gametes, conditions would be

intermediate between parental heredity (via seeds) and cytoplasmic heredity via chloroplasts and mitochondria (Figure 3). In addition to this adaptationist explanation, we cannot rule out the possibility that some plant–microorganism associations result from genetic or ecological drift.

Relevance of the Functional Core Microbiota for Agroecological Systems

Promotion of biodiversity and biotic interactions represents a major tool in agroecology for developing agricultural systems that will be more sustainable and less dependent on chemical inputs [43]. In this context, acquiring better knowledge about the functional core microbiota and ways to promote it is essential for the development of agroecological systems. This is a major paradigm shift because plant domestication may *in contrario* have affected the ability of plants to establish beneficial associations with soil microorganisms [44]. More particularly, during the green revolution the selection of useful genotypes for crop productivity was usually conducted under fertile conditions, and reduced exposure to soilborne pathogens may have selected against plant traits that are essential for hosting and sustaining beneficial microorganisms [45]. This is supported by the diversity differences between the rhizosphere microbiota of domesticated and ancestral barleys (*Hordeum vulgare*) [21] and by the greater dependence on mycorrhizal symbiosis of ancient cultivars as compared to more recent cultivars [46]. The implementation of a 'back to the roots' paradigm shift [45] would consist in the integration of plant traits involved in the recruitment and activities of the functional core microbiota into breeding programs aimed at improving agronomic cultivars. Research has identified plant traits involved in beneficial interactions between plants and particular PGPR strains. Thus, the seminal paper by Smith *et al.* [47] highlighted the identification of tomato quantitative trait loci (QTLs) associated with the disease-suppressive capacity of a strain of *Bacillus cereus*. More recently, candidate genes related to root architecture adaptation and shoot growth promotion in *Arabidopsis* inoculated with a pseudomonad strain were identified by genome-wide analysis of more than 300 wild *Arabidopsis* accessions [48]. Although these types of plant traits remain to be identified for the functional core microbiota, these results imply that the approach is indeed promising and opens exciting perspectives. The ultimate goal will be to select plant genotypes fitting into agroecological systems that will rely more on biodiversity and biotic interactions (e.g., microbial functions) and less on the abiotic environment (Figure 4), including chemical inputs.

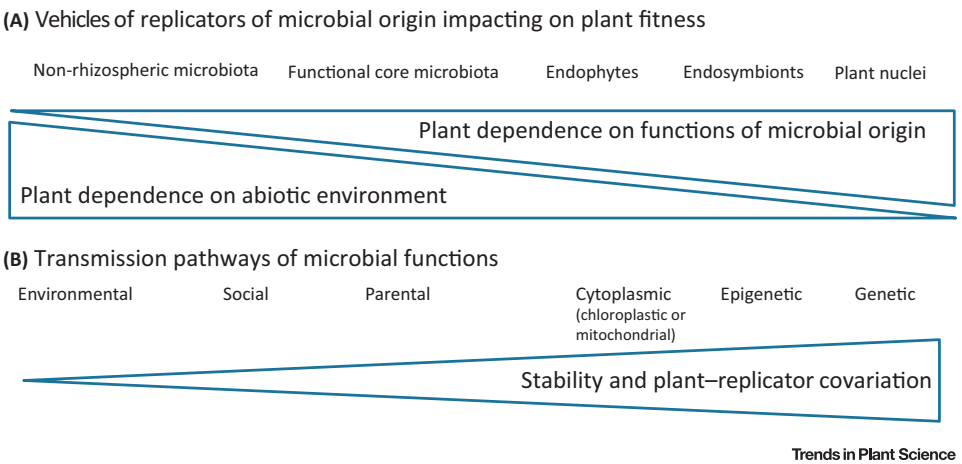

Figure 4. Vehicles and Transmission Pathways of Replicators of Microbial Origin Impacting on Plant Fitness. (A) Ranking of vehicles based on their level of association with plants, from free-living microorganisms to plant nuclei. Replicators with a large impact on plant fitness can be transferred from microbial vehicles to the plant nucleus through natural selection. According to the more-or-less close association between vehicles and plants, plants are more dependent on replicators carried by the vehicles, or are more dependent on abiotic constraints (e.g., mineral resources). (B) Transmission of the replicators impacting on plant fitness may follow different types of inheritance pathways ranging from environmental to genetic. The faithfulness of these transmission pathways across generations may vary and account for the strength of the covariation between plants and replicators.

Concluding Remarks and Future Perspectives

Plants recruit functional microbiota related to each plant genotype that can be called functional core microbiota. The plant–functional core microbiota associations with reciprocal beneficial effects will benefit from a selective advantage as compared to associations with neutral or deleterious effects. The functional core microbiota may thus ensure a better fitness of the holobiont (i.e., plant plus functional core microbiota). The core microbiota functions are coded by replicators distributed among microbial vehicles that can vary according to soils, so that a functional core microbiota is likely to be associated to a given host whatever the soil. The recruitment of a functional core microbiota by plants results from an increase in the abundance of the replicators implicated in beneficial functions (see Outstanding Questions). The stability of the association between plant and functional core microbiota depends on the way replicators are passed on, implying genetic and non-genetic heredity. Better knowledge of the plant traits that mediate the recruitment of beneficial replicators will open onto exciting perspectives for plant breeding that are expected to better value soil biotic resources so as to reduce chemical inputs in agroecology (see Outstanding Questions).

Outstanding Questions

Which strategies can we implement to unveil new replicators contributing to essential functions for the plant (e.g., nutrition, pathogen antagonism, elicitation of defense reactions)?

Will a given functional core microbiota be recruited by a given plant genotype irrespective of the microbial reservoirs (vehicles) harbored by the soils?

How can we demonstrate the beneficial effects of the functional core microbiota on holobiont fitness?

How are holobiont key functions distributed between plants and their associated microbiota? Does this depend on their cost for plants/microorganisms, or on the evolution rates of the partners and the environmental constraints?

What is the relative importance/weight of each strategy (enrichment in vehicles/allele selection/HGT) that increases the frequency of replicators beneficial for holobiont fitness? Does this distribution depend on plants, on microorganisms, and/or on environmental conditions?

Which plant traits are involved in the recruitment of replicators beneficial to the holobiont? How can we include these traits into plant breeding programs aimed at better promoting biotic interactions?

Is the functional core microbiota part of a continuum of plant–microbe interactions ranging from free-living microorganisms to microbial genes integrated into plant genomes? Is this gradient associated with decreasing dependency on the abiotic environment?

Acknowledgments

The authors are grateful to Annie Buchwalter for helping with the English text.

References

1. Bais, H.P. *et al.* (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266
2. Rudrappa, T. and Bais, H.P. (2008) Genetics, novel weapons and rhizospheric microcosmal signaling in the invasion of *Phragmites australis*. *Plant Signal. Behav.* 3, 1–5
3. Violle, C. *et al.* (2007) Let the concept of trait be functional! *Oikos* 116, 882–892
4. Vandenkoornhuyse, P. *et al.* (2015) The importance of the microbiome of the plant holobiont. *New Phytol.* 206, 1196–1206
5. Theis, K.R. *et al.* (2016) Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *MSystems* 1, e00028
6. Ludenberg *et al.* (2012) Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488, 86–90
7. Bulgarelli, D. *et al.* (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488, 91–95
8. Coleman-Derr, D. *et al.* (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave* species. *New Phytol.* 209, 798–811
9. Beijerinck, M.W. (1913) *De Infusies en de Ontdekking der Backteriën. Jaarboek van de Koninklijke Akademie voor Wetenschappen,* Müller
10. Ranjard, L. *et al.* (2013) Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. *Nat. Commun.* 4, 1434
11. Dawkins, R. (1976) *The Selfish Gene,* Oxford University Press
12. Walker, B.H. (1992) Biodiversity and ecological redundancy. *Conserv. Biol.* 6, 18–23
13. Yachi, S. and Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1463–1468
14. Beckers, B. *et al.* (2016) Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome. *Proc. Natl. Acad. Sci. U. S. A.* 113, 2312–2317
15. Bouffaud, M.-L. *et al.* (2016) Is plant evolutionary history impacting recruitment of diazotrophs and nifH expression in the rhizosphere? *Sci. Rep.* 6, 21690
16. Louca, S. *et al.* (2016) High taxonomic variability despite stable functional structure across microbial communities. *Nat. Ecol. Evol.* 1, 15
17. Wilson, D.S. and Sober, E. (1989) Reviving the superorganism. *J. Theor. Biol.* 136, 337–356
18. Zilber-Rosenberg, I. and Rosenberg, E. (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32, 723–735
19. Mougel, C. *et al.* (2006) Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol.* 170, 165–175
20. Shi, S. *et al.* (2016) The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecol. Lett.* 19, 926–936
21. Bulgarelli, D. *et al.* (2015) Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17, 392–403
22. Burke, C. *et al.* (2011) Bacterial community assembly based on functional genes rather than species. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14288–14293
23. Ofek-Lalzar, M. *et al.* (2014) Niche and host-associated functional signatures of the root surface microbiome. *Nat. Commun.* 5, 4950
24. The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214
25. Kwak, Y.S. *et al.* (2012) Factors impacting the activity of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* against take-all of wheat. *Soil Biol. Biochem.* 54, 48–56
26. Raaijmakers, J.M. and Mazzola, M. (2016) Soil immune responses: soil microbiomes may be harnessed for plant health. *Science* 352, 392–1393
27. De La Fuente, L. *et al.* (2006) phlD-based genetic diversity and detection of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens*. *FEMS Microbiol. Ecol.* 56, 64–78
28. Blouin, M. *et al.* (2015) Levels and limits in artificial selection of communities. *Ecol. Lett.* 18, 1040–1048
29. Swenson, W. *et al.* (2000) Artificial ecosystem selection. *Proc. Natl. Acad. Sci. U. S. A.* 97, 9110–9114
30. Panke-Buisse, K. *et al.* (2015) Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J.* 9, 980–989
31. Polz, M.F. *et al.* (2013) Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends Genet.* 29, 170–175
32. Bruto, M et al. (2014a) Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. *Sci. Rep*. 5, 16825.

33. Wisniewski-Dyé, F. *et al.* (2011) *Azospirillum* genomes reveal transition of bacteria from aquatic to terrestrial environments. *PLoS Genet.* 7, 12
34. Mølbak, L. *et al.* (2007) Root growth and exudate production define the frequency of horizontal plasmid transfer in the rhizosphere. *FEMS Microbiol. Ecol.* 59, 167–176
35. Ling, J. *et al.* (2016) Plant nodulation inducers enhance horizontal gene transfer of *Azorhizobium caulinodans* symbiosis island. *Proc. Natl. Acad. Sci. U. S. A.* 113, 13875–13880
36. Lopes, L.D. *et al.* (2016) Bacterial abilities and adaptation toward the rhizosphere colonization. *Front. Microbiol.* 7, 1341
37. Venturi, V. and Keel, C. (2016) Signaling in the rhizosphere. *Trends Plant Sci.* 21, 187–198
38. Lebeis, S.L. *et al.* (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science* 349, 860–864
39. Danchin, E. *et al.* (2011) Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nat. Rev. Genet.* 12, 475–486
40. Bonduriansky, R. *et al.* (2012) The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.* 5, 192–201
41. Jablonka, E. and Raz, G. (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84, 131–176
42. Johnston-Monje, D. and Raizada, M.N. (2011) Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One* 6, e20396
43. Lemanceau, P. *et al.* (2015) Understanding and managing soil biodiversity: a major challenge in agroecology. *Agron. Sustain. Dev.* 35, 67–81
44. Perez-Jaramillo, J.M. *et al.* (2015) Impact of domestication on rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* 90, 635–644
45. Philippot, L. *et al.* (2015) Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* 11, 789–799
46. Hetrick, B.A.D. *et al.* (1993) Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Can. J. Bot.* 71, 512–518
47. Smith, K.P. *et al.* (1999) Genetic basis in plants for interactions with disease suppressive bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 96, 4786–4790
48. Wintermans, P.C.A. (2016) et al., Natural genetic variation in *Arabidopsis* for responsiveness to plant growth-promoting rhizobacteria. *Plant Mol. Biol.* 90, 623–634
49. Spaepen, S. and Vanderleyden, J. (2011) Auxin and plant-microbe interactions. *Cold Spring Harb. Perspect. Biol.* 3, a001438
50. Fuchs *et al.* (2011) Microbial degradation of aromatic compounds – from one strategy to four. *Nat. Rev. Microbiol.* 9, 803–816
51. Loper, J.E. *et al.* (2012) Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genet.* 8, e1002784
52. Bottini, R. *et al.* (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl. Microbiol. Biotechnol.* 65, 497–503
53. Nett, R.S. *et al.* (2017) Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. *Nat. Chem. Biol.* 13, 69–74
54. Morris, R.O. (1987) Genes specifying auxin and cytokinin biosynthesis in prokaryotes. In *Plant Hormones and their Role in Plant Growth and Development* (Davies, P.J., ed.), pp. 636–655, Netherlands, Springer
55. Kudoyarova, G.R. *et al.* (2014) Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. *Plant Physiol. Biochem.* 83, 285–291
56. Melotto, M. *et al.* (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. *Annu. Rev. Phytopathol.* 46, 101–122
57. Ribaudo, C.M. *et al.* (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. *J. Plant Growth Reg.* 25, 175–18
58. Glick, B.R. (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiol. Lett.* 251, 1–7
59. Bruto, M et al. (2014b). Frequent, independent transfers of a catabolic gene from bacteria to contrasted filamentous eukaryotes. *Proc. R. Soc. Lond. B Biol. Sci.* **60**, 77-85.
60. Creus, C.M. *et al.* (2005) Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. *Planta* 221, 297–303
61. Ryu, C.-M. *et al.* (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4927–4932
62. Bakker, P.A.H.M. *et al.* (2014) Rhizobacterial salicylate production provokes headaches! *Plant Soil* 382, 1–16
63. Dhakal, R. *et al.* (2012) Production of GABA (γ-aminobutyric acid) by microorganisms: a review. *Braz. J. Microbiol.* 43, 1230–1241
64. Dodd, I.C. *et al.* (2010) Rhizobacterial mediation of plant hormone status. *Ann. Appl. Biol.* 157, 361–379
65. Cusano, A.M. *et al.* (2011) *Pseudomonas fluorescens* BBc6R8 type III secretion mutants no longer promote ectomycorrhizal symbiosis. *Environ. Microbiol. Rep.* 3, 203–210
66. Viollet, A. *et al.* (2017) *Pseudomonas fluorescens* C7R12 type III secretion system impacts mycorrhization of *Medicago truncatula* and associated microbial communities. *Mycorrhiza* 27, 23–33
67. Rezzonico, F. *et al.* (2005) The type III secretion system of biocontrol *Pseudomonas fluorescens* KD targets the phytopathogenic chromista *Pythium ultimum* and promotes cucumber protection. *Mol. Plant Microbe Interact.* 18, 991–1001
68. Dong, Y.-H. *et al.* (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3526–3531
69. Richardson, A.E. *et al.* (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321, 305–339
70. Singh, B. and Satyanarayana, T. (2011) Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiol. Mol. Biol. Plants* 17, 93–103
71. Stein, L.Y. and Klotz, M.G. (2016) The nitrogen cycle. *Curr. Biol.* 26, R94–R98
72. Vansuyt, G. *et al.* (2007) Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. *Mol. Plant Microbe Interact.* 20, 441–447
73. Shirley, M. *et al.* (2011) Comparison of iron acquisition from Fe-pyoverdine by strategy I and strategy II plants. *Botany* 89, 731–735
74. Scavino, A.F. and Pedraza, R.O. *et al.* (2013) The role of siderophores in plant growth-promoting bacteria. In *Bacteria in Agrobiology: Crop Productivity* (Maheshwari, D.K., ed.), pp. 265–285, Springer
75. Suárez, R. *et al.* (2008) Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. *Mol. Plant Microbe Interact.* 21, 958–966
76. Alami, Y. *et al.* (2000) Rhizosphere Soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. *Appl. Environ. Microbiol.* 66, 3393–3398
77. Couillerot, O. *et al.* (2009) *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Lett. Appl. Microbiol.* 48, 505–512
78. Haas, D. and Keel, C. (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* 41, 117–153
79. Vacheron, J. *et al.* (2016) Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Front. Plant Sci.* 7, 1212
80. Nowak-Thompson, B. *et al.* (2003) 2,5-Dialkylresorcinol biosynthesis in *Pseudomonas aurantiaca*: novel head-to-head condensation of two fatty acid-derived precursors. *J. Bacteriol.* 185, 860–869
81. Robin, A. *et al.* (2007) Diversity of root-associated fluorescent pseudomonads as affected by ferritin over-expression in tobacco. *Environ. Microbiol.* 9, 1724–1737

82. Tsuge, K. *et al.* (2001) Cloning, sequencing, and characterization of the iturin A operon. *J. Bacteriol.* 183, 6265–6273

83. Steller, S. *et al.* (1999) Structural and functional organization of the fengycin synthetase multienzyme system from *Bacillus subtilis* b213 and A1/3. *Chem. Biol.* 6, 31–41

84. Raaijmakers, J.M. (2010) et al., Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol. Rev.* 34, 1037–1062

85. Ma, Z. *et al.* (2016) Biosynthesis, chemical structure, and structure–activity relationship of orfamide lipopeptides produced by *Pseudomonas protegens* and related species. *Front. Microbiol.* 7, 382

86. Newman, M.-A. *et al.* (2007) Priming, induction and modulation of plant defense responses by bacterial lipopolysaccharides. *Innate Immun.* 13, 69–84

87. Gomez-Gomez, L. and Boller, T. (2002) Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci.* 7, 251–256

88. Meziane, H. *et al.* (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Mol. Plant Pathol.* 6, 177–185

89. Salas-Marina, M.A. (2015) et al., The Epl1 and Sm1 proteins from *Trichoderma atroviride* and *Trichoderma virens* differentially modulate systemic disease resistance against different life style pathogens in *Solanum lycopersicum*. *Front. Plant Sci.* 6, 77

90. Iavicoli, A. *et al.* (2003) Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Mol. Plant Microbe Interact.* 16, 851–858

91. van Loon, L.C. *et al.* (2008) Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. *Mol. Plant Microbe Interact.* 21, 1609–1621

92. Höfte, M. and Bakker, P.A.H.M. (2007) Competition for iron and induced systemic resistance by siderophores of plant growth promoting rhizobacteria. In *Soil Biology: Microbial Siderophores* (Varma, A. and Chincholkar, S., eds), pp. 121–133, Springer

93. Audenaert, K. *et al.* (2002) Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. *Mol. Plant Microbe Interact.* 15, 1147–1156