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POSITION PAPER

Optimize the plant microbiota to increase plant growth and health

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The "Groupement d'Intérêt Scientifique Biotechnologies Vertes" (GIS BV) organized on November 13th, 2018 in Paris, a scientific workshop on "Metagenomics for agro-ecosystems management and plant breeding". Thirty-four scientists, including eight from the private sector attended the workshop. General discussion was organized around the presentations related to plant, seeds and soil microbiota, and data treatment to reconstruct interaction networks. This article gathers the current French research strengths, relative to the international context and highlights the research priorities between the public and the private sectors, using plant genetics and plant-microbiota interactions for the benefit of future agricultures.

Socio-economic context, scientific challenges and opportunities

Plants live in association with a wide diverse and complex assembly of viruses and microorganisms including bacteria, archaea, oomycetes, fungi and protists [1,2]. These microbial assemblages, collectively referred to as the plant microbiota, impact plant fitness through the modification of a number of traits including: biomass production [3], acquisition of nutrients [4], flowering time [5] or resistance to number of abiotic [6] and biotic stresses [7–10]. Hence, maximizing the plant-beneficial potential of these microbial assemblages could ultimately result in enhancing crop yield and reducing pesticides and fertilizers [11].

However, management of plant microbiota composition for developing sustainable agroecosystems still remains incredibly challenging. Gaining a basic understanding of the main biological, evolutionary and ecological processes involved in the assembly and dynamics of plant microbial communities should help to deploy microbiota-based strategies to improve plant productivity. Hence, over recent years a number of research groups explored the impact of environmental factors and host genetic variation on the composition and dynamics of plant microbiota [12–19]. Overall, these studies acknowledged an important influence of the environment on plant microbiota composition and a restricted but often significant effect of the host genotype. Gaining basic knowledge of processes involved in plant microbiota composition represents also an economic opportunity at the worldwide level for deploying biostimulant- or biocontrol-based solutions. A growing number of startups (e.g. AgBiome, Aphea.bio, Biome Makers, Concentric, Gingko Bioworks, Indigo, Pivot Bio, Trace Genomics), agrochemical (e.g. Basf, Bayer, Corteva AgriScience) and seed compagnies (e.g. Limagrain, KWS) as well as institutional platforms (e.g. Cirad-MetaHealth) are embarking on the plant microbiota adventure.

Current research strategies

- **Assessing the composition of plant microbiota through metagenomics-based approaches**

The taxonomic structure of microorganisms communities is nowadays frequently estimated using culture-independent DNA high throughput sequencing (HTS). While most microorganisms cannot be isolated and cultivated under standard laboratory conditions, HTS technologies, including metabarcoding and shotgun metagenomics approaches, are routinely employed for assessing the taxonomic and functional profiles of microbiota. Metabarcoding relies on amplification and sequencing of a portion of gene/intergenic regions that serve as barcodes for species identification. These markers, which must be ubiquitous, are ideally composed by conserved and highly variable regions. In addition, these markers should be in single copies in all target genomes, which is usually not the case, meaning that exact quantification is still out of reach. Because of their sequence polymorphisms and ubiquities within prokaryotes and eukaryotes, the hypervariable regions of the 16S/18S rRNA genes and internal transcribed spacer (ITS) are widely employed, respectively. The success of these microbial markers is also largely due to comprehensive international publicly available databases containing many sequences of specimen [20–23]. Among the many obstacles for exact quantification are the so-called "compositionality issue", as sequencing only provides access to relative abundances [24], amplification bias, where some sequences of the markers are amplified more than others during the first step [25] and extraction bias. These obstacles can be mitigated to some extent by spiking [26], total DNA quantification [27] and positive controls. The final limitation of metabarcoding is the limited phylogenetic resolution of the marker: 500 base pairs are enough to identify organisms at the genus level, but not always at the species and rarely at the strain levels. It only gives access to Operational Taxonomic Units (OTU), Amplicon Sequence Variants (ASV) or oligotypes, not directly organisms [28–30]. Efforts are underway to define alternative universal markers (e.g. rpoB [31] and gyrB [32] for eubacteria, GH63 [33] and RPB2 [34] for dikarya) and non-universal markers (e.g. available only on some branches of the bacterial tree) with better resolutions but those efforts are hampered by the limited content of corresponding taxonomic databases. With all those limitations in mind, there is a link, however imperfect, between taxa abundance and number of sequences and metabarcoding remains a fast, cheap and relatively effective way of assessing the taxonomic composition of plant microbiota.

By contrast with bacteria and fungi, viruses do not even all encode their genomes with the same classes of nucleic acids let-alone have genes or other fragments of sequences universally found across all genomes. Therefore, virus metagenomic studies have generally relied on methodologies that firstly enrich for virus-derived nucleic acids in a sample, and then amplify and/or directly sequence these in a sequence independent manner. Consequently, plant viral metagenomic approaches have targeted five main classes of nucleic-acids: (i) total RNA or DNA; (ii) virion-associated nucleic acids (VANA) purified from viral particles; (iii) double-stranded RNAs (dsRNA); (iv) virus-derived small interfering RNAs (siRNAs) and (v) opportunistic mining of publicly accessible plant transcriptomics databases (reviewed in [35]). Interestingly, virus metagenomic studies have enabled the direct testing of hypotheses relating to the impacts of host diversity, host spatial variations and environmental conditions on plant virus diversity and prevalence [12,36,37].

The functional content of microbial assemblages could be either indirectly predicted according to its taxonomic profiles (e.g. PICRUSt [38]; Tax4fun [39]; FunGuild [40]) or directly estimated via random shotgun DNA sequencing. Although this latter approach, coined metagenomics, produces datasets of higher complexity in comparison to metabarcoding, it offers a more robust representation of the functional profiles of a microbiota. In addition to sequence cleaning, the typical pipeline uses meta-assembly to reconstruct contigs, binning to find clusters of contigs coming from the same organism and reconstruct metagenomic species (MGS) or pangenomes (MSP) or gene prediction and clustering to reconstruct a catalog of genes present in the ecosystem. The final step involves mapping sequences to the catalog of genes or MGS to quantify the abundance of each gene / MGS in the ecosystem. Unlike well studied ecosystems such as the human gut, there is no pre-computed gene catalog for plant microbiota. Metagenomics forgoes the amplification step and therefore is not affected by amplification bias but it suffers from its own afflictions: it is expensive compared to metabarcoding, the lack of targeted amplification makes it harder to separate bacterial DNA from the host plant DNA and the catalog has millions of

genes, many of which have no or low-quality annotation. Assembly-independent approaches can be used to extract specific markers or to measure dissimilarity/similarity distance. The potential of metagenomics remains powerful to find functions and metabolic pathways represented in the system.

Assessing the composition of plant microbiota, using either a taxonomic or a functional point of view, is the first step towards correlating them with plant traits of interest (e.g. plant yield, resistance to plant pathogens). These studies can also provide useful information to isolate candidates taxa or genes associated with the trait of interest.

- **Reinoculation of synthetic communities or microbiota fraction to assess the impact of microbiota on plant fitness**

Correlation between plant microbiota structure and host phenotypes provide useful indications for discovering which member(s) of the microbial assemblages influence specific plant traits. Reconstructing synthetic communities (SynCom) that are composed of defined microbial strains is an interesting approach to infer causal relationship between microbiota membership and host phenotype. This experimental strategy was recently employed to increase phosphate concentration in Arabidopsis thaliana shoots [41] or for plant protection against plant pathogenic fungi [42]. The pre-requisite for performing SynCom reconstruction is the availability of a culture collection that is representative of the microbial diversity [43]. In most cases, obtaining a good coverage of this diversity is challenging, especially when the sampled habitat possesses a high microbial richness. An alternative approach is therefore to use washes of plant tissues (e.g. roots, leaves, and seeds), rhizosphere soil suspensions, or soil spore extractions as representatives of plant microbiota composition. Such microbiota inocula are then inoculated in soil or sprayed on surface-sterilized plant seeds before monitoring the trait of interest. Such experimental designs highlighted for instance plant protection mediated by leaf or rhizosphere microbiotas against bacterial plant pathogens [10,44]. Similarly, transferring tiny amounts of disease suppressive soil to a conducive soil conferred plant protection to fungal pathogens [45].

Scientific and technical bottlenecks

- **How to predict the microbial traits involved in plant growth and the corresponding plant traits that would be affected?**

While the importance of a wide range of microbial processes on soil nutrient cycling was deeply investigated [46,47], the functional potential of the microbial communities associated with plants remains a challenge. The roles of specific functions (e.g. nitrogen fixation, phosphate solubilization, phytohormone synthesis, and control of ethylene levels) were generally demonstrated independently. Random metagenome and metatranscriptome sequencing approaches provide an interesting starting point for predicting the whole functional diversity. However, many microbial genes families do not have established biological activity. Microbiota effect on plant growth and plant fitness has to be specified by traits amenable to measurement in large environmental variations, but robust investigations still remain rare [48].

- **What are the mechanisms for plant recognition of microbial assemblage processions?**

The way in which a plant genotype determines the composition and structure of its microbiota is relatively unknown compared with the interactions of human genetic variations with its microbiome [49]. Unfortunately, biological and methodological difficulties limit any mechanism transposition from one system to another [50]. Biological difficulties include reduced host genotype diversity of both cases and interactions of uncontrolled environmental factors with host genotypes and microbes. Technical difficulties lie in the transformation of molecular data into phenotypes and in the different statistical approaches used according to the system. Anyway, genome-wide associations studies (GWAS) include statistical models that compare and quantify how the effects of genotype on phenotype vary across environmental conditions to understand how factors may shape the microbiome [51]. GWAS of plant-microorganisms interactions initially focused on binary studies involving a plant genotype and a single plant pathogen [52]. An analogy to the functioning of the plant's immune system conceptualized how this, by discriminating pathogens from other microorganisms,

determines the composition and structure of the microbiota, but this approach remains restricted in scope, although it involves molecular signalling between the host-plant and microorganisms, particularly through microbe-associated molecular patterns (MAMPs) and MAMP-triggered immunity (MTI) [53]. On the other side, mapping microbiota traits in natural situations is complex because environmental variations mask relationships between host genes and microbiota traits; therefore, it is preferable to create synthetic communities composed of the most abundant microorganisms found in natural situations and of increasing complexity to then, test how plant genotypes shape the microbiota [50]. The discovery of a genetic basis in host plant for interactions with microbiotas suggests new opportunities to exploit natural genetic variation in plant crops to enhance our understanding of beneficial plant-microbiota interactions and develop agroecological strategies for disease control or/and plant growth and development in agriculture.

Heritability of the plant microbiota.

Although its effect is more limited than environmental fluctuations, the plant genotype is a significant driver of plant microbiota composition. For instance, host genetics significantly affects the abundance of bacterial taxa associated to the maize rhizosphere is [54]. This broad-sense heritability (H2) is probably linked to host genes that are either related to plant metabolites [55] and/or linked to plant immunity [53,56,57]. Whether heritable plant-associated bacterial taxa are transmitted from the environment (i.e. horizontally) or from maternal plant to its progeny (i.e. vertically) remains to be investigated. Despite a probable limited vertical transmission in plants in comparison to horizontal transmission [58], a fraction of the plant microbiota could be transmitted in clonal offsprings of *Glechoma hederacea* [59] or in seeds of various plant species [32,60–62].

Studying the relative impact of vertical versus horizontal transmission could have important implications for the design of agronomical practices. Vertical transmission might be considered for the selection of desirable microorganisms through plant breeding, and horizontal transmission be integrated into agro-ecological approaches (e.g. selection of key production areas or agricultural practices) for the benefit of crops.

Prediction of microbial interaction through design of co-occurence networks

Plant microbiota are complex systems with hundreds of actors that interact with each other and are affected by environmental conditions. Networks are a simple way to model those interactions to find groups of taxa that interact preferentially with each other, hubs and keystone (systematically important) species [63]. Six keystone taxa were identified among plant-associated taxa [64]. Unfortunately, interactions are not directly observed and must be reconstructed from the footprints they leave in abundance data. Most network inference methods are based on co-occurrence or co-abundance data and rely on some variant of correlation to reconstruct ecological interactions. Ecological interactions such as mutualism, commensalism, and competition can reasonably be inferred from co-abundance data but others such as amensalism, and syntrophy are almost impossible to recover [65]. Many methods based on correlation thresholding [66] or graphical models [67] were proposed to infer interactions. However, they do not account for environmental variables so that shared habitats preferences can be mistaken for direct interactions [68]. Recent methods control for environmental variables [69] and integrate organisms assessed with different markers (such as fungi and bacteria) into the same network [70]. It is however important to remember that network reconstruction framed that way is in essence a statistical problem with severe limitations attached. For optimal performance, the number of samples should be roughly similar to the number of taxa in the network: this requires either (i) large sample sizes or (ii) a focus on dominant taxa, excluding de facto rare but potentially interesting taxa from the network. Finally, no matter how sophisticated are the methods, inferred edges may not represent ecological interactions and/or miss genuine ones [71]. Whenever feasible, experimental validations (for example, based on microorganisms isolated through culturomics) should be performed to validate the strongest edges.

- **Progress must be made in the isolation and culture of microorganisms representing the microbial diversity associated with plants and in the evaluation of their environmental impact**

Despite variability in post-inoculation plant growth promoting effects [72], the production and market of microorganisms know a considerable increase and should account for more than $ 10 billion in 2025 [73]. Current limitations for efficient production of stable microbial inoculants include limited large-scale cultivability and shelf life of some taxa (e.g. mycorrhizal fungi; [74,75]) as well as extensive data requirements for the registration of new strains [76]. This latter obstacle currently hampers the development of complex inoculants composed of multiple strains, although these complex inoculants showed better efficiency than single strains [77].

While the range and sustainability of inoculum-mediated plant benefits (biomass, yield, and survival) are the main demand for end-users [79], their environmental impact are poorly considered whereas it remains a critical issue. Three levels of environmental impacts were identified for mycorrhizal fungi, but this also stands for other microorganisms [77,80]: First, the alteration of composition and structure of native microbial community. Second, the exchange of genetic material with native community, and third the persistence and/or spread of inoculants, increasing consequently the first two impacts.

Cartography of ongoing international initiatives

Over recent years, a number of international initiatives emerged in the field of plant microbiota. The most widely recognized international action is probably the Phytobiomes Alliance (http://www.phytobiomesalliance.org/Pages/default.aspx), which is an industry-academic collaborative initiative composed of more than 20 sponsors. While studying plant microbiota interactions is a key priority of Phytobiomes Alliance, other aspects of the plant phytobiome, i.e. targeting or not the plant immediate environment including micro- and macro-organisms [1]. A more recent initiative, the Ag Microbiomes Research Coordination Network, was funded by the National Science Foundation (https://agmicrobiomercn.umn.edu/). The goal of Ag Microbiomes RCN is to promote cross-disciplinary collaborations in the field of the Plant and Soil microbiome. Others international actions in plant microbiomes were recently released and included; they are, for instance, the EU-funded project Microbiome Support (https://www.eufic.org/en/collaboration/article/the-microbiome-saga-what-does-research-need-to-do-better), the Australian Microbiome Initiative (https://www.australianmicrobiome.com/) and the UK Plant Microbiome Initiative (https://www.cabi.org/news-and-media/2017/cabi-and-rothamsted-research-launch-uk-plant-microbiome-initiative/). Another initiative is the working group on Plants and Microbiomes that is part of the European Plant Science Organization (EPSO) network. EPSO is an independent academic organization federating more than 220 public research organizations in Europe and beyond. In 2017, the working group published a report defining a strategy for plant microbiome research in Europe. A second working group meeting in 2019 is to define the needs and to provide advice to current and future EU framework programs.

Overview of the French public and private research on the topic

The PhytoMic network was created in 2016. It is supported by the INRA (French National Institute for Agronomical Research) divisions i.e. EA (Environment and Agronomy), EFPA (Forest, Grassland and Freshwater Ecology), and SPE (Plant Health and Environment) as well as the metaprogram MEM (Meta-omics and microbial ecosytems). The network is currently composed of 20 research units interested in plant microbiota. The primary objective of the network is to bring together the skills (e.g. plant genetics, community ecology, microbiology, plant pathology and agronomy) required for a comprehensive understanding of the plant microbiota.

The PhytoBioM network was created in 2018. It is supported by the LabEx AGRO (coordinated by the University of Montpellier and Agropolis Fondation). The network is currently composed of 14 research units in close interaction with institutional structures for research and training in Southern countries IRD's (Research Institute for

Development) international joint laboratories, CIRAD's (Agricultural Research and International Cooperation for Development) platforms in partnership, INRA's international laboratories). The primary objective of the network is to create a unify taskforce to address the scientific and societal challenges emerging from the phytobiome concept and provide innovations for sustainable agriculture. The network relies on a wide range of experts (i.e. ecophysiologists, plant physiologists, breeders, plant pathologists, molecular ecologists, microbiologists, virologists, entomologists and computer scientists) investigating the soil-plant-atmosphere continuum and the whole plant system (rhizosphere, endosphere and phyllosphere).

Conclusion: strategic research targets for public-private research, at French and European levels

Over the past few years a growing number of studies highlighted that environmental selection and plant genotype partly drove the structure of the plant microbiota. Identifying at a finer grain resolution the management practices and/or host genes involved in this selection required a high level of replications over space and time along with a wide range of accessions. Public-private partnerships (PPP) should provide opportunities for developing such ambitious experimental on important agronomical crops. For instance, private partner provides access to experimental plots and inbred lines, while public partner provides expertises in genomics/metagenomics. This type of PPP would potentially identify suit of genes/practices that change the structure of plant microbiota. The impact of such changes on host fitness should be latter evaluated. This could be performed in controlled or semi-controlled conditions through emerging public plant phenotyping platforms that can monitor, in a high-throughput manner, number of plant traits such as germination rate, plant architecture or chlorophyll content.

Acting on this idea to develop PPP in plant microbiome basic and applied knowledge, the March 2019 workshop was one major step and must be followed by partnership implementation.

This paper is endorsed by the strategic committee of the GIS BV.

Annex 1. List of French laboratories and implication in research funded projects

Unit	City	Habitat	Plant	Topic
AGIR	Toulouse	Soil Spermosphere	Agricultural Crops	Abiotic stress - Biotic Stress
Agroecologie	Dijon	Soil Rhizosphere	Model species, Agricultural Crops	Mutualism (AMF) - Biotic stress - Biogeochemical cycles - Biocontrol
BFP	Bordeaux	Carposphere Phyllosphere	Agricultural Crops	Biotic stress (viruses)
BGPI	Montpellier	Phyllosphere Endosphere Rhizosphere	Tropical and Mediterreanean Agricultural Crops	Biotic stress (Xanthomonas ; Magnaporthe ; viruses) – Mutualism (AMF) - Biocontrol
BIOGECO	Bordeaux	Phyllosphere	Forest	Abiotic stress - Biotic stress -
BIOGER	Grignon	Residuesphere	Agricultural Crops	Biotic stress (Zymoseptoria, Leptosphaeria)
Ecobio	Rennes	Rhizosphere	Model species, Agricultural Crops	Mutualism (AMF, Rhizobia)
Eco&Sols	Montpellier	Soil Rhizsophere	Tropical and Mediterrean Agricultural Crops	Abiotic stress - Biogeochemical cycles
GDEC	Clermont	Ear	Agricultural Crops	Biotic stress (Fusarium)
IAM	Nancy	Rhizosphere	Forest	Mutualism (EMF) - Abiotic stress
IGEPP	Rennes	Rhizosphere	Agricultural Crops	Abiotic stress - Biotic stress (Leptosphaeria – Delia; Plasmodiophora; nematodes)
IPME	Montpellier	Rhizosphere Phyllosphere	Agricultural Crops	Mutualism-Abiotic stress - Biotic stress - Biocontrol
IPS2	Saclay	Rhizosphere	Model species	Mutualism (Rhizobia)
IRHS	Angers	Seed Spermosphere	Agricultural Crops	Biotic stress (Xanthomonas, Alternaria)
ISA	Sophia-Antipolis	Rhizosphere	Agricultural Crops	Biotic stress (Phytophtora)
LEM	Lyon	Rhizosphere	Agricultural Crops	Biotic stresses – Biogeochemical cycles - Biocontrol
LIPM	Toulouse	Rhizosphere Phyllosphere	Model species	Abiotic stress - Biotic stress (Xanthomonas - Ralstonia)
LRSV	Toulouse	Rhizosphere	Model species	Biotic stress (Aphanomyces - Phytophtora)
LSTM	Montpellier	Rhizosphere	Mediterranean and Tropical plants	Mutualism (Rhizobia - AMF/EMF)
SAVE	Bordeaux	Phyllsophère	Agricultural Crops	Biotic stress

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