REVIEW

Dissection of plant microbiota and plant-microbiome interactions

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Plants rooted in soil have intimate associations with a diverse array of soil microorganisms. While the microbial diversity of soil is enormous, the predominant bacterial phyla associated with plants include Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia. Plants supply nutrient niches for microbes, and microbes support plant functions such as plant growth, development, and stress tolerance. The interdependent interaction between the host plant and its microbes sculpts the plant microbiota. Plant and microbiome interactions are a good model system for understanding the traits in eukaryotic organisms from a holobiont perspective. The holobiont concept of plants, as a consequence of co-evolution of plant host and microbiota, treats plants as a discrete ecological unit assembled with their microbiota. Dissection of plant-microbiome interactions is highly complicated; however, some reductionist approaches are useful, such as the synthetic community method in a gnotobiotic system. Deciphering the interactions between plant and microbiome by this reductionist approach could lead to better elucidation of the functions of microbiota in plants. In addition, analysis of microbial communities' interactions would further enhance our understanding of coordinated plant microbiota functions. Ultimately, better understanding of plant-microbiome interactions could be translated to improvements in plant productivity.

Keywords: plant microbiota, plant microbiome interaction, synthetic community

Introduction

Plants have evolved in close association with their microbial inhabitants, and thus, now regarded as plant holobionts (Vandenkoornhuyse *et al.*, 2015). The holobiont may be regarded as the host plant and its associated microbiota (Margulis and Fester, 1991). The plant-associated microorganisms are diverse, including bacteria, archaea, fungi, and protists, that collectively constitute the complex plant microbiota. Plant microbiota comprise the microbial communities living on the plant surface as well as the inside of plants. The surface niches of plant microbes include that of the root surface (rhizosphere) and the above-ground part of the plant (phyllosphere). Microbes also colonize the inside of plants (endosphere), in both the below-ground and above-ground parts. While plant functions are mostly controlled with the expression and regulation of their own genes, plants also depend on their microbial colonizers to modulate certain functions (Turner *et al.*, 2013). The plant host and its microbiota are interdependent. Plant hosts provide niches with nutrients to the partner microbes, and the microbes help their hosts with various beneficial functions (Hassani *et al.*, 2018). The microbiota can vary in their relationship with plant hosts: they can be mutualistic, commensal, parasitic, or pathogenic to plant hosts. In this review, we use the term "plant-microbiome" interactions to refer to the functional traits of plant microbiota interacting with plant hosts.

Although the microbial interaction with host plants has been recognized as an important component for both plant fitness and microbial evolution in plant niches, detailed plant microbiome structures relevant to plant function are not fully understood yet. Recent advances in culture-independent approaches and high-throughput analysis of plant microbiota have started to reveal the plant-microbiome interactions to an extent. This review focuses on bacterial members of the plant microbiota, their assemblage on various plants, and their functional relevance to beneficial traits in plants. We also discuss a reductionist approach to microbiota analysis, i.e., the synthetic community (SynCom) approach. SynCom refers to a controlled community of culturable microbes that represent the function and structure of the original plant-associated microbiome, and thus can provide inputs regarding the latter. In addition, we focus this review on the plant-microbiome interactions in model plants such as Arabidopsis, rice, and tomato.

Plant-associated microbiome structure

The diverse range of microbes inhabiting healthy host plants is referred to as the plant microbiota (Lindow and Brandl, 2003; Delmotte *et al.*, 2009; Bulgarelli *et al.*, 2012; Knief *et*

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al., 2012; Lundberg *et al.*, 2012). The seemingly healthy host plant with its associated diverse microbes is home to several mutualistic interactions, including unintended or intended symbiotic ones and various commensal ones. Numerous mutualistic and commensal microbes reside in several distinct habitats provided by host plants, such as the endosphere, the rhizosphere, and the phyllosphere. Advanced technologies such as next-generation sequencing technologies, advanced statistical methods, and tools of bioinformatics render it possible to reveal the plant microbiota structures and to dissect plant-microbiome interactions. Here, we describe the microbiota structures discovered in different microbial niches in plants (Table 1).

Rhizosphere

Ten to forty-four percent of fixed carbon and lignocellulosic cell wall materials of plants are released from the roots (Guttman *et al.*, 2014). These root exudates of the plants represent an energy and signaling source for soil microorganisms, leading to recruitment of rhizosphere microbiota from the abundant microbiota of the surrounding soil (Badri *et al.*, 2009; Dennis *et al.*, 2010; Uroz *et al.*, 2010; Hirsch and Mauchline, 2012; Badri *et al.*, 2013; Bulgarelli *et al.*, 2013). The constituents of root exudates fluctuate qualitatively and quantitatively depending on the growth stage and the nutritional status of the plant, and the spreading space of the root (Hartman *et al.*, 2009; Malusà *et al.*, 2016). Thus, the root exudates bring about the creation of a specific rhizosphere microbial community. Only 2–5% of the rhizosphere microbiota is in-

volved in the promotion of host plant growth, especially under constrained conditions (Lareen *et al.*, 2016). The root may just be a gateway for the rhizosphere microbiota to the upper part of the plant (phyllosphere) and inside the plant (endosphere) (Thapa and Prasanna, 2018). The comparison between microbial profiles of the bulk soil and the rhizosphere revealed a distinct rhizosphere effect on the plant microbiota (Uroz *et al.*, 2010; Peiffer *et al.*, 2013). In addition, several studies have demonstrated that the soil type more strongly affects the community of rhizosphere microbiota than the host species (Table 1) (Uroz *et al.*, 2010; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012).

Taxonomy profiling generated from shotgun metagenomic data in rice plants revealed that strictly anaerobic or facultative anaerobic microbes were abundant in the rhizosphere. Anaerobic microbes are thought to play a crucial role in maintaining the rhizosphere microbial community in rice plants; moreover, anaerobic and aerobic microbes can co-exist under aerobic conditions (Akasaka *et al.*, 2003; Tonouchi, 2009; Qiu *et al.*, 2014; Kim and Lee, 2020). Alpha, Beta, and Deltaproteobacteria were the most enriched in the rhizosphere, and methanogens belonging to archaea were also detected (Knief *et al.*, 2012). The microbial profile of rice rhizosphere displayed more diversity as compared to that of the rice phyllosphere. Recently, bacterial communities were differentiated in two tomato cultivars: a susceptible one and a resistant tomato cultivar against bacterial wilt caused by *Ralstonia solanacearum*. In particular, members of *Flavobacteriaceae* were more abundant in the rhizosphere of the resistant cultivar

Table 1. Microbiome structure and function of plant microbiota studied in major model plants

Host plant	Features	References
Rhizosphere		
Arabidopsis thaliana	• Rhizosphere- and endosphere-inhabiting bacterial communities are more strongly influenced by soil type than host genotype. • The taxonomy of leaf and root microbiota were extensively overlapped.	Bulgarelli *et al.* (2012), Lundberg *et al.* (2012), Schlaeppi *et al.* (2014), Bai *et al.* (2015)
Rice	• Microbial community is influenced by genotype, geographical effect, and cultivation practice. • Microbial consortia were involved in methane cycling.	Edwards *et al.* (2015)
Tomato	• Bacterial community between resistant and susceptible cultivars differs. • Plant could recruit "bodyguard" native microbiota to protect itself.	Kwak *et al.* (2018)
Endosphere		
A. thaliana	• Composition of root endophytes was affected by soil type, however, host genotype was limited to influence the profiling of endophytic community. • Diversity of the root endosphere was less than the rhizosphere. • Among core microbiome in endosphere, many OTUs were overlapped in rhizosphere microbiota.	Bulgarelli *et al.* (2012), Lundberg *et al.* (2012)
Rice	• Gammaproteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were commonly found in the rice endosphere. • Many root endophytes were associated with growth promotion using phytohormones and siderophores and induced systemic resistance.	Ding *et al.* (2019)
Tomato	• Genotypes affected the composition of endophytes. • *Bacillaceae* and *Rhizobiaceae* were enriched in root endosphere.	French *et al.* (2020)
Phyllosphere		
A. thaliana, clover, soybean	• Dominant phyla in bacterial phyllosphere community were Proteobacteria, Actinobacteria, and Bacteroidetes. • Genes involving cuticle formation and ethylene signaling influenced the composition of phyllosphere microbial community.	Delmotte *et al.* (2009), Vorholt (2012), Chen *et al.* (2020)
Rice	• In the analysis of microbial proteome, *Methylobacterium* related to methanol-based methylotrophy was enriched in phyllosphere microbiota. • Proteins related to stress response, methanol utilization, the fasciclin proteins or invasion-associated locus B protein were dominant.	Knief *et al.* (2012)
Tomato	• Soil may be a reservoir of microbiota in the phyllosphere.	Ottesen *et al.* (2013)

than in the rhizosphere of the susceptible cultivar, based on the analysis of 16S rRNA gene sequencing and shotgun meta-genomic sequencing (Table 1) (Kwak *et al.*, 2018).

Endosphere

Several recent studies have demonstrated that the diversity of endophytic microbial communities is mostly dependent on the host plants. Endophytic microbial colonization is also determined by their specific competencies such as the presence of flagella, cell-wall degrading enzymes, detoxification mechanisms, type IV pili, twitching motility, and lipopoly-saccharides (LPS) (Compant *et al.*, 2010). Pathogens have been known to colonize the insides of plants and obtain nu-trients using carbohydrate-utilizing enzymes. The same stra-tegies have also been adopted by non-pathogenic, endophytic bacterial strains (Brader *et al.*, 2017). In a study, most of the endophytic bacteria from a population range of 10^5–10^7 CFU/g plant were derived from the root systems (Hallmann, 2001; Sessitsch *et al.*, 2002; Idris *et al.*, 2004; Krechel *et al.*, 2004; Berg *et al.*, 2005; Compant *et al.*, 2005; Hardoim *et al.*, 2008; Kandel *et al.*, 2017).

The distinct composition of endophytes was found to be affected by the plant species, genotype, and the growth stage. Moreover, the environmental conditions of soil and other abiotic and biotic stresses of the host plant contributed to the diversity and abundance of the endophytic community in the roots (Brader *et al.*, 2017; Correa-Galeote *et al.*, 2018; Sasse *et al.*, 2018; Xu *et al.*, 2018). Generally, endophytes were more often associated with plant growth-promoting effects than the rhizosphere inhabitants (Conn *et al.*, 1997; Chanway *et al.*, 2000). A core endophyte in *Arabidopsis thaliana* and *Populus deltoides* grown in different areas was found not to be affected by the plant genotype and growth stage (Gottel *et al.*, 2011; Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). Three phyla, including Betaproteobacteria, Bacteroidetes, and Acti-nobacteria, were observed in the endosphere of *A. thaliana*, while no functional analysis was reported between these fa-milies and the host plant (Bulgarelli *et al.*, 2012). The most abundant phyla in grapevine roots were Proteobacteria, Aci-dobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Chloroflexi, Firmicutes, and Gemmatimo-nadetes (Samad *et al.*, 2017). In the roots of maize, Proteo-bacteria, Firmicutes, and Bacteroidetes were observed as the most dominant phyla (Correa-Galeote *et al.*, 2018). Among different *Arabidopsis* species, the composition of endophytes differentiates quantitatively rather than qualitatively (Schla-eppi *et al.*, 2014). Interestingly, a recent study in rice revealed that rice domestication affected the seed microbiota, includ-ing epiphytes and endophytes (Kim *et al.*, 2020). This sug-gests that human activities to improve plant productivity could influence the plant microbiota structure.

Phyllosphere

The phyllosphere provides a relatively harsh microbial ha-bitat because of exposure to ultraviolet radiation, low water and nutrient availability, and fluctuating temperature. Never-theless, the leaf surface provides the habitat for diverse mi-croorganisms. Bacteria represent the most abundant group of microbiota in the phyllosphere, and show a dense popu-lation of up to 10^8 cells/g of leaf material (Remus-Emsermann *et al.*, 2012). Several phyllosphere bacterial profiling studies have been conducted to compare the relative significance of phytochemicals secreted among different plant species (Whipps *et al.*, 2008) and the environmental effects (Redford *et al.*, 2010; Kim *et al.*, 2012) in terms of shaping the bacte-rial composition. It was observed that the biogeographical effects had a greater influence on shaping the microbial com-munity than species-dependent association (Knief *et al.*, 2010; Finkel *et al.*, 2011). It has been reported that the struc-ture of the microbial community in rice phyllosphere is dis-tinct from that of other plants (Knief *et al.*, 2012; Vorholt, 2012), whereas that of *A. thaliana* was similar to most plants (Delmotte *et al.*, 2009; Vorholt, 2012). Interestingly, the func-tional analysis of phyllosphere using metaproteogenomics revealed functional and structural similarities among dif-ferent plant species, including *A. thaliana,* grown in different locations (Delmotte *et al.*, 2009).

Holobiont view of plant-microbiome interaction

Plants harbor an enormous number of microbes within and on the surface (McNear, 2013), and the importance of these microorganisms for plant growth and survival is well esta-blished. For instance, it has been reported that in the absence of certain bacteria, it is difficult to culture transplants of some plant species (Leifert *et al.*, 1989). Plant growth and survival (fitness) are the consequence of the plant itself as well as the associated microbiota, which are collectively termed as the holobiont (Margulis and Fester, 1991; Vandenkoornhuyse *et al.*, 2015). Further, the holobiont should be regarded as the collective functions/interactions that exist between the host and its corresponding microbiome (Vandenkoornhuyse *et al.*, 2015). Similarly, the holobiome represents the collec-tive genomes of the host and its microbiome and is regarded as the genomic reflection of the multipartite interactions that exist among individuals constituting the holobiont (Guerrero *et al.*, 2013).

Plant functions modulated by microbiome

A variety of complex interactions exist among the plant-asso-ciated microbial community members (Hassani *et al.*, 2018). These interactions not only affect the overall microbial com-munity structure but also have the potential to influence plant health and fitness in a positive or deleterious way (Fig. 1). These include plant disease suppression (Mendes *et al.*, 2011; Cha *et al.*, 2016; Ritpitakphong *et al.*, 2016), plant priming (Van der Ent *et al.*, 2009), elicitation of systemic resistance (Zamioudis *et al.*, 2015), improved nutrient acquisition (Van Der Heijden *et al.*, 2016), abiotic stress tolerance (Rolli *et al.*, 2015), enhanced adaptation to ecological variations (Haney *et al.*, 2015), or assistance in plant-mycorrhizal associations (Garbaye, 1994). Below, we discuss how the microbial inter-actions (within the plant microbiota) and the host plant-mi-crobiome interactions affect plant functions, with particular emphasis on microbiome-mediated (i) protection against pathogens, (ii) resistance to biotic and abiotic stresses, and (iii) plant growth promotion.
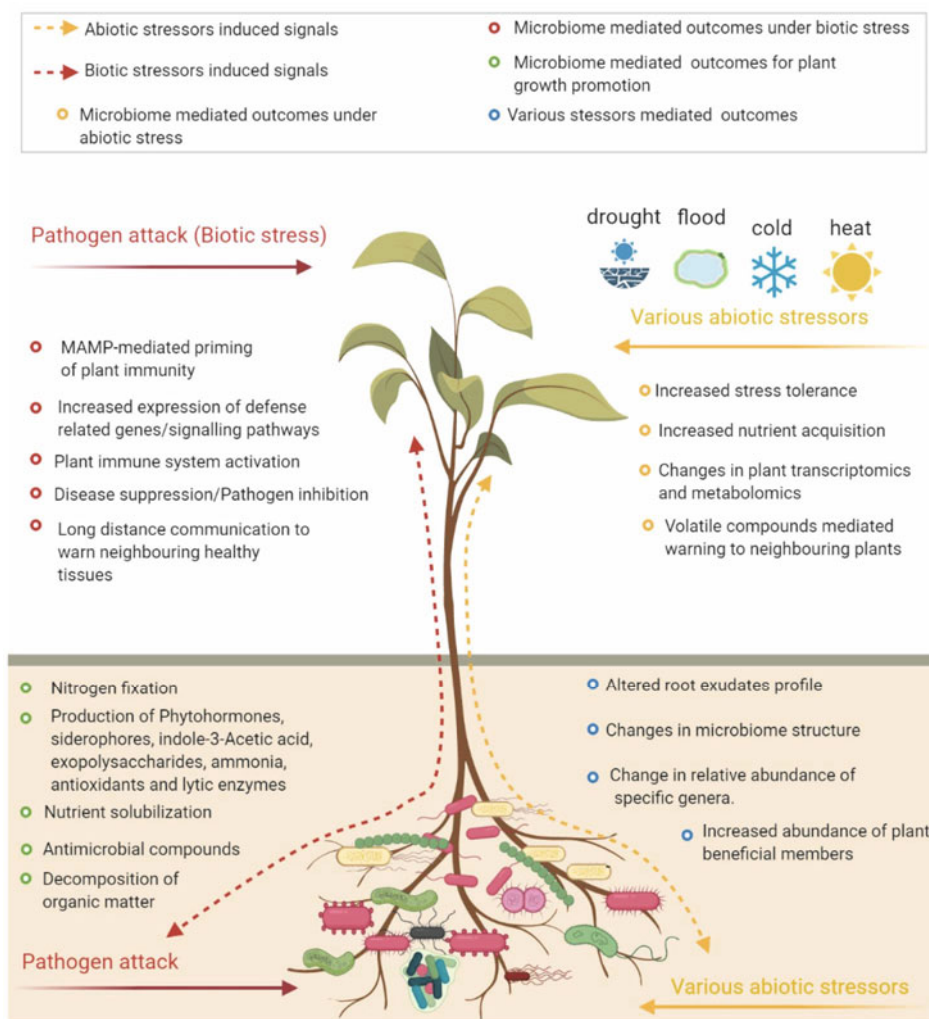
Fig. 1. Modulation of various plant functions by plant-associated microbiome. The plant and its associated microbiome are usually in a mutually beneficial association where the plant provides common goods for the microbial community, and in return the associated microbiota promote plant health and growth. A variety of biotic and abiotic stresses can elicit changes in the plant-associated microbiome. These changes in the microbiome in turn benefit the plant via a variety of mechanisms enabling the host plant to respond better to those stresses.

Microbiome-mediated disease resistance

The role of soil microbiota in protecting plants against various diseases has long been reported. Soil with beneficial microbial content has been described as disease-suppressive soil, with regard to its biocontrol potential (Schroth and Hancock, 1982). In disease-suppressive soils, there has been mounting evidence of plant microbiota-mediated protection against a variety of diseases, such as potato scab disease (Meng *et al.*, 2012), Fusarium wilt (Chialva *et al.*, 2018), damping-off disease by *Rhizoctonia solani* (Mendes *et al.*, 2011), Fusarium wilt of strawberry (Cha *et al.*, 2016), and take-all disease of wheat (Weller *et al.*, 1988). This protection against disease is either a direct outcome of the microbiome or its modulation of the plant's immune system (Millet *et al.*, 2010; Mendes *et al.*, 2011). For instance, a variety of microbes in the rhizosphere have been known to produce antimicrobial compounds, thereby protecting plants (Hassani *et al.*, 2018). For example, the volatile organic compound- and thiopeptide-

producing *Streptomyces* strains protect plants against certain fungal pathogens such as *Fusarium oxysporum* (Hassani *et al.*, 2018). Additionally, during invasion by a pathogen, the plant is capable of releasing specific compounds from the roots, leading to microbial community shifts in the rhizosphere that ultimately result in destruction of the pathogen (Chapelle *et al.*, 2016). Similarly, salicylic acid, a plant defense hormone, can modulate the selection of specific microbial members on plant roots, thereby affecting the overall microbial community structure of the rhizosphere (Lebeis *et al.*, 2015). Furthermore, recent studies with tomato plants and bacterial wilt as a model system showed that rhizosphere microbiota between tomato cultivars are different between susceptible and resistant varieties, and that bacterial members of the microbiota seem to be critical for disease suppression (Kwak *et al.*, 2018) and resistance modulation (Choi *et al.*, 2020).

Plants are capable of not only exploiting their indigenous microbiome for protection against pathogens but also attrac-

ting and selecting microbes from the environment to help them cope with certain types of stresses. This ability of the plant was termed as the "cry for help" strategy (Liu and Brettell, 2019). Some types of stresses could potentially result in inheritable beneficial interactions between microbes and plant hosts, which enable the future generations' survival and growth (Berendsen *et al.*, 2018). For instance, some soils that previously were a source of the fungus causing take-all disease in wheat, were found to cause a less severe disease in future generations (Raaijmakers *et al.*, 1999).

Microbiome-mediated stress tolerance

There is growing evidence that microorganisms are also capable of rescuing plants from various types of stresses (Lau and Lennon, 2012; Lee *et al.*, 2012; Chen *et al.*, 2018; Lombardi *et al.*, 2018). For example, certain bacterial members are often enriched when plants are exposed to pathogen/pest attacks. Thus, plant-associated microbes might have evolved to relieve plant biotic stresses via different mechanisms, including antibiotic production (Raaijmakers *et al.*, 1999), induction of systemic resistance (Berendsen *et al.*, 2018), histone acylation (Chen *et al.*, 2018), abundance of specific bacterial genera (*Flavobacterium* TRM1) (Kwak *et al.*, 2018), and production of nonribosomal peptide synthetases and polyketide synthases (Carrión *et al.*, 2019). Plants are also subject to a variety of abiotic stresses, which have been known to elicit changes in the host transcriptome and metabolome, which could ultimately lead to variations in the plant root exudate profile and thus alter the plant microbiota. Exposure of plants to certain stresses can significantly change the associated microbiota, which in turn can be beneficial not only for the host plant survival but also for the fitness of future generations (Fig. 1).

Microorganisms have the ability to respond to and adapt quickly to variation in the plant root exudate profile and other root-originated stimuli. Various abiotic stresses such as drought (Fitzpatrick *et al.*, 2018), light intensity/limitation, metal toxicity (Timm *et al.*, 2018), and nutrient deficiency (Ham *et al.*, 2018), can lead to remarkable variations in plant microbiota. The stress-mediated changes in microbiota (enrichment of specific genera) are usually beneficial for plant hosts and lead to stress tolerance (Xu *et al.*, 2018). For instance, drought is known to increase the population of some specific phyla such as Actinobacteria both in the plant root rhizosphere and the endosphere (Santos-Medellín *et al.*, 2017; Fitzpatrick *et al.*, 2018; Xu *et al.*, 2018), indicating that plants and their associated microbes use a co-adaptive strategy under a particular stress (Lau and Lennon., 2012). Similarly, phosphate/nitrogen deficiency in plants results in an increase in specific microbial members capable of nutrient acquisition (Ham, 2018). Therefore, there is increasing evidence suggesting that plants utilize the 'cry for help' strategy to interact with and benefit from its microbial counterparts under stress. However, it is still unclear whether this change in the microbial community under stress is mediated actively by the plant or is the result of the stress itself. Despite a growing number of studies exploring how specific members of the rhizosphere microbial community have the ability to promote plant growth, comprehensive understanding of how this microbiome helps the plants cope with various stresses is lacking.

Plants use hormone-mediated signaling to communicate across distant locations within the plant body, which ultimately leads to systemic resistance to various kinds of stress (Pieterse *et al.*, 2009). Such induced resistance in the plant phyllosphere (by leaf damage or hormonal application) can further elicit changes in the rhizosphere microbiota (Pineda, 2017). Similarly, plants challenged with foliar feeding insects or chemical inducers were found to attract certain beneficial microbes, which ultimately enhance plant defense (Lee *et al.*, 2012). Although we know that stressed tissue acts as an alarm for healthy tissue against danger, the detailed nature of these signals and their effect on various plant tissue microbiomes is yet to be understood (Hammerbacher *et al.*, 2019). Volatile organic compounds are the plausible candidates for long-distance communications and are capable of affecting the plant microbiome (Kong *et al.*, 2021), but we do not have sufficient knowledge of the nature and repercussions of the changes that they bring to the plant microbiota (Farré-Armengol *et al.*, 2016; Farag *et al.*, 2017). Overall, a more precise approach is needed to demonstrate and explore the underlying mechanisms that govern plant-microbiome interactions when plants are under stress.

Microbiome-mediated growth promotion and development

A variety of plant-associated bacteria confer plant fitness benefits with a direct or indirect impact on plant growth. Various mechanisms for plant growth promotion include nitrogen fixation; phytohormone (auxins, cytokinins, and gibberellins) production; phosphate solubilization; siderophores, ammonia, and various lytic enzyme production (Kour *et al.*, 2019). Diverse interactions exist among the host plant and its microbiota, which ultimately lead to plant growth promotion and development (Fig. 1). For instance, the tripartite interactions between mycorrhiza, bacteria, and plants have long been known to have a direct positive impact on plant health (Bonfante and Anca, 2009). Specific rhizobacteria (helper bacteria) have been known to interact with mycorrhiza to enhance plant-mycorrhizal interactions and promote symbiosis between the fungus and plant host (Artursson *et al.*, 2006; Labbé *et al.*, 2014). It has been shown that helper bacteria can enhance the receptiveness of plant roots for only the preferred fungi and exclude other fungi (Frey-Klett *et al.*, 2007). Some bacteria that are either endosymbionts of mycorrhizal fungi or just other nitrogen-fixing bacteria can directly influence plant health and growth in a positive way (Glaeser *et al.*, 2016). Plants harbor a variety of microbes, including symbiotic bacteria and fungi, as well as pathogenic microbes (Bonito *et al.*, 2014). Microbial interactions (among the plant microbiota members) are also crucial to support plant growth and health (Durán *et al.*, 2018). Furthermore, studies suggested that symbiotic nodule formation in legumes by rhizobia was coordinated by a broad range of plant root microbiota that contributed to plant fitness benefits (Zgadzaj *et al.*, 2016). It has been also reported that rhizobia are in fact, the important group of bacteria responsible for root growth in non-legume plants, in addition to symbiotic nitrogen fixation in legume plants (Garriodo-Oter *et al.*, 2018). Recent metagenomic approaches have revealed that rhizobia are not the only inhabitants of nodules, and that they are accompanied by other non-rhizobia members (extensively reviewed by

Martínez-Hidalgo and Hirsch, 2017). Although most non-rhizobia bacteria lack nitrogen fixation as well as nodule formation potential, they are still capable of increasing legume survival, especially under stressful conditions (Martínez-Hidalgo and Hirsch, 2017). However, it is yet to be understood how the plant host maintains a balance between the competing demands of these diverse microbial community members and benefits in terms of growth, from their interactions.

Synthetic community approach to dissect plant-microbiome interaction

Because plants harbor an enormous number of microorganisms, it is challenging to determine the role of individual microbes in the complex plant microbiota and to understand microbiome function in a mechanistic way. Conventional approaches have utilized a number of *in vitro* screening strategies and plant-bacterium binary association assays to identify individual interactions; however, these approaches have limitations in understanding plant-microbiome interactions (Bulgarelli *et al.*, 2013; Finkel *et al.*, 2017). This suggests that conventional screening methods are inefficient when it comes to deciphering the complexity of plant microbiota and microbiota-mediated functions. The synthetic bacterial community (SynCom), on the other hand, is a structurally defined/controlled community composed of cultured microbial members that can act as a representative of the function and structure of the original plant-associated microbiome (Vorholt *et al.*,

2017). A great advantage of the SynCom approach is that this community can be manipulated by the addition, elimination, or substitution of strains, to perform desired functions such as growth promotion, and render disease and stress resistance in plant hosts (Fig. 2). Moreover, genetic manipulations can also be carried out; for instance, functions of individual microbes in the SynCom can be removed or enhanced via gene silencing or increased expression, respectively. Bacterial members in a specific SynCom are defined as culturable microbes that lend themselves suitable for research and reductionist approaches to dissect the complexity of the community. When the SynCom approach combines with a plant host in a gnotobiotic condition, one can quantitatively and qualitatively assess plant-microbiome interactions. In addition, the practical application of SynCom has become more important in agricultural ecosystems to overcome the limitations of traditional microbial applications. These include better compatibility, efficient competitiveness with indigenous plant microorganisms, and better adaptability to the environment of the applied SynCom (Hart *et al.*, 2018).

The SynCom approach has been widely adopted in various studies. Extensive cultivation of bacterial members in the rhizosphere and phyllosphere of Arabidopsis dramatically increased the recovery of cultured bacterial isolates among community members (Bai *et al.*, 2015). The SynCom approach with cultured members of leaf and root microbiota revealed that microbial niche specialization and reciprocal relocation between root and leaf microbiota are apparent to have functional overlap of niche-specific microbial members
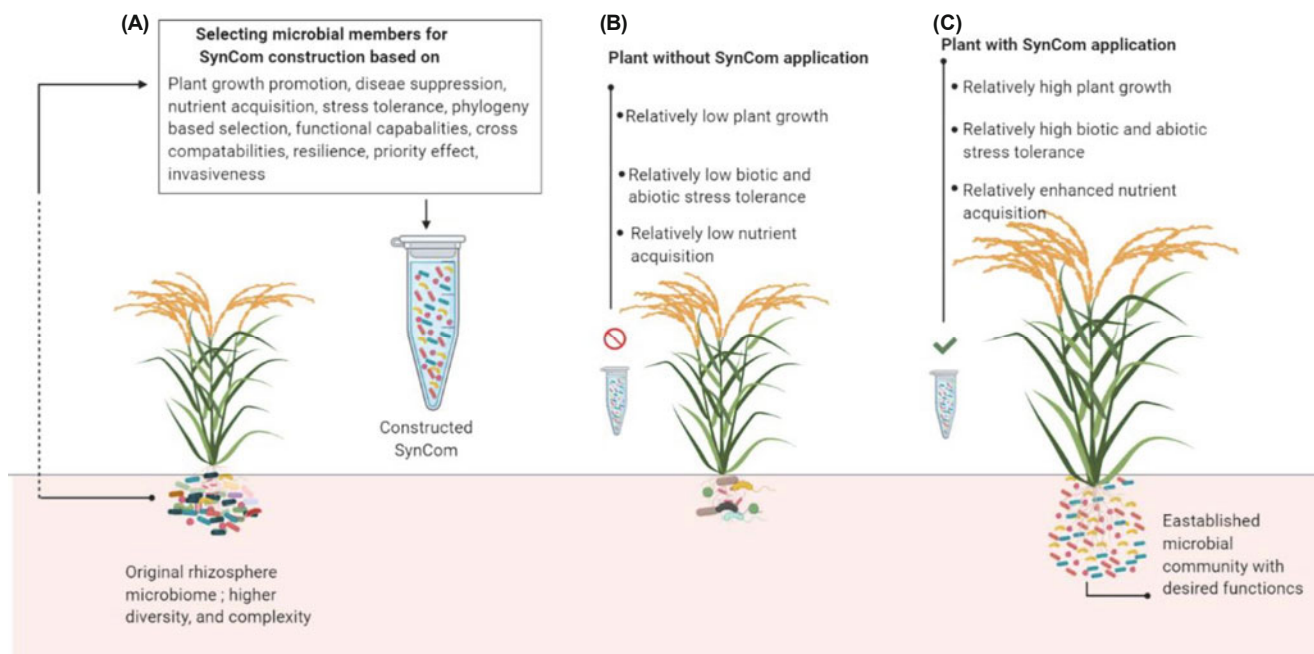

Fig. 2. Construction of synthetic microbial community (SynCom) with desired functions to enhance plant growth and tolerance to biotic and abiotic stressors. (A) The SynCom members are selected (in the above depiction from rhizosphere) based on various criteria such as growth promotion, tolerance to various kinds of biotic and abiotic stress, and nutrient acquisition. SynCom is defined and has a relatively simple community structure and low microbial diversity as compared to the original rhizosphere microbial community. (B) SynCom can be designed and potentially applied to target plants which are either nutrient-deprived or under various kinds of biotic or abiotic stress. (C) Applying the SynCom comprising of defined microbial members with desired functions could potentially affect plant growth and health by either promoting nutrient acquisition or providing tolerance against biotic and abiotic stress via various mechanisms.

(Bai *et al.*, 2015). This pioneering work suggested that the SynCom approach would be a powerful strategy to dissect plant-microbiome interactions. SynCom was further adopted to define core members of the plant microbiota related to specific plant functions, such as phosphate stress and immunity (Castrillo *et al.*, 2017), salicylic acid-mediated root microbiota assemblage (Lebeis *et al.*, 2015), plant root growth (Finkel *et al.*, 2020), plant growth and health (Niu *et al.*, 2017), and disease suppression (Carrión *et al.*, 2019). SynCom itself has wide application in the dissection of plant-microbiome interactions; however, it is also likely that SynCom can reinforce and prove the results obtained in various plant microbiota studies using the cultured microbial members. Therefore, cultivation of a wide variety of community members representing the plant microbiota is a prerequisite for conducting an experiment with SynCom (Vorholt *et al.*, 2017). So far, most SynCom experiments have been restricted to Arabidopsis-microbiome interactions due to the limitations of bacterial culture collection. Several microbial members need to be cultivated from other model plants to study plant-microbiome interactions and also establish the cross-utility of SynCom over various plant species (Roy *et al.*, 2019). Various studies have used the small-scale SynCom approach to examine the contribution of microbial members to plant growth and phosphate mobility (Baas *et al.*, 2016), better nutrient uptake in wheat (Dal Cortivo *et al.*, 2018), and improved drought tolerance (Molina-Romero *et al.*, 2017).

Perspective: To understand plant-microbiome interactions

Advanced multi-omics technologies such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics, and tools for large-scale biological data analysis have been rapidly developed in the field of human and plant microbiome research. Long-term research goals have been facilitated by the European Community's Seventh Framework Programme, and the Earth Microbiome Project (EMP), through provision of a huge database of microbes, and a standard protocol of microbiome analysis (Qin *et al.*, 2010; Gilbert *et al.*, 2014). Likewise, a well-organized long-term research plan for plant microbiome analysis that can be applied to modern agricultural practices is indispensable for the impending global agricultural challenges (i.e., rapid growth of human population and climate change).

  Several studies have reported that a core microbiome is present in plant microbiota. Single or several bacteria as a core microbiome play a crucial role in retaining root growth (Finkel *et al.*, 2020), plant growth promotion (Berendsen *et al.*, 2018), and the incidence of disease in tomato plants (Lee *et al.*, 2020). Model systems shed light on the role of the core microbiome with multifaceted perspectives in terms of establishing causality of observed phenotypes in plants. These empirical approaches provide a better understanding of plant-microbiome interactions, resulting in the discovery of co-inciding interactions among many microorganisms. Mechanisms influencing the composition of beneficial microbiota have not been fully understood because of the inherent complexity of biological interactions; they need to be investigated

using model systems among microbes and those between microbiota and host plants. The systematic cultivation of plant microbiota decreases the fickleness caused by the inheritance of a complex community and makes it possible to test principles of plant-microbiome interactions under controlled circumstances of a specific community. An advanced strategy to dissect the plant microbiota may be needed to determine the mechanisms influencing not only microbial interactions but also the community structure and function *in vivo* and *in planta*. The SynCom approach with several culturable bacteria associated with plants, grown in well-defined environments, may provide an interpretation of the role of microbial compositions; microbial interactions; and microbial genes, proteins, and metabolites. This approach would elucidate the functional mechanisms and the specific interactions between the microbiota and its host plants. Therefore, SynCom enables not only the validation of the dissected mechanism in plant-microbiome interactions *in vivo*, but also the transfer of an intriguing discovery generated from natural conditions to an empirically consistent system in the laboratory. Moreover, the SynCom experimental output can be verified in natural field conditions for practical purposes.

  In the model crop plants under natural and agricultural environments, the structural and functional analyses of plant microbiomes have been investigated. The complexity and dynamics of microbiome analysis can be addressed using recent advancements in artificial intelligence (AI), including machine learning algorithms that integrate comprehensive data of plants *in vivo* and in natural field conditions (Murphy, 2012). Nevertheless, this comprehensive data has not been sufficiently generated in agricultural science as compared to medical science (Toju *et al.*, 2018). Comprehensive efforts to integrate the AI-based approach with a sufficient amount of plant microbiome data, are required for wholesome understanding of the plant-microbiome interactions and for further application of the research output to agricultural practice.

  Although the structure of plant microbiota and the complex interplay between plant host and microbiota have been revealed to a certain extent, there are still several important questions to be answered. Here, we enlist some of them: 1) What are the principles of plants and microbes to have a unique microbiota structure in the endosphere compared to the rhizosphere? Are there certain plant gate-keeping systems evolved to selectively allow certain microbes to become endophytes? Do endophytic microbes have unique traits to dwell inside the host plant? 2) What are the plant's genetic principles to differentiate their foes (i.e., pathogens) from their friends (the large assemblage of commensals)? How do plants recognize their friends to nurture and maintain the community successfully in a certain defined population? 3) How does the plant microbiome modulate plant function? Do they work in a syntropic way (i.e., in a way to mimic multi-cellular organisms) to ameliorate plant function? Much remains to be determined; however, both systematic and reductionist approaches need to be combined to thoroughly analyze the plant microbiome function. For instance, AI-based refining of the microbiome data, multi-omics analysis of microbial community *in planta*, and SynCom approach could be complementary strategies that help our understanding of the complex plant microbiome and its function. A thorough understanding

of plant-microbiome interactions and the proper translation of that understanding to practice, would definitely help us to sustain healthier plant ecosystems with higher productivity.

Box 1.	Terminology and definitions
Microbiota:	All microbes in a particular environment.
Microbiome:	The collection of the genomes of microbes in a particular environment.
Holobiont:	Totality of host organisms and its symbiotic microbiota
Mutualism:	Beneficially ecological interaction between two organisms
Symbiosis:	A close relationship between two species in which at least one species benefits.
Commensal:	Biological interaction in which one organism gains benefits without influencing the other
Phyllosphere:	The microbial habitat defined by the surface of aboveground plant organs.
Rhizosphere:	The microbial habitat around plant roots in which physical, chemical, and biological composition is influenced by the root's growth, respiration, and nutrient exchange.
Endosphere:	The microbial habitat inside plant organs including both above- and below ground.
Core microbiome:	Microbes play pivotal roles in assembling plant-associated microbiomes inside and around host plants.
Synthetic community (SynCom):	The combination of culturable microbes for the reductionist approaches to enable testing of hypotheses by targeted manipulation.
Gnotobiotic:	Condition of a germ free organism
Disease-suppressive soils:	Soil in which crop plants suffer less from specific soil-borne pathogens than expected because of the presence and activities of microorganisms (other than pathogens) in that soil.

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Conflict of Interest

We have no conflicts of interest to report.

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