Root surface as a frontier for plant microbiome research

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Plants associate—analogous to animals or us humans—with a multitude of microorganisms, which collectively function as a microbiome. A major discovery of the last decade is that numerous organisms of a microbiome (aka microbiota) are not unpretentious background actors. Instead, some microbiota members influence host processes including behavior, appetite, and health in animals (1) and contribute to nutrition and health of plants (2–4). Recently, the compositions of the plant root-associated microbiota from numerous plant species, including major crops, were revealed using high-throughput DNA sequencing. Factors such as soil type or host genotype influence the root-associated microbiota. However, the processes that determine the acquisition of the root microbiota, its resistance to stress, and its ecological function remain poorly understood. Edwards et al. (5) present the third publication in a recent series of PNAS articles about the bacterial microbiota associated with plant roots of maize (6), related Brassicaceae (7), and now *Oryza sativa* (rice). It comprises a comprehensive characterization of three microbial habitats that are in the proximity of, on, and inside plant roots, which are named rhizosphere, rhizoplane, and root endosphere (Fig. 1).

A major advance of Edwards et al. is the description of the acquisition process of the root endosphere microbiota (5). In contrast to the gut microbiota, which is partly inherited from the mother, the root endosphere microbiota is largely reestablished every time a plant germinates. Until now, the acquisition of the endosphere microbiota was proposed to occur in two steps: root exudates, presenting a complex mixtures of organic compounds that are secreted by plant roots, trigger a first general enrichment in the rhizosphere, followed by a host genotype-dependent differentiation of the microbiota "thriving on the rhizoplane and within plant roots" (2). Edwards et al. (5) reveal the early steps of root microbiota acquisition based on a high spatial resolution of root habitats in time series experiments. The authors transplanted sterile germinated seedlings into soil and sampled the root-associated habitats from time points between 1 and 13 d after transplantation. The microbiota comparison in space and time revealed that the habitat-specific community structures were largely established after 1 d. Although the composition of the root endosphere was organized within 1 d, the steady-state size of the bacterial population was reached in 13 d. This work permitted refinement of a two-step model of root microbiota assembly (2) to a model with at least three selective steps, with the rhizoplane as a key component that serves a critical gating role for controlling microbial entry into the host tissue (Fig. 1).

Model

The root endosphere microbiota results from gradual community shifts including enrichment and, mainly, depletion processes from the surrounding soil microbiota presenting the start inoculum (Fig. 1). The enrichment process begins to act at a distance in the rhizosphere, continues at the rhizoplane, and is likely to be largely driven by root exudation. Unlike enrichment, the exclusion process appears to operate more intimately: first on the rhizoplane and then more pronounced in the root endosphere. One explanation for the first step of exclusion is that the rhizoplane selects for bacteria which are able to form biofilms and successfully compete in the presence of elevated nutrient levels. Presumably, the second step of exclusion results from the selection of the rhizoplane bacteria which possess traits enabling the colonization of the root endosphere. Such microbial traits may allow the bacteria e.g., to evade recognition or to manipulate host defense reactions. We assume the involvement of microbial traits that subvert host immune processes because

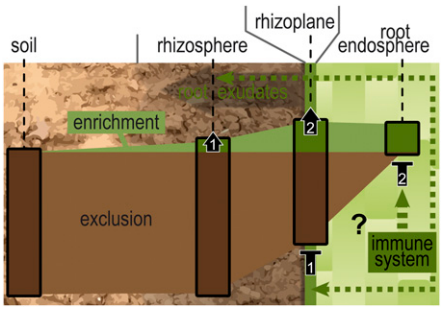

Fig. 1. Acquisition of the plant root endosphere microbiota from soil. Gradual shifts in microbiota composition occur in the root-associated habitats including a zone of soil surrounding the roots (rhizosphere, gray shading) to which root exudates are secreted, the root surface (rhizoplane), and the inner host tissue (root endosphere). Bars illustrate compositional changes in each microbial habitat due to enrichment (black arrows) and exclusion (black T-symbol) processes. Bars are scaled using the number of DNA sequences that change in abundance (enrichment/exclusion) in the rhizosphere (152/17), the rhizoplane (422/730), and the root endosphere (394/1,961) compared with soil (see ref. 5).

it appears, paradoxically, that plants permit endosphere colonization, whereas host cells initiate defense responses on the detection of molecular epitopes, which are conserved throughout the bacterial kingdom (8). Hence, we speculate that the host immune system influences microbiota selection in general and that it has a strong impact at the second step of exclusion from the rhizoplane to the root endosphere microbiota (Fig. 1).

Host genotype-dependent variation in microbiota composition may occur where host physiological processes (e.g., root exudates or immune system) are involved. The different rice cultivars varied noticeably in their rhizosphere communities, whereas the rhizoplane or root endosphere microbiota was little affected by the host genotype (5). Also, maize inbred lines exhibited quantitative differences in rhizosphere microbiota composition (6). This contrasts observations in *Arabidopsis* where endosphere and not rhizosphere communities were affected by

Author contributions: M.G.A.v.d.H. and K.S. wrote the paper.

The authors declare no conflict of interest.

See companion article on page E911.

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the host genotype (9, 10). It appears that host genetic differences in microbiota composition can occur in all root-associated habitats, but that the habitat where the host genotype-dependent variation emerges depends on the plant species. Note that the host genotype-dependent effects discussed here affect community composition at best at the level of microbial species because the 16S profiling technique (used in refs. 5, 6, 9, and 10) does not resolve subspecies variation between microbiota members, and this is the level of variation that determines the outcomes of most plant–microbe interactions (8).

Frontiers

Numerous key questions emerge for further plant microbiome research including the following. What is the ecological function of the plant microbiome? What are the roles of core microbiota members that are shared between many plant species? How do plants interact with the microbiota and what is the molecular cross-talk between host and associated microbes? Can we localize the microbiota in the endosphere by, for example, using in situ hybridization methods? Finally, can we capitalize on the plant microbiome to enhance yield and sustainability in agriculture? Below we detail three frontiers.

The majority of microbiota studies including Edwards et al. (5) focus on a single group of microbes, e.g., prokaryotes. However, many plant species including major crops such as rice, maize, and cereals are colonized by a wide range of fungi, including mutualistic arbuscular mycorrhizae (4, 11). Clearly, the simultaneous examination of bacteria and fungi in plant roots deserves further attention. The study of multitrophic interactions between bacteria, archaea, and fungal members of the root microbiota is currently in its infancy and is likely to reveal additional insights in microbiota functionalities.

Beyond the examination of community composition, a major frontier is to investigate the functions of the plant microbiota. Individuals of the plant microbiota can provide a number of beneficial services to the host plant including delivery of nutrients, protection against disease and tolerance to abiotic stress (2, 4, 11, 12). However, our knowledge of such plant growth-promoting microbes is largely biased to studies conducted on individual isolates under laboratory conditions, and we have a limited understanding of how entire microbial communities contribute to plant growth. Recent observations that a high diversity of microbial communities in soil, including major members of the plant root microbiota, has a positive impact on a range of plant species and on the functionality of an ecosystem, exemplifies the benefits from complex diverse microbial communities (13).

The advances in basic microbiome science in the human and animal fields have triggered efforts for using the microbiome to improve human health (1). Likewise, can we exploit the microbiome to enhance plant productivity in agricultural settings (14)? The control of pathogen burden or the increase of nutrient use efficiency would permit the reduction of agrochemical inputs, thereby promoting a more sustainable agriculture. We may be able to manipulate the host side of the interaction, as done during breeding for disease-resistant cultivars, by selecting lines with enhanced responsiveness to beneficial services of the root microbiota (15). Complementarily, we might try to improve plant performance through the active manipulation of the root microbiome of crops (e.g., by coating seeds or preinoculating seedlings with particular microbes).

Edwards et al. reveal the early steps of root microbiota acquisition based on a high spatial resolution of root habitats in time series experiments.

Tools

One approach for new insights in the plant microbiome relies on the systematic isolation of root microbiota members (16). Reference stocks of pure isolates together with the information on community composition allow the reconstruction of at least the cultivable fraction of the root microbiota. Inoculation experiments should make it possible to examine the interaction of such synthetic communities with the host plant to unravel the functions of whole communities and their individual members. Here, genome and transcript sequencing is expected to reveal the microbial traits which are expressed and relevant in the interaction with the plant. Complementary to such cultivation approaches, the direct sequencing of complex mixtures of DNA from various organisms of a habitat (metagenomics) reveals the metabolic capacity of a microbiome. In rice, numerous microbial traits such as nitrogen fixation, the flagellum, protein secretion systems, or quorum sensing, and their habitat specificity were predicted using metagenomics (17, 18).

Conclusion

The study by Edwards et al. (5) unifies innovative basic science, insights into applied aspects, and stimulating elements such as the exploration of coabundance networks. As in this study, the use of state-of-the-art methodology in well-designed experiments and testing clearly formulated hypotheses with a high degree of scientific rigor advance the field of plant–microbiota interactions and contribute to a more holistic understanding of the plant microbiome.

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