COMMENTARY

Leaf-derived bacterial communities adapt to the local environment

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Plants host a diverse community of microbes known as the microbiota. A number of studies have used culture-independent sequencing methods to describe the composition of these communities (1–3). While the microbiota originates from the broader environment in which the plant is growing, it is composed of only a subset of microbial taxa and is remarkably consistent at higher taxonomic levels among plants from diverse species and geographic locations (4, 5). Given the robustness of the microbiota community structure, the plant is thought to act as a filter, selecting for specific taxa that can thrive under close association with the plant host (6). At lower taxonomic levels, the microbiota of roots and aerial plant parts differ, which suggests further adaptation of the microbiota for specific local conditions of the respective plant organs. While the microbiota is known to change under different environmental conditions, little is known about the dynamics of these changes, and whether they represent adaptation of the community members (7–9). In PNAS, Morella et al. (10) adopt an approach similar to experimental evolution to study changes in the leaf microbiota over successive plant inoculations and the adaptation of the resulting communities to their environment.

An initial inoculum collected from leaves of field-grown tomato plants was used to spray-inoculate tomato plants in the greenhouse. After several weekly inoculations, the microbial communities from aerial plant parts were again collected and used to inoculate a second round of plants. This process was repeated for a total of 4 rounds of inoculation and collection, a process referred to as passaging (Fig. 1*A*). The starting inoculum and those collected after each passage were profiled based on DNA sequencing of bacterial and fungal marker genes, which were assigned to operational taxonomic units (OTUs) based on 97% sequence similarity.

Analysis of the bacterial OTU profiles after successive passages revealed interesting changes. The overall diversity of the microbiota was strongly reduced, with many OTUs of the original inoculum being lost. However, the vast majority of the OTUs in the final community could still be traced back to the original inoculum. Control plants, which had been sprayed with sterile solution or heat-killed bacteria, had much lower absolute bacterial load, and many OTUs from the original inoculum were detected. Thus, even though the plants were grown in nonsterile conditions that allowed microbial contamination, bacteria from the greenhouse were outcompeted by the leaf-derived inoculum. This suggests that the inoculated microbes were better adapted to the leaf environment. The fungal component of the microbiota also showed a reduction in diversity after passaging. However, after the fourth passage, the fungal community was dominated by an OTU that was not detected in the initial inoculum.

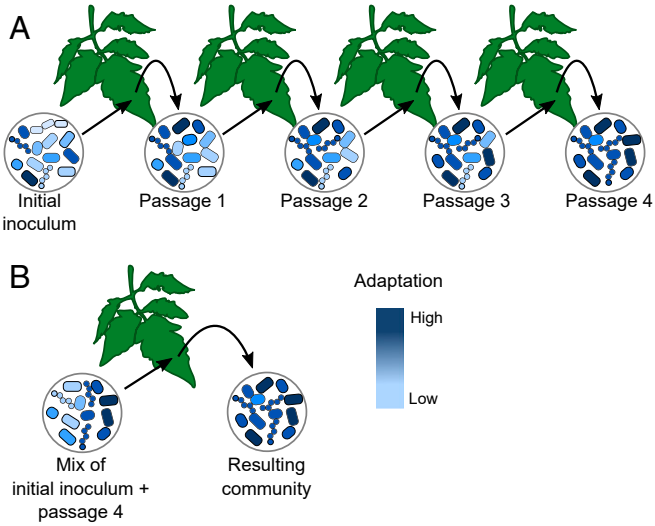

Fig. 1. Adaptation of leaf microbial communities over successive rounds of inoculation. (*A*) A diverse initial inoculum is sprayed onto tomato plants, and the microbial community that develops is collected, in a process known as passaging. Over several passages, the diversity of the community decreases. (*B*) The initial inoculum is mixed with the community after the fourth passage and used to spray tomato plants. The resulting community strongly resembles that of the fourth passage, indicating that the selected microbes are adapted to the leaf environment.

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To test how plant genotype influences the microbiota, a number of tomato lines that differed in their suite of disease resistance loci were included. After the first 2 passages, the plant genotype did exert a significant effect on community composition. However, the genotype-driven variation in community composition did not correlate with disease resistance or susceptibility designation. This is perhaps not surprising, as resistance genes mediate immunity to very specific pathogen strains (11). Although the plant immune system likely exerts some control over microbiota members, previous studies have found only subtle changes in the microbiota composition of even strongly immunocompromised plants (12). The early differences in microbiota among different genotypes in the study are therefore likely due to other, as-yet-unknown differences between the genotypes. Following further passages, the effect of genotype became much weaker, and the genotype did not explain a significant amount of the variance in the final communities.

The authors then analyzed which specific OTUs changed in relative abundance over the successive passages. The 100 most abundant bacterial OTUs belonged almost exclusively to the 4 phyla considered to make up the core plant microbiota, namely Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (1, 2). The shift in community composition after passaging was not due to changes in the relative abundances of large taxonomic groups. Rather, there seemed to be changes in the relative abundances of closely related OTUs. For example, 2 OTUs within the Pseudomonadaceae were highly abundant in the initial inoculum but much less abundant after the fourth passage, while a third Pseudomonadaceae showed the opposite pattern. However, care must be taken in the interpretation of OTU-level changes, as a single OTU may represent multiple closely related strains that nevertheless show important functional differences (13).

To rule out the possibility that the passaging process merely represents a bottleneck event in which relatively abundant taxa are more likely to be propagated, the authors examined the relationship between relative abundance of the OTUs and their occupancy, or the proportion of plants in which they were present. After the first passage, they observed a strong correlation between relative abundance and occupancy, suggesting that abundant taxa indeed have a higher probability of being collected and subsequently reinoculated. However, this relationship became weaker after multiple passages. Thus, lower abundance OTUs could be transferred consistently during passaging, while many abundant OTUs were unable to persist.

To confirm that the observed change in microbial community reflected an adaptive process, plants were sprayed with a mixed inoculum, containing an equal number of viable cells from both the initial inoculum and the community recovered after the fourth passage. Interestingly, the resulting bacterial community on these plants was indistinguishable from that of the fourth passage (Fig. 1*B*). Successive passages on the greenhouse tomatoes therefore seemed to select for strongly adapted OTUs, which prevent less adapted OTUs from becoming established.

It is notable that such a strong adaptive change would occur after 4 passages, given that the initial inoculum was collected from the same plant species and organ. One possibility is that the resultant microbial community becomes adapted for the specific conditions of the plants in the greenhouse. The initial inoculum was collected from the leaf surface of field-grown tomato plants located in multiple fields. Thus, it may be composed of microbes adapted to various environmental conditions, as well as non-adapted microbial tourists introduced by atmospheric drift such as soil dust and liquid aerosols. It would be interesting to see how the trajectory of the community shift during passaging is affected by altering the conditions under which the plants are grown.

The experimental design may also have allowed the community shift to be observed. Carlström et al. (14) recently showed that a few keystone bacterial strains can strongly influence the leaf microbial community through their effects on other strains. However, once the microbiota is established, it is remarkably robust, with later addition of single bacterial strains or even larger taxonomic groups having little effect on the existing community structure. By providing multiple opportunities for microbiota establishment, the passaging approach may therefore have allowed nascent differences in community structure to manifest. As the plants were grown in nonsterile conditions, they would have contained a standing microbial community prior to the first spraying. Although the inoculated community was apparently able to invade the standing community, this may have affected the final community structure.

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The timing of bacterial inoculation is also relevant for the community formed from the mixed inoculum, as both groups of microbes were added to the plant simultaneously. It would be interesting to test whether the microbes from the fourth passage would be capable of supplanting a community previously allowed to establish from the initial inoculum. In addition, microbiota samples were collected relatively soon after the final spray inoculation, which may favor faster-growing strains that are able to quickly achieve high abundances. It is possible that a longer incubation time would allow more slow-growing OTUs to increase in number, reverting the community structure to one more resembling the initial inoculum.

It is unclear why particular OTUs from the initial inoculum were able to grow more competitively than others under the experimental conditions. Identification of the functional traits required for microbes to successfully colonize and persist on plants remains an active area of research (15). Previous studies have sought to identify bacterial genes associated with root colonization through genomic analysis. Levy et al. (16) compared the genomes of plant-associated and non–plant-associated bacteria, and identified a number of genes that contribute to survival on plant roots. Since they analyzed bacteria isolated from diverse plant species, however, the genes that they identified are unlikely to be involved in adaptation to specific conditions.

Another strategy to identify traits required for adaptation to the plant host is to examine genes with differential frequency after colonization compared to the starting inoculum (15, 17). While Morella et al. (10) do not report metagenomic data for their samples, a comparison of the gene frequencies between the initial inoculum and the adapted community would offer an excellent opportunity to identify genes and gene pathways that are found in a higher proportion within the community after passaging, and therefore may be important for persistence on the plant leaf.

The plant microbiota can have important beneficial functions for plant health, including resistance to abiotic stress or protection from pathogens (8, 9, 18). Since humans are reliant on plants

for survival, translation of these functions to crops could be hugely beneficial. In many cases, however, the favorable effects are mediated by specific members of the microbial community. Without further physiological experiments, it is impossible to determine whether the change in leaf microbial communities observed by Morella et al. (10) confers any adaptive advantage to the plant host. Increased knowledge of the factors driving how and when microbiota composition changes is essential to allow for rational intervention of the microbiota such that the desired members of the community are present.

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