

Researchers cultivate the majority of bacteria in the laboratory that colonize Arabidopsis plants in nature

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Arabidopsis thaliana. Credit: Wikipedia.

No organism is an island - a fact that also applies to plants. Healthy plants host complex microbial communities comprising over 100

bacterial species which presumably play important roles in plant growth and health. Plants allow access only to a select community of bacteria, designated the plant microbiota, that originate mainly from a vast diversity of microorganisms present in natural soil. Researchers from the Max Planck Institute for Plant Breeding Research in Cologne together with scientists in Switzerland cultivated over half of the bacteria found on and in the leaves and roots of the model plant *Arabidopsis thaliana* (thale cress). Using this representative collection of over 400 bacterial strains in pure culture, the researchers can now reconstitute any microbial community in the leaves and roots of *Arabidopsis* under laboratory settings. This development marks the beginning of a new era in plant-microbe ecology using defined microbiota.

One aim of the relatively young field of plant microbiota research has been to generate an inventory of plant-associated microbial communities. Paul Schulze-Lefert from the Max Planck Institute for Plant Breeding Research in Cologne and Julia Vorholt from the ETH Zurich and their colleagues have now taken another important step towards this goal. The team cultivated far in excess of half of the [bacterial species](#) colonizing leaves and roots of *Arabidopsis* plants grown in nature and established a collection of [bacterial strains](#) with which the [leaf](#) and root microbiota can be reconstituted on germ-free plants.

The astonishing similarity between the [bacterial communities](#) produced in the laboratory and those found in nature opens the door to microbiota reconstitution biology. The use of such defined communities enables for the first time controlled perturbation of the microbiota under controlled environmental conditions without the vagaries that are inevitable in nature due to environmental fluctuations.

The two scientists cultivated up to 65 percent of the bacterial species found in the root microbiota and up to 54 percent of the species of the leaf microbiota as pure cultures. Yet according to the received wisdom

in microbial ecology states, it is not possible to cultivate more than one percent of the bacteria from natural environmental samples. "This is simply incorrect," says Schulze-Lefert. "We know from our bacterial cultivation efforts that our core culture collection contains the majority of the bacterial species that is present in the communities and provides a very good representation of the taxonomic diversity of the natural leaf and root microbiota. The collection may not be perfect but it provides a very good starting point for microbiota reconstruction experiments," he adds.

Upon closer inspection of bacterial species profiles, Schulze-Lefert and Vorholt observed a considerable similarity between the microbial communities found in the Arabidopsis leaf and root. "Almost half of the species are identical," explains Schulze-Lefert. "Despite the fact that the samples for the root microbiota were collected in Cologne and those for the leaf microbiota in Zurich and Tübingen, if you consider the root and leaf microbiota from a higher taxonomic perspective, that is from the level of the bacterial families and classes present, there are no differences at all. So the microbiota are highly robust in different natural environments," he adds.

The Research Groups determined the genomes of 432 bacterial isolates from their collection by DNA sequencing and compared them. "Not only do we have pure cultures for the reconstitution experiments, we also know the complete genome of each community member in our core collection," says Schulze-Lefert. The scientists were then able to compare the biochemical capabilities of the leaf and root microbiota. To do this they combined the genes from the genomes with a computer into functional networks. The similarity of the profiles of bacterial species present in the leaf and root communities corresponds to an extensive overlap in functional capabilities encoded by the corresponding genomes. Such experiments are possible today because the biochemical functions of many genes are known, as are the cellular networks and

metabolic reactions in which they are involved.

Due to the extensive overlap of genome-encoded functional capabilities and the similarities of the species profiles, Vorholt and Schulze-Lefert concluded that the majority of the leaf- and root-associated bacteria originate from the extraordinarily diverse soil microbiota. This suggests that a plant's leaves are colonized mainly by soil-derived bacteria via the root as stopover site.

Nevertheless, it is also possible to observe functional differences encoded by the genomes of leaf- and root-associated bacteria. These relate to apparent differences in the ecological niches of the leaf and root. This can be construed, for example, from the bacterial genes needed to feed on complex carbohydrates that are present on leaves and roots; the root microbiota needs fewer of these, as only roots exude large amounts of simple sugars.

The scientists also carried out microbiota reconstitution experiments. To do this, they used a closed artificial environment with sterile clay as a soil substitute, a sterile liquid nutrient medium without organic carbon, and germ-free *Arabidopsis* seeds. Cultivation was carried out in transparent sterile chambers which were inoculated with defined bacterial communities at different time points. "Although the system is highly artificial, the communities that populate the leaves and roots are remarkably similar to the communities on plants grown in nature," says Schulze-Lefert.

Even if the defined microbiota inoculum was added to the clay or on leaves alone, the leaf or root microbiota were able to populate not only their corresponding plant organ but, to a considerable extent, also the remotely located leaf and root organ. If the leaf and root microbiota were mixed and then applied to the clay, the root microbiota outcompeted the leaf-derived bacterial community in the root. A

corresponding competitive advantage exists for the leaf microbiota in leaves. This points to a specialization of bacterial communities to leaf and root ecological niches despite the extensive functional overlap between the leaf and [root](#) microbiota.

The scientists are at an early stage in these microbiota reconstitution experiments. They can now leave out individual bacterial species or entire families from the defined microbiota and test these under different environmental stress conditions. They expect that such controlled perturbation experiments will provide molecular insights into how the bacterial communities mobilize and supply soil nutrients for plant growth and how the microbiota protects its host against microbial pathogens.

More information: Yang Bai et al. Functional overlap of the Arabidopsis leaf and root microbiota, *Nature* (2015). [DOI: 10.1038/nature16192](#)

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