

Shining a light on the dark world of plant root-microbe interactions

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Interactions between bacteria and roots are critical to the terrestrial ecosystem. The zone of soil immediately surrounding roots is known as the rhizosphere and the surface of the root the rhizoplane (1, 2). This region is of paramount importance to the growth and productivity of plants because it is the main area where they interact with an enormously complex microbial community; the microbiome. Soil may contain up to 10^4 bacterial species and 10^9 bacteria per g as well as a diverse archaeal and eukaryotic population (3, 4). While the diversity of bacteria in the rhizosphere is less than in soil, it is much more active because it is under strong plant selection and has access to root exudation. Plants may release up to 20% of their photosynthate via their roots, which is critical in shaping and possibly farming the root microbiome. This microbiome consists of symbionts, commensals and pathogens that will interact with roots, the soil and one another to form a root holobiont that determines much of biological and agricultural productivity. In spite of the immense importance of the root environment, it is often overlooked because it is challenging to image and study roots that are underground and are difficult to observe without disturbance. For example, compare the ease of sampling shoots relative to roots. One of the principal ways of overcoming this is to image roots with X-ray computed tomography (X-ray CT scanning), a powerful way of building three dimensional images of root growth in a spatial and temporal fashion (5). However, this approach is not suited to examine the critical interaction of microbes with roots. To overcome this, plants can be grown between thinly separated glass sheets, known as rhizotrons, on agar plates or similar artificial media and imaged by a wide range of physical and microscopic techniques. This tends to be done at one time point and does not allow dynamic mapping of the spatial and temporal interaction of microbes with roots. A further recent advance is the use of a microfluidics based RootChip to monitor root development (6). However, a breakthrough in the live imaging of roots and bacteria, is the report of Massalha et al 2017 using a technique called TRIS (Tracking Root Interactions System). For TRIS analysis, roots are grown into a narrow root chamber (160 μ m high) contained in a microfluidics device. Bacteria are introduced into this chamber and imaged with a wide variety of techniques, including dark-field and confocal microscopy. The microfluidics chamber was made by using soft lithography to etch a single sheet of polydimethylsiloxane with 9 separate chambers, each for a single *Arabidopsis* root, with separate input and output channels for bacteria. Using *Bacillus subtilis*, there was a remarkable accumulation of bacteria observable in just 20 min, behind the root tip in the root elongation zone. This could be further imaged every 30 min up to 12 hours, with later colonisation visible further up the root. The root elongation zone was elegantly and precisely mapping as the region of initial accumulation using Gfp marked lines of *Arabidopsis* (*COBL9*, *CORTEX*, *WER*, *SCR* *WOL* and *PET111*) and mKate labelled bacteria. Thus the root elongation zone appears to be a special hotspot in the initial interaction of roots and microbes. Some

of this may result from it being a major site of exudation. Such rapid accumulation of bacteria at the root elongation zone must require chemotaxis of motile bacteria to plant metabolites (7). Given the broad range of metabolites secreted by *A. thaliana* roots it is not possible to say whether this is a reaction to a specific or multiple metabolites. However, preliminary metabolomics conducted on root secretions in this study revealed the presence of several amino acids that are known chemoattractants. Following on from this, an exciting use of TRIS will be to screen mutant bacteria to determine whether specific chemotactic signals are required and whether there are specific recognition and signalling mechanisms involved. It is known that early attachment of *B. subtilis* requires the chemoreceptors McpB and McpC, and the orphan receptor TlpC (8), therefore it will be of interest to test these mutants in the TRIS system.

Bacterial chemotaxis is almost certainly involved in bacterial migration to roots, followed by attachment and biofilm formation (9-11). Many processes in initial attachment and biofilm formation are conserved amongst bacteria. *B. subtilis* requires exopolysaccharide (EPS) and proteins such as TasA to form a biofilm matrix on roots and it has also been shown that induction of a biofilm is stimulated by the plant polysaccharides pectin, xylan and arabinogalactan, with the latter important in *A. thaliana* (9). Furthermore, the regulatory proteins SinA and SpoA and up to five Kin sensor kinases appear to mediate the stimulation of biofilm formation by plant polysaccharides. Likewise, in pseudomonads, biofilms also require EPS but in addition involves the 6310-amino acid LapF protein to adhere to plant roots (12). In the well-studied case of *Rhizobium*, attachment to root hairs at mildly acid pH (6.5) is initially mediated by end on attachment of polar-located glucomannan to pea lectin (13). *Rhizobium* adhering proteins (RAPs), then become essential, possibly by binding to EPS to initiate a biofilm complex (14). In addition, cellulose microfibrils are made which reinforce the biofilm matrix. In rhizobia, binding to root hairs can lead to root hair curling, entrapment and infection thread development through which bacteria traverse the developing nodule before being engulfed by plant cells in the nodule cortex (15). The details of rhizobial attachment have been intensively studied because N₂ fixation by rhizobia contributes a substantial portion of the biospheres available nitrogen. A critical point concerning attachment in different bacteria is that it occurs at later stages in root colonisation than accumulation at the root elongation zone. For example, while much attention has been focussed on attachment in rhizobia which occurs on differentiated root hair cells in contrast to the root elongation zone, which contain undifferentiated cells. This implies there are likely to be multiple stages in attraction to roots, initial colonisation and biofilm formation, as well as attachment to root hairs. The work of Massalha (16) suggests we may have underestimated the spatial and temporal separation of steps in root colonisation. Indeed, the first step in colonisation may be to the newly divided cells of the root elongation zone which is not already colonised by bacteria that have formed a highly resistant biofilm.

A further, exciting development in the work of Massalha (16) was the introduction of *B. subtilis* (labelled with mKate) and *Escherichia coli* (labelled with Gfp) and the demonstration that *B. subtilis* actively excluded *E. coli* from colonisation of the root. It is known that *B. subtilis* can secrete the anti-adhesion compound surfactin that inhibits biofilm formation by pathogens such as *E. coli* CFT073 (17) and Massalha et al (2017) speculate that it might induce the exclusion zone around roots. This opens up a large area of biology with the ability to look at a myriad of combinations of bacteria that are differentially marked to look for antagonistic or

symbiotic interactions. This could even be extended beyond two or three bacteria to larger synthetic communities, where keystone species are marked with fluorescent labels and the effects on root colonisation by multiple bacteria investigated. Furthermore, it would be of interest to see if TRIS could be used to study potential three-way interactions between bacteria, mycorrhizae and roots.

Finally, Massalha (16) developed a two-root chamber where roots from two plants are separated by a semipermeable membrane (pore size 50 μM) that permits solute and bacteria to move but prevents roots on different plants from contacting one another physically. This allows bacterial preference for specific plant root genotypes to be assayed in one chamber. It was observed that the hairless *caprice/triptychon* (*cpc/trt*) and the excessive root hair *werewolf/myb23* (*wer/myb23*) mutants had peak attraction of bacteria occurring about one hour earlier than wildtype plants. There are obviously a large range of *Arabidopsis* mutants that could be tested in this way including those involved in immune signalling (18) and root secretion such as ABC exporters (19). The alternative approach will be to use bacterial labelled with inducible fluorescent-, luminescent- (e.g. lux) (20-22) or FRET-biosensors (23) to monitor root secretion and the bacterial response to roots.

TRIS has manifold uses in plant and microbial science to examine interactions of both partners. It is particularly valuable because it can give a spatial and temporal map to the earliest events in root attachment and colonisation. While it has a vast range of applications to classical reductionist biology, where the interactions of a single microbe and plant are considered, it has powerful application to the new wave of integrative community biology. This has been led by high-throughput sequencing that has shown how plants can shape their rhizosphere and root-associated communities (4, 24, 25), and how specific or groups of bacteria can suppress pathogens (26). However, much of this work to date has been descriptive, where community composition is counted. Techniques such as TRIS, where single or multiple bacteria can be assayed with a range of plants, offers an exciting way forward to investigate more complex dynamic community level interactions. It will be particularly informative to move to other plant species, particularly those which can be infected by mycorrhizae (most plants) and/or nodulate (legumes) via the common SYM signalling pathway, which has also recently been shown to alter the microbial community (27). With a light like TRIS the future of root-microbe interactions is bright.

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