Meetings

Culturing a plant microbiome community at the cross-Rhodes

28th New Phytologist Symposium: Functions and ecology of the plant microbiome, Rhodes, Greece, May 2012

Plants live in close association with microbial communities consisting of a variety of bacteria and fungi. Individual members of this microbiota can contribute to plant growth and development, plant productivity and phytoremediation (Weyens *et al.*, 2009). These microbes prosper in close proximity of plant roots (the rhizosphere), on root and leaf surfaces (the rhizoplane and phyllosphere (Lindow & Brandl, 2003), respectively) as well as within plant tissue as leaf or root endophytes (Hardoim *et al.*, 2008). Interactions of plants with their associated microbes span a broad continuum of symbioses from mutualistic through commensalistic, including parasitic at the extreme. Moreover, soil bacteria influence each other in complex networks, which influence plant fitness directly or indirectly as described for disease suppressive soils (Berendsen *et al.*, 2012). Thus far, molecular genetic research has focused mainly on binary microbial interactions of plants with individual pathogens or symbiotic mutualists that cause macroscopically visible plant phenotypes such as disease symptoms for pathogenic bacteria or plant growth promotion for mycorrhizal fungi and nitrogen fixing bacteria.

However, a large portion of the plant-associated microbial diversity has not been isolated as pure cultures and underlying first principles of how plants establish and interact with their associated microbiota as a whole remain largely elusive: Which factors influence plant–microbe interactions at the community level? How do plants discriminate friends from foes? How do beneficial microbes escape the sophisticated plant innate immune system? Can the low heritability of plant growth be accounted for by environmental interactions with large microbial assemblies? Now, with new tools like next-generation DNA sequencing it is possible to take a deep census of the plant microbiota in a culture-independent manner and use this as a springboard for more detailed analyses of both the host and microbiota contributions to an extended plant phenotype (Dawkins, 1978). This is typically achieved by massive sequencing of bacterial 16S rRNA genes or of internal transcribed spacer regions between rRNA genes of fungi or by untargeted metagenomic sequencing of a microhabitat.

Importantly, new technologies raise new challenges including analysis of huge amounts of data and the need to establish best practice standards for an emerging research field (Knight *et al.*, 2012). To do so, while trying to bridge the gaps between phyllosphere and rhizosphere research, as well as between plant and soil science, was the remit of the 28th New Phytologist Symposium on functions and ecology of the plant microbiome held in Rhodes, Greece in May 2012. This symposium brought together researchers working in genetics/genomics, soil science, microbiology, computational biology, and plant and microbial physiology. Over 130 participants from these highly diverse disciplinary backgrounds presented 94 posters and 27 talks over five sessions: Rhizosphere microbiology and biochemical cycling; Soil microbial communities; Phyllosphere and endophytic microbial communities; Genetic control and selection of plant microbiomes; and Characterization and ecology of plant microbiota. An important feature of the meeting was the inclusion of both structured discussions and the ever critical informal discussions, which were made easier by the lovely location, which provided ample opportunity for participants to discuss and debate the issues raised in individual presentations. Here, we present some of the emerging and unresolved issues that formed the basis of many lively debates.

'As sequencing technologies continue to evolve, it is imperative that a substantial effort be placed onto the analysis step of these studies, which has so far not kept pace with data generation.'

The meeting was organized by Jeff Dangl (University of North Carolina, USA) and Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Cologne, Germany), who had independently launched projects to profile the *Arabidopsis thaliana* root-associated bacterial microbiota by pyrosequencing bacterial 16S rRNA gene fragments, but soon coordinated experimental standards to maximize comparability of data between different laboratories. Despite the use of four different soils from two continents and different PCR primer sets, but under similar controlled environmental conditions and root sampling procedures, both groups found that the Arabidopsis root-associated bacterial microbiota is significantly different from the surrounding soil. The soil type was one of the main determinants of the composition of root-inhabiting communities, indicating that these bacteria are recruited from soil. Of note, many abundant soil bacteria were essentially excluded from entering roots, underlining the role of root cells as gatekeepers for soil bacteria that will eventually become enriched in the root endophytic compartment. The consistency between the results of both studies underlines the

robustness of the methodology as such, if rigorous quality standards are applied (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012).

Furthermore, ribotype profiles are sufficiently dependent on host genotype to vary between inbred Arabidopsis accessions (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012). Similar results for bacterial communities associated with Arabidopsis leaves were reported at the meeting by James Kremer (Michigan State University, USA). He and his coworkers also used 16S ribotyping to profile bacteria from the phyllosphere of selected Arabidopsis ecotypes as well as from mutants defective in their innate immune detection. He potentially uncovered a role for microbe-associated molecular pattern-triggered immunity in shaping the Arabidopsis phyllosphere microbiota as specific proteobacterial operational taxonomic units (OTUs). were enriched in *fls2*, *efr1* and *fls2/efr1* double mutants compared to wild type. Microbiota profiles of systemic acquired resistance (SAR)-induced plants and the natural accession Belmonte, which expresses naturally elevated levels of salicylic acid, also indicate that the phyllosphere microbiota is influenced by salicylic acid-related signaling.

Jeff Bennetzen (University of Georgia, USA) reported promising early results defining an experimental path to determine novel host factors contributing to the control of plant–microbe interactions by mapping using segregating populations of the pitcher plant *Sarracenia* and the model C_4 grasses *Setaria* and maize. He and his collaborators also recently published the genome sequence of *Setaria* and of its wild relative (Bennetzen *et al.*, 2012), including transcriptome data, widening the set of available reference plants for microbiome studies. Together, the aforementioned studies suggest that it will be possible to define and ultimately isolate, host genetic loci involved in shaping plant associated bacterial communities. However, the complexity of these bacterial communities and the unavoidable noise in the experimental system must not be underestimated, as became evident by other presentations at the meeting. For example, Joy Bergelson (University of Chicago, USA) warned about the difficulties in identifying from Arabidopsis field experiments host loci shaping the composition of phyllosphere bacterial communities.

Plant–microbial interactions uncovered by integrated '-omics' studies

An example of how a metagenomic 16S rRNA gene survey provides the basis for future host genetic studies was given by Gerry Tuskan (Oak Ridge National Laboratories, USA). After an initial culture-independent survey of the rhizosphere and endophytic microbial communities associated with 25 *Populus deltoides* genotypes, 1200 isolated bacteria were systematically screened for plant growth promotion capability. One of the plant growth promoting isolates was used in follow-up experiments in Arabidopsis, which combined physiological data with metabolite production and transcript profiles to determine how it influences overall plant health via RNA regulation, protein degradation, and hormonal metabolism (Weston *et al.*, 2012). This type of study is helpful in better defining the molecular mechanisms underlying the widespread phenomenon of induced systemic resistance (ISR) in aerial parts of plants following a local infection of roots with *Pseudomonas fluorescens* or other ISR-inducing rhizobacteria (Berendsen *et al.*, 2012).

Another example of how metagenomics and proteomics can converge to allow better understanding of the mechanisms and processes occurring in plant-associated microbial assemblages was presented by Julia Vorholt (ETH Zürich, Switzerland). For these experiments, leaves were collected from plants grown in nature and revealed that the alpha-proteobacterial genera *Sphingomonas* and *Methylobacterium* are dominant candidate leaf commensals. These studies also identified unusually high levels of *Sphingomonas* TonB-dependent receptors that might have a role in scavenging plant metabolites present in low amounts on the leaf surface for bacterial growth. In follow-up work, the Vorholt laboratory first reconstructed a simplified Arabidopsis phyllosphere under laboratory conditions and showed that *Sphingomonas* bacteria provide indirect plant protection against the leaf pathogen *Pseudomonas syringae* DC3000. This system was then employed in a forward genetic screen *in planta* to identify *Sphingomonas* mutants that fail to provide protection against co-inoculated *P. syringae* DC3000 (Vogel *et al.*, 2012). Together these studies are elegant examples of how new technologies can merge to uncover molecular determinants underlying community-scale traits such as this indirect protection against pathogenic invaders.

Data analysis strategies

It is important that the data yielded by the aforementioned multi-scale '-omics' studies is vast to the point that it creates analysis and cross-referencing challenges, costing precious time and money. The technologies utilized to perform this research, especially at the nucleic acid level, are getting faster and cheaper, further increasing the amount of data produced. This will become acute as the field generates hundreds or thousands of whole genome sequences from cultured of bacterial and fungal isolates to extend the limited information given by 16S sequence based ribotyping. As sequencing technologies continue to evolve, it is imperative that a substantial effort be placed onto the analysis step of these studies, which has so far not kept pace with data generation. Between July 2007 and July 2011, the cost of sequencing a human genome decreased by > 800 fold; whereas, the cost of computing has only decreased *c.* four-fold (Pollack *et al.,* 2011). The advent of cloud computing and the availability for high quality open-source software may allow for an increase in the speed and quality of analysis without driving up the cost. For example, new open-source software called Grinder can be used to simulate sequencing libraries, such as 16S ribotyping, by a variety of platforms (Angly *et al.*, 2012). These simulated community datasets allow for the testing of new analysis software, α and β diversities calculating, and discovery of sequencing biases inherent to the experiment.

Future studies

At the meeting, a long-term future research strategy became apparent in which after an initial culture-independent survey of the plant microbiota, the corresponding community members are isolated in collections of pure cultures. This approach was for

example successfully used to identify a *Pseudomonas* strain that protects sugar beet seedlings from infection with *Rhizoctonia solani* in disease suppressive soil, as reported at the meeting by Jos Raaijmakers (Wageningen University, the Netherlands; Mendes *et al.*, 2011). These culture collections can also be used to compose defined communities for inoculation experiments of gnotobiotic plants. Such recapitulation communities decrease the noise inherent to any complex community and allow testing of well-studied principles of binary plant–microbe interactions in a community context (Fig. 1). In order to perform such experiments, our research community requires comprehensive and indexed collections of cultured plant-associated microbiota on a variety of media. For example, by constructing microbe libraries in a high-throughput limiting dilution approach, such as been recently used in gut microbiota studies (Goodman *et al.*, 2011). As microbiology techniques are evolving, for example, by adopting next generation sequencing, it will be possible to grow and screen many more microbes than we originally thought in the laboratory (Bomar *et al.*, 2011). To this end, there was a call at the meeting for significant collaboration within the field to share culturing techniques and media, so that a greater diversity of microbes can be captured and be placed into a collection.

Beyond such synthetic community studies under laboratory conditions, other promising directions discussed at the meeting included examining microbial communities that might have adapted to live in association with high yield and low input perennials that are potential biofuel crops such as sugarcane (presented by Phil Hugenholtz, University of Queensland, Australia) and *Miscanthus* grown in fields with differing abiotic conditions. Specifically, Angela Kent (University of Illinois at Urbana-Champaign, USA) described the *Miscanthus* microbiome including diazotrophs, dominated by Bradyrhizobium, which contribute to the plant's nitrogen supply, and demonstrated that habitat (endophytic or rhizosphere occupation), plant genotype, pH and soil fertility factors can shape the microbial assemblages associated with this candidate bioenergy crop. More broadly, knowledge on the evolution of the microbiota within defined plant species phylogenies will be helpful to learn whether particular sets of microbial taxa have co-evolved together with a given host species to colonize extreme environmental niches, for example, serpentine soils. These studies represent exciting new lines of research, which are eagerly anticipated. Additionally, *in situ* ecological genomics approaches will need to be designed that must take into consideration robust and standardized procedures in order to yield useful long-term contributions to understanding plant-associated metagenomes (Knight *et al.*, 2012).

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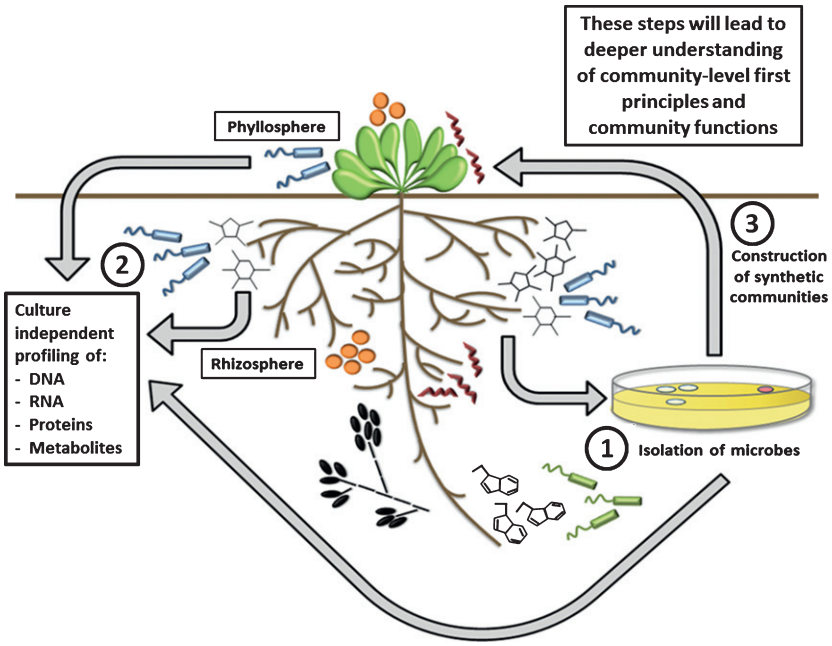

Fig. 1 Strategies and evolution in the plant microbiome research field. Both above- and below the ground, plants live in close association with microbial communities consisting of a variety of bacteria and fungi. First, molecular genetic research relied on culturing of individual isolates to study binary microbial interactions of plants with model pathogens or symbiotic mutualists (1). New tools of profiling whole microbial communities at the level of nucleic acid, proteins and metabolites allow characterization of plant–microbe interactions in a culture-independent manner and their contributions to an extended plant phenotype (2). Results and experiences of the first two approaches can be combined to isolate previously identified key community members in pure culture to compose defined communities for inoculation experiments of gnotobiotic plants. The noise inherent to any complex community is significantly reduced, which enables testing of well-studied principles of binary plant–microbe interactions in a community context and deducing community-level first principles and community functions (3).

the best poster awards. The authors apologize to all speakers whose talks were not explicitly mentioned, but length considerations forced them to focus on very few of all the exciting presentations given at the meeting.

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