drawer. But the stitch or two of alterations by Schmidt and colleagues[1,2] has ensured that gravitational lensing will still be on the hot list next season. ■

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MICROBIOLOGY

Life on leaves

Johan Leveau

The surface of plant leaves — the phyllosphere — is home to many microbes. A 'community proteogenomics' approach offers a fresh look at what it takes to survive and thrive in this unique habitat.

Under the microscope, aerial plant leaves resemble eerie landscapes, with deep gorges, tall peaks and gaping pits that riddle the waxy surface. Add to this scenery a climate that features temperature highs of 50 °C or more, exposure to harmful ultraviolet rays, erratic periods of drought and limited access to nutrients, and one gets the picture that this is a hostile environment. Still, many bacteria, fungi, yeast and other microorganisms dwell in great abundance in this 'phyllosphere'[1], which is the subject of a new investigation by Delmotte and colleagues[2]. In their paper, published in *Proceedings of the National Academy of Sciences*, they bring twenty-first-century tools to bear on the phyllosphere, with special reference to bacteria.

Much is known about microbial adaptations to the leaf surface — for instance the production of pigments to avoid DNA damage from solar radiation or the accumulation of compatible solutes to deal with water stress. However, most of this knowledge has been inferred from single microbial species, from cultivating representative isolates in the laboratory and from exposing isolates artificially to plant foliage for an assessment of which genes contribute to microbial fitness in the phyllosphere. Delmotte and colleagues' investigation[2] is an exercise in 'community proteogenomics'. This approach does not rely on cultivation, does not focus on a single species, and does not suffer from the controlled conditions that typify lab experiments. The result is a snapshot-like, culture-independent insight into the diverse mechanisms that underlie the success of leaf-surface microbial colonists — in this case bacteria, the most abundant of the colonists at estimated densities of 10^6–10^7 cells per square centimetre (ref. 3).

Community proteogenomics[4] arose from the marriage between metagenomics and metaproteomics. Metagenomics involves analysis of the mix of all microbial DNA present in a particular environmental sample, whereas metaproteomics does the same for all proteins. The metaproteomic portion of Delmotte and colleagues' approach involved collection of microbial biomass from leaf surfaces, protein extraction and digestion, separation of the fragments by liquid chromatography and analysis by mass spectrometry. The result was a mixed bag of nearly half a million spectra, each corresponding to a short peptide sequence. Linking these spectra to proteins with a possible function and evolutionary origin is a challenge and is possible only with a proper frame of reference. Typically, this frame is provided by the publicly available databases of annotated DNA and protein sequences.

However, if a microbial community has few representatives in the public database, the chances are that many of the sequences in the database will be too dissimilar to allow positive matching with short peptide sequences from the environmental proteome. This is where the metagenomic part of the proteogenomic approach comes in: it increases the probability of protein identification by metagenomic profiling of the same sample from which the proteins were extracted. In the case of Delmotte *et al.*[2], pyrosequencing was used to construct a representative library of DNA sequences from the leaf samples: by including these metagenomic data on top of the sequences in the public database, up to 87% more proteins could be identified in the bacterial leaf communities. This suggests that many bacteria from the leaves of the plants that were investigated — soya bean, clover and *Arabidopsis* — are indeed genetically distinct from the bacteria for which genomic data are currently available. This was especially true for members of the genus *Sphingomonas*, which were among the most numerous bacteria present. Were it not for the metagenomic data, none of the abundant proteins assigned to this genus would have been identified.

The phyllosphere metaproteogenome reveals that many of the highly expressed bacterial proteins — porins, TonB-like proteins and components of ABC-type transporters, for example — are apparently involved in scavenging what little food there is available on the leaf surface. This possibility is consistent with studies showing the limited access to nutrients in the phyllosphere, such as the products of photosynthesis that leak from the leaf interior[5]. Proteins for using methanol, a plant waste product, were also abundant and could be assigned to *Methylobacterium* species — leaf colonizers of many different plants[6]. Stress proteins were over-represented as well, revealing a need to protect the bacterial cells from oxidative and osmotic damage, and to prevent them from becoming desiccated. One of the surprising finds was the prominence of a protein containing a fasciclin domain, possibly involved in cell adhesion, but with no previously suspected role in survival in the phyllosphere.

The wider context for this line of research is illustrated by considering the significance of microbial populations on leaves. They play a part in the global nitrogen and carbon cycles; they participate in removing airborne pollutants[7]; and they contribute to the decomposition of leaf litter and to the production of plant and animal feed by composting and silaging. The traditional focus of phyllosphere research has been on microorganisms that are of agricultural relevance, in particular plant pathogens and their antagonists. But the discovery of archetypal leaf bacteria such as the plant-pathogenic *Pseudomonas syringae* in non-agricultural environments[8], and the detection of human enteropathogens such as *Escherichia coli* O157:H7 on leaf surfaces[9], are inviting a more expansive view of the phyllosphere as a source and sink of environmental bacteria.

One value of the study by Delmotte *et al.*[2] is that it will help to draw this microbial habitat to the attention of a broader audience of researchers and into the field of comparative '-omics'. It will also serve as a baseline for further proteogenomic excursions into the phyllosphere, which are likely to involve studies at higher resolution, both temporally and spatially. Among the issues to be addressed are the dynamics of microbial protein expression relative to changes in community composition, and the role of the plant and its environment in driving the functional plasticity of foliage-associated microorganisms. ■

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