Utilising the Plant Microbiome in Agriculture

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Scientia

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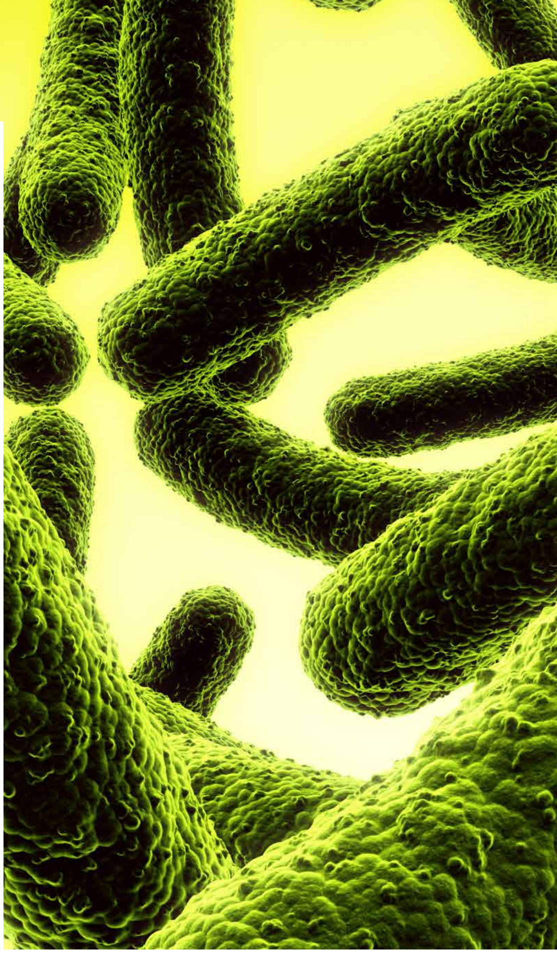

Dr Philippe Rolshausen and his team at the University of California, Riverside, aim to understand the microbial communities that live on and in fruit trees, with implications for crop yield and plant disease. With backing from Californian tree crop industries and funding from state and federal institutions, his group uses a suite of 21st century 'omics' technologies to delve into the mysteries of the plant 'microbiome' and develop farming practices to support plant health.

Fruit is considered an important component of a healthy diet. This assumption forms the basis of government guidelines, official health and dietary advice, conventional wisdom and food marketing. In recent years, the global fruit market has increased significantly. Global production of fresh fruits grew by 50% between 2004 and 2014, with 5.4 million hectares dedicated to cultivation in 2014, up from 3.8 million in 2004 (FAOStat, http://www.fao.org/faostat/). With 2 billion more people to feed by 2050, this trend will need to be sustained, and we will also need to find alternative ways to grow food. On the 'supply' side, this has been driven by advances in cultivation and processing technologies, globalisation and sophisticated global supply chaining, allowing on-demand availability of a wide diversity of fruits, all year round. On the 'demand' side, this has been driven by greater affluence and health consciousness of consumers, increased demand for healthier and organic food products, and changing consumer tastes. The production of fresh and processed fruit products is important to the economies of several countries, as well as the dietary health of billions.

Fruit cultivation for many invokes imagery of family-run farms, postcard scenes of idyllic vineyards and orchards, and grandparents' tales of Saturday afternoon apple scrumping escapades. While these images may bear some resemblance to fact, fruit farming and processing has become increasingly industrialised, with mechanisation and automation standard within this sector. However, even in the era of 21st century agribusiness, yield and quality in fruit production are very much dependent on the prevailing environmental conditions, both biotic and abiotic.

There is a complex web of biotic factors influencing crop cultivation that agriculturists must understand and control. As well as 'macroscopic' and easily observable organisms, such as birds and insects, invisible micron-scale organisms, so small that they cannot be detected without advanced microscopy, can also influence crop yields and quality (positively or negatively). Plants host complex communities of microorganisms, including bacteria, fungi and viruses, within their tissues (endophytes) and on their surfaces (epiphytes). This is known as the plant-associated microbiota, the plant microbiome or the phytobiome. We are only just beginning to scratch the surface of this invisible yet important world of plant-associated microbes, with important implications for crop production.

The human microbiome is a field of intense investigation, particularly the influence of human-associated microbes on disease, immunity, nutrition, health and wellbeing. This research has spawned the multi-billion dollar probiotics industry, worth over $36 billion in 2015, and projected to grow to $64 billion in 2023 (http://www.prnewswire.com/news-releases/probiotics-market-size-to-exceed-usd-64-billion-by-2023-global-market-insights-inc-578769201.html). While the health benefits of probiotic foods and supplements are still subject to scientific validation, the media and the internet often propagate misinformed half-

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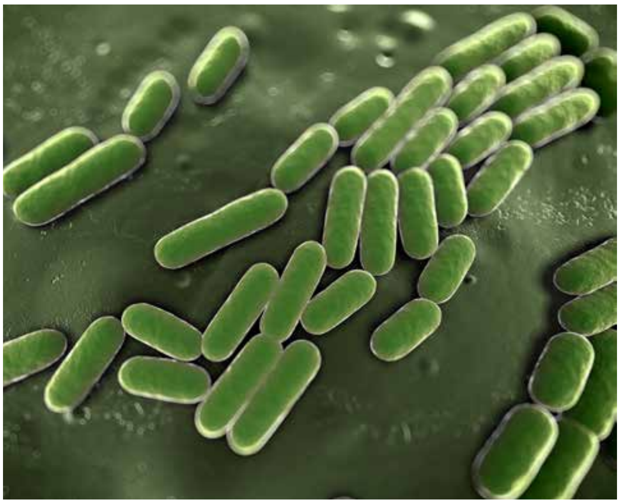
truths on their efficacy. In contrast, little such commercial or media attention is given to the plant microbiome for agriculture, despite its importance to global food production.

Dr Philippe Rolshausen, a researcher in the Department of Botany and Plant Sciences, University of California, Riverside, hopes to raise the profile of the microbiome in crop production. ‘Microbiome research is an exciting developing field that has received a lot of attention and publicity especially for human research, but for agriculture we are far behind,’ says Dr Rolshausen. Some microbes are demonstrably beneficial to the plant host, such as root-associated mycorrhizal fungi that sequester phosphates, nitrogen-fixing rhizobacteria, plant growth promoting rhizobacteria and fungi and bacteria that stimulate plant defences. The majority of microbes are considered to be commensals, deriving benefit from the host without causing obvious harm or help. A number of microbes are pathogenic and can severely impact crop productivity that can lead to devastating socio-economic outcomes. The most notorious example is the Irish potato famine caused by the soil-borne fungus *Phytophtora infestans*, the causal agent of the potato blight disease that occurred in Ireland in the mid-19th century https://www.britannica.com/event/Great-Famine-Irish-history. Dr Rolshausen works on economically detrimental vascular plant fungal and bacterial diseases affecting specifically grapevine and citrus. Only when such crop diseases are understood can effective strategies be implemented for their prevention and mitigation, and Dr Rolshausen’s group aims to elucidate the pathogenesis of these diseases on a microbial and molecular level.

Dr Rolshausen liaises closely with commodity groups, to carry out microbiome research mainly with tree crops – crops of particular significance to the Californian economy and culture. ‘Today, one of the most important problems that the California tree crop industries have to cope with are invasive pests and diseases,’ he explains. ‘I am working with these industries to develop and implement innovative yet practical solutions to address these challenges.’ Using a suite of 21st century ‘omics’ technologies, his group aims to elucidate tree crop microbiota, and use these insights for diagnostic purposes and to devise strategies to fight pathogens and boost yields. In particular, the group’s use of DNA profiling of host-associated microbial communities has led to some interesting and novel insights regarding the complex interactions between the microbiota, the plant host and the environment. It appears that host plants selectively recruit specific microbial species to colonise particular tissues, and the prevailing environmental conditions, (such as irrigation, fertilisers and pesticides application) can influence microbial colonisation and community dynamics.

Biotic stress factors (i.e., plant pathogens) greatly influence the host microbiota, a research topic of great significance to Dr Rolshausen and his team. One potential outcome of this research, which the group is keen to pursue, is the development of microbiota-based diagnostic tools for early detection of plant diseases, following on from their current focus on DNA-based pathogen diagnostics. In the framework of a project funded by the grape industry, and in collaboration with UC Davis, the team are investigating next generation (DNA) sequencing (NGS) approaches to identify and quantify fungal pathogens associated with grapevine trunk diseases. Trunk diseases are caused by a complex of fungal pathogens, and applying NGS technology will allow the accurate and early diagnosis of infection, identifying the fungal taxa responsible for the disease so that adapted management strategies can be implemented in a timely manner. Another yet more exciting outcome would be to promote the plant’s ‘friendly’ microbiota to confer tolerance and resistance against pathogens. The group hypothesises that some the plant-associated microbes



operate on a molecular level, producing natural antimicrobial compounds that inhibit the growth of pathogens. Furthermore, they hope to develop probiotic approaches to encourage the growth of symbiotic microbial communities to improve plant health. 'Just like for humans, I believe we can develop and commercialise probiotics and prebiotics for plants,' Dr Rolshausen explains.

Management of Pierce's Disease in Grapevine using a Microbiome Approach

Few natural phenomena strike the fear in the hearts of Californian winegrowers like Pierce's disease (PD). In the 1940s and 1990s, vineyards in southern California were decimated by PD, raising concerns among grape stakeholders. PD causes proliferation of diseased vines that leads to vine death in as fast as two years, reducing availability of high-quality grapes for winemaking. The disease is caused by *Xylella fastidiosa*, a bacterial pathogen that resides in the xylem vessels of affected plants. It spreads between plants by insect vectors, especially the native blue-green sharpshooter (*Graphocephala atropunctata*). However, the 1990s blight was spread mainly by an invasive species – the glassy-winged sharpshooter (*Homalodisca coagulata*). The problem in southern California was exacerbated by the rapid intensification of both grape and citrus industries, and citrus groves and vineyards in close proximity to each other. Citrus trees prove an ideal feeding and breeding host for *H. coagulata*, which experience a population explosion and then fly into neighbouring vineyards to feed on the vines. Up to now, the problem has been managed by intensive insecticides, increasing the risk of resistance among insect populations. Yet, this invasive insect vector has moved its way to northern California, at the epicentre of the wine grape industry.

Upon observation that certain grapevines in natural vineyard settings express reduced susceptibility to the disease, Dr Rolshausen's group is investigating the role of the microbiome associated with those specific vines. They carried out microbial community analysis and metagenomic screening to identify microbes that apparently confer tolerance to PD. These identified two bacteria, *Pseudomonas fluorescens* and *Achromobacter xylosoxidans*, that were associated with less PD symptoms in affected vineyards. The mode of action of the two bacteria is currently being investigated in plants. A number of microbes isolated from these vines were also found to inhibit *X. fastidiosa* growth in in vitro inhibition assays. Analysis of metabolites from one fungus, *Cochliobolus* sp., revealed that a small organic compound, radicinin, was the causative agent of antimicrobial activity. This is currently being developed into a formulation for downstream commercial curative applications.

Citrus Huanglongbing and the Search for New Control Strategies

Huanglongbing (HLB) is one of the most destructive diseases of citrus globally. It is caused by colonisation of the citrus tree's phloem vessels for sugar transport, by the bacterial pathogen *Candidatus* Liberibacter asiaticus (*C*Las), and is spread by the invasive insect vector, the Asian citrus psyllid (ACP), *Diaphorina citri*. Native to southern Asia, *D. citri* has spread worldwide, including the US, and has been particularly destructive to the citrus industries in Florida. The ACP vector is now established in California, where several HLB infected trees have been found.

*C*Las cannot be cultured, greatly hampering research into HLB. Despite this, Dr Rolshausen's group is using culture-independent DNA-based techniques to investigate the associations between *C*Las and citrus plants. Importantly, robust diagnostic tools are necessary for early detection of *C*Las, especially at the early stages of an epidemic like in California, so that infected trees that would otherwise serve as a *C*Las inoculum reservoir, can be removed early on. Conventional PCR-based methods are not always reliable, and can fail to detect *C*Las due to spotty distribution in trees, giving false-negatives. A number of more reliable alternative diagnostics are being developed, based on the biochemical changes to the host upon *C*Las infection, such as those that detect changes in volatiles emitted from tree foliage. Research teams are also using metagenomics and microbial community analysis of citrus tree tissues, to look at changes in the microbiome as a function of HLB infection. Thus, organisms associated with citrus trees may turn out to be promising biomarkers of *C*Las infection. Similar to PD in grapevines, some citrus trees appear to cope with the disease (a.k.a. survivor trees). Dr Rolshausen's team is mining this survivor tree phenotype in order to identify beneficial microbes and/or compounds that could confer increased tolerance to HLB.

Tree Crop Microbiomics: The Way Forward

Dr Rolshausen and his colleagues have shown that what is needed is a more holistic approach to tree crop agriculture. Crops must not be merely viewed as static, sterile biological production units. Indeed, it would be wrong to think of crop plants as 'just plants', but as hosts for a diverse and dynamic communities of microbes, constantly interacting with the host and the environment in interesting ways. Our knowledge of these fascinating and important biological interactions is still fairly limited, and the team's mission is to elucidate what constitutes a healthy plant microbiome and identify beneficial microbes that promote plant health and provide protection against biotic and abiotic stresses.

The economic significance of this research cannot be underestimated. As global tree crop farming expands and becomes ever more intensive, the threat of pathogenic plant diseases increase, especially when cultivation practices are not conducive to optimum plant health. There is a need for a deeper understanding of pathogenic plant disease, reliable diagnostics for early and quantitative detection, disease-fighting strategies based on the plant's natural microbiome-mediated 'immunity', and the implementation of such insights into routine tree crop management.

Meet the researcher

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Dr Philippe E. Rolshausen completed his BSc in Plant Biology at the University of Tours, France, in 1994, and went on to study for an MSc in Ampelography (Viticulture) at INRA, University of Bordeaux, France, in 1995. Being instilled with a passion and fascination of the science of winemaking, he went on to gain a PhD at the Department of Plant Pathology, University of California, Davis, in 2004. His dissertation topic was 'Biology and control of Eutypa dieback of grapevine in California'. Apart from a brief stint as a Post-doctoral Researcher at the University of Connecticut, he has held a number of extension, outreach and research posts at the University of California, almost continuously to the present day. His main research interest has been the biology and management of pathogenic diseases of grapevines, particularly in the context of the plant microbiome. He is now applying microbiomic approaches to other tree crops, including citrus, almond, pistachio and avocado.

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