Investigating plant-pathogen interactions with FT-IR metabolomic fingerprinting and ESI-MS metabolomic profiling

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Metabolomics is perhaps the ultimate level of post-genomic analysis as it can reveal changes in metabolite fluxes which are controlled by only minor changes within gene expression measured using transcriptomics and / or by analysing the proteome which elucidates post-translational control over enzyme activity. Plant-host interactions represent one of the most biochemically complex and challenging scenarios which are currently being assessed by metabolomic approaches. For example, the mixtures of pathogen colonised and non-challenged plant cells represents an extremely heterogeneous and biochemically rich sample, there is also the further complication of identifying which metabolites are derived from the plant host and which are from the interacting pathogen.

Our approach to metabolomics involved a first round analysis with Fourier-transform Infrared spectroscopy (FT-IR) due to its high-throughput nature. Chemometric analysis leading to the clear clustering of experimental classes within cluster plots indicated that the experimental approach used produced highly reproducible data and would be suitable for further more costly metabolomic profiling. Metabolomic profiling allows the detection, quantification, and identification of pre-defined target compounds within the metabolome, for which we employed Electrospray ionisation mass spectrometry (ESI-MS).

Our metabolomics approach has been applied to several plant-pathogen systems each of varying complexity. The first was based upon the resistant and susceptible interactions between *Brachypodium distachyon* (an emerging model pooid) and *Maganaporthe grisea* (Rice blast disease). The second system employed *Arabidopsis thaliana* cell suspension cultures which were challenged by virulent, avirulent and non-pathogenic isogenic strains (having just a single gene difference) of *Pseudomonas syringae* pv. *tomato* (*Pst*). At 12 h post-challenge, the plant and pathogen cells were separated by differential filtration, permitting a dual metabolomic analysis of both host and pathogen individually. The third and final system involved engineering a dexamethasone induced gene promoter switch along with *avrPtoB* (a *Pst* avirulence gene) into *A. thaliana*. In tomato, the AvrPtoB protein is recognised by the host's cognate resistance gene. *A. thaliana* does not posses this resistance gene and thus the virulence functions of AvrPtoB are displayed without being overcome by the host's resistant response. Thus, this system permits the dexmethasome induced expression of a single pathogen avirulence protein, providing a homogeneous source of material for analysis. Our approach has been extremely successful for the investigation of all three systems.