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Use of Rhizosphere Metabolomics to Investigate Exudation of Phenolics by Arabidopsis Roots

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The rhizosphere is a specialised micro-niche for bacteria that have an active exchange of signals and nutrients with the host plant. Nearly 20% of photosynthates are released as root exudates, which consist of primary metabolites and products of secondary metabolism which are largely phenolic in nature. Previously, using rhizosphere metabolomics, we showed that nearly 50% of organic carbon in the exudates is in the form of phenolic compounds, of which the largest fraction is from the phenylpropanoid synthesis pathway. Using *Arabidopsis* as a model, we have demonstrated that a biased rhizosphere can be created using plants with varying levels of phenylpropanoids due to mutations in the biosynthetic or regulatory genes. These phenylpropanoids levels are reflected in the exudates, and exudates from lines with regulatory gene mutations, *tt8* and *ttg*, have higher levels of phenylpropanoids, whereas biosynthetic mutant line, *tt4*, has very low and undetectable levels of phenylpropanoids. The biased rhizosphere of *tt8* and *ttg* lines provides a nutritional advantage to rhizobacteria that can utilize these phenylpropanoids such as quercetin. With such a strategy to increase the competitiveness of plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas putida*, this system can be applied to improve plant performance.

In order to better understand the metabolic basis of the nutritional advantage behind the competitiveness of the favoured *P. putida*, we elucidated its quercetin utilization pathway. We have recently cloned the gene for quercetin oxidoreductase (*QuoA*) and expressed it in transgenic *Arabidopsis* lines to alter the plant phenylpropanoid metabolism, using a gain of function approach. Since phenylpropanoid biosynthesis in plants involve formation of quercetin from naringenin, we envisaged that *QuoA* expression in plants will provide us with a genetic tool to "reverse" this biosynthetic step. This perturbation led to a decrease in flavonoids and an increase in lignin and anthocyanin metabolites. We describe here the metabolites present in the root exudates using high resolution accurate mass (HRAM) metabolomics approach. Using this approach, biased rhizosphere for another class of PGPR strains can now be created. In this case, lignin- and anthocyanin- utilizing strains will be selectively preferred.

We have set up a platform to perform metabolomics of exudates at the root surface. This has allowed us to use the liquid extraction surface analysis (LESA) system using a Thermo Velos Pro Orbitrap-MS to identify differences in exudate profiles along the root system of Arabidopsis. This platform enables direct sampling and measurement from plant roots grown aeroponically. As the metabolites are extracted from root surface and directly injected into the mass spectrometer, there is minimal loss of sample in this process. This method will now allow us to further dissect rhizosphere properties from places such as young root apex, as well as from the more mature base of roots. Taken together, these resources of altered rhizosphere, nutrient utilization pathways in microbes and surface analysis technology will help in extending our understanding of the processes in the plant rhizosphere.