Plant-Microbe Interfaces: Developments in integrated omics to link microbial metabolism to community structure/function in plant/microbial systems

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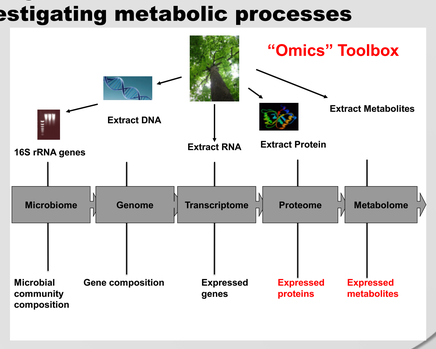
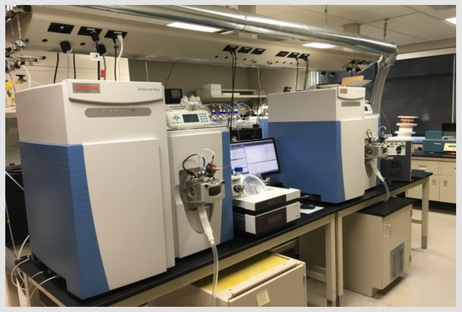
Abstract

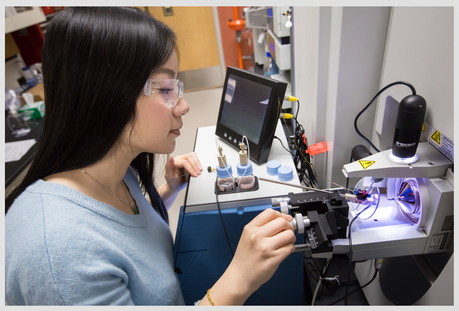
- **Research needs within the PMI project necessitate experimental measurements of the molecular machinery essential for understanding the symbiotic relationships of microbes and plants. To this end, a systems-biology approach affords measurement and characterization of the DNA (genome), RNA (transcriptome), protein (proteome), and metabolite (metabolome) components and relationships within plant-microbe systems.**
- **Integration of omics approaches provides a powerful approach to investigate how molecular information is coordinated and regulated in complex biological systems. This wealth of information is essential for investigating metabolic processes and inter-species signaling.**
- **Highlighted here are new capabilities for integrated metabolomics and proteomics, and how this approach can be used to examine microbe-microbe interactions in a co-feeding experiment.**



Experimental omics MS platform

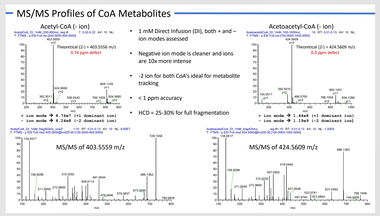
- **High performance LC-MS/MS methodology is essential for extensive metabolomic and proteomic measurements.**
- **The availability of advanced MS platforms, such as the QExactive-Plus-Orbitrap instruments, afford dramatic improvements for both small molecule and proteome characterizations**
- **This platform has been implemented to provide automated nano-LC with multi-dimensional LC, improved measurement throughput, and wider dynamic range, which collectively provide improved reproducibility and reduced variance.**

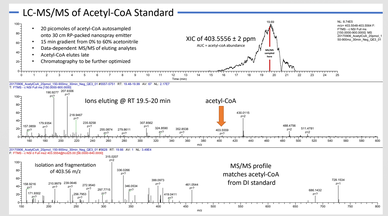




Enhanced MS capabilities for metabolomics and proteomics

- **ThermoFisher QExactive-MS systems provide high performance capabilities for nano-LC-MS/MS measurements, enabling a range of 1D- and 2D-LC-MS capabilities. This system provides high resolution (up to 240K), high accuracy (< 3 ppm), and high speed (up to 12 Hz) measurement metrics for on-line LC-MS campaigns.**
- **We have designed and implemented an automated "mini-MudPIT" approach (2D-LC-MS/MS) that provides unprecedented depth, reproducibility, and precision for a variety of microbial and plant proteome measurements.**
- **We have adapted an automated 1D-LC-MS/MS approach for metabolomic studies.**

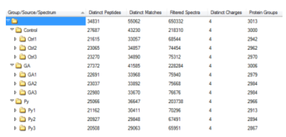




- **Interest in microbial metabolite control and redox balancing has prompted a need to develop and evaluate the QExactive system for molecules such as acetyl-CoA. This instrumental approach provides a mass measurement accuracy of better than 1 ppm along with detailed fragmentation information, as shown on the top left figure. Measurement of this analyte by 1D-LC-MS/MS (reverse phase) revealed detection sensitivity of at least 20 pmol, as shown on top right figure.**

Deeper 2D 'Mini-MudPIT' 8 hr runs

- *P. putida* example: Improved peptide and protein discovery/quantitation at a fraction of the time – and autosampled for improved reproducibility



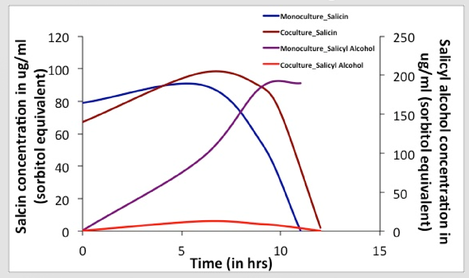
- **9 samples – 3 days of MS analysis**
- **Ctrl vs. Glyceraldehyde vs. Pyrolysis Oil**
- **3 salt-cuts per fraction / autosampled**

- Increased # peptide matches → more data points to infer protein abundance ➔ higher proportion of identified proteins are quantifiable.

- **For microbial and plant proteomics, we have focused on advancements in three critical areas: sample preparation, peptide separation, and accurate identification of modified and unmodified peptide sequences. These improvements now afford reproducible and deep measurement depths, with significantly improved throughput, as shown in the table above. For plant proteomics, the improved protein extraction and recovery facilitates deep proteome measurements on samples with limited biomass (apical meristem sections) or recalcitrant biomass (root). Additionally, these LC-MS platforms are now being used to detect and track how nitrogen fixed by bacteria becomes imported into plant biomass.**

Integrated omics reveals details of salicin cross-feeding in a microbial co-culture

- **The bacterial strains *Rahnella* sp. OV744 and *Pseudomonas* sp. GM16, both isolated from *Populus,* are involved in the degradation of higher order salicylates (phenolic glucosides) through uncharacterized mechanisms. We hypothesize that these two bacteria can co-metabolize a simple phenolic glucoside (salicin) by utilizing individual pathways for degradation of salicin and salicyl alcohol respectively.**
- **To test this hypothesis, we employed an integrated omics approach to examine a co-culture of OV744 and GM16 grown on salicin.**



- **A time-course metabolite study of monoculture revealed that OV744 was able to grow on salicin, and subsequently secretes salicyl alcohol into the media, as shown above. Over time, the salicin concentration decreased. Overall, the results suggested that the salicyl alcohol released by OV744 was utilized by GM16 in the co-culture, as shown in the figure above and data table below.**

	Avg	ug/ml (sorbitol equivalent)			2-furanacetic acid	
Time	Sample ID	salicin	salicyl alcohol	2-isopropylmalic acid	2-furaglycolic acid	citramalic acid
0	SN Rahnella sp.OV744	79.19	**0.89**	0.00	0.01	0.01
6	SN Rahnella sp.OV744	90.11	**93.62**	0.04	0.01	0.07
9	SN Rahnella sp.OV744	54.86	**184.74**	0.12	0.01	0.24
11	SN Rahnella sp.OV744	0.11	**189.90**	0.14	0.01	0.28
0	OG SN Rahnella sp. OV744 and Pseud. GM16	47.38	0.54	0.00	0.01	0.01
6	OG SN Rahnella sp. OV744 and Pseud. GM16	97.56	12.61	0.03	0.12	0.09
9	OG SN Rahnella sp. OV744 and Pseud. GM16	89.01	9.06	0.06	0.77	0.30
10	OG SN Rahnella sp. OV744 and Pseud. GM16	72.52	7.02	0.06	1.23	0.41
12	OG SN Rahnella sp. OV744 and Pseud. GM16	2.09	0.44	0.02	1.69	0.32

- **Proteomics suggested that OV744 transports salicin using the PTS based permease, which converts salicin to salicin 6-phosphate during transport. Salicin 6-phosphate then is hydrolyzed to glucose 6-phosphate and salicyl alcohol by the phosphoglucosidase enzyme. Glucose 6-phosphate then is channeled into central carbon metabolism for production of ATP and energy conversion, and salicyl alcohol is released into the environment. The resulting salicyl alcohol then is imported by GM16, and converted to catechol by three sets of enzymes (aryl-alcohol dehydrogenase, acyl-CoA reductase and salicylate hydroxylase). Catechol goes through ortho- cleavage step to convert to muconic acid, which is then fed into the β-ketoadipate pathway resulting in the formation of succinyl-CoA and acetyl-CoA that are channeled into the central carbon pathways.**

Conclusions

- **Instrumentation advancements in high performance LC-MS afford enhanced capabilities for both metabolomic and proteomic measurements of microbial and plant systems. In particular, nano-LC-MS/MS with reverse phase chromatography affords a somewhat universal platform for both small molecule and protein measurements.**
- **Methodology advances with a QExactive-Plus-MS platform have achieved high throughput, automated 1D- and 2D-LC/MS/MS measurements with high resolution, high mass accuracy, and enhanced reproducibility.**
- **An integrated omics approach involving metabolomics and proteomics revealed detailed information about the metabolic pathways and handoff of metabolic products between two plant-related microbes.**

Acknowledgement:

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