Accelerating Strain Phenotyping with Desorption Electrospray Ionization-Mass Spectrometry Imaging and Untargeted Molecular Analysis of Intact Microbial Colonies

Authors: Berkley M. Ellis,[1] Hawkins S. Shepard[1]* (hawkins.s.shepard@vanderbilt.edu), Jody C. May,[1] Piyoosh Babele,[1] Carl H. Johnson,[1] Brian F. Pfleger,[2] **Jamey D. Young**,[1] and John A. McLean[1]

Institutions: [1]Vanderbilt University, Nashville; [2]University of Wisconsin-Madison, Madison

Project Goals: In an effort to accelerate the timeframe associated with the screening of genetic edits, an analytical workflow using desorption electrospray ionization mass spectrometry imaging was developed that allows for the rapid analysis of genetically engineered biological systems in-situ. The method has demonstrated the ability to differentiate individual phenotypes in co-cultures and can provide comparisons of the relative levels of metabolites produced.

Developments within the field of synthetic biology have allowed for the production of genetic variants to outpace the screening capabilities of traditional analytical workflows. The acceleration of strain phenotyping is a critical need for effective determination of optimal target sequences for genetic engineering. In order to increase the throughput associated with this process, we have developed a workflow implementing desorption electrospray ionization-mass spectrometry imaging (DESI-MSI) that reduces sample preparation and allows for the rapid characterization of biosynthetic systems. DESI-MSI is unique in its ability to perform detailed spatially-resolved chemical mapping *in situ* that enables broadscale molecular phenotyping, including the analysis of labile and volatile small molecules. Through untargeted acquisitions and unsupervised segmentation, this multiplexed method is able to efficiently determine the metabolic phenotypes of bacterial colonies by analyzing their molecular profiles. The effectiveness of the workflow was demonstrated through the analysis of *Escherichia coli* strains engineered to overproduce free fatty acids (FFAs), wherein a single acquisition is able to distinguish individual phenotypes present within a co-culture and identify strains with the highest FFA production. Because the untargeted nature of the DESI-MSI approach, global metabolic information related to off-target production, product sinks, and membrane lipid compositions is also garnered, providing a more holistic screening of engineering efficacy. Further developments are currently being undertaken in order to optimize the workflow for the characterization of genetic edits associated with the production of high-value chemicals by cyanobacteria, with products such as FFAs having the potential to provide the basis of a renewable feedstock for biodiesel.

This work was supported by Department of Energy awards DE-SC0019404 and DE-SC0022207.