Rapid Strain Phenotyping and Metabolic Flux Analysis to Accelerate Engineering of Microorganisms.

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Project Goals: This project aims to comprehensively enhance microbial production of commodity chemicals. While portions of this project are directed towards increasing titers by engineering pathways and manipulating the circadian clock, this work is focused on the technologies evaluating strain production. We describe the development and application of analytical methods measuring metabolism that comprehensively characterize the outcomes of specific editing events and elucidate targets for further optimization. By increasing throughput and molecular breadth of analytical measurements assessing phenotypes, we aim to accelerate engineering and ultimately commercial utility of bacterial constructs.

Reading and editing DNA sequences are no longer the rate-limiting steps in microbial strain engineering, rather it is characterizing the metabolic outcomes of a specific genetic editing event.[1,2] To address this issue, we have developed a desorption electrospray ionization- imaging mass spectrometry (DESI-IMS) method that simultaneously samples various strains and biosynthetic products. Our DESI-IMS workflow is performed under ambient conditions with minimal sample preparation. The sole sample preparation step is adhering a membrane scaffold to a glass slide on which microorganisms and their metabolites are retained. By preparing many strains (samples) in a single step, we facilitate a robust interrogation of relative metabolite abundances and reduce the time and error associated with sample preparation by alleviating the need for pipetting and extractions. We demonstrate the inherent multiplexing capabilities of IMS by simultaneously characterizing various Escherichia coli strains engineered for free fatty acid (FFA) production and their respective biosynthetic products.

Using the developed workflow, we can phenotype engineered strains on the basis of biosynthetic products and also across the measured metabolome via untargeted IMS acquisitions. This is a necessary innovation in analytical measurements of phenotypes, because annotating global metabolism in addition to targeted metabolites typically require additional experiments.[2] To enable comprehensive analyses, we developed an unbiased data analytics workflow using unsupervised segmentation. With this workflow, we establish the ability to phenotype various engineered E. coli strains and differentiate them on the basis of comprehensive metabolomic measurements. We demonstrate the advantages of both untargeted acquisitions and unsupervised data analytics by characterizing secondary fatty acid production and providing insight into the

prevailing biology of microorganisms via variations in membrane lipid saturation. In sum, we establish that the developed workflow can accelerate synthetic biology strategies with applications in directed evolution, functional genomics, and metabolic flux studies.

Work within this project has also been directed towards developing novel FFA producing cyanobacteria, which fix atmospheric CO_2 and does not compete with arable land. We developed strains of Synechococcus sp. PCC7002 that produce up to 860 mg/L octanoic acid (C8) titers. Our future plans are to determine the metabolic bottlenecks inhibiting higher FFA production. Stable isotope (13C) based nonstationary metabolic flux analysis (INST-MFA) analyses of FFA producing Synechococcus PCC 7002 strains will help us to identify the bottleneck pathway for further genetic modifications to improve titers.[3] We will also use the developed DESI-IMS method to quickly determine high performing fatty acid strains and identify favorable mutations.

Our studies demonstrate the importance of DESI-IMS and INST-MFA. The development of these technologies has implications in the field of synthetic biology by providing rapid analytical readouts on metabolic production and efficiency. Further, future work incorporating MFA within DESI-IMS workflows presents a technology capable of rapid annotation of metabolism which might be crucial for the engineering of metabolically efficient microorganisms for industrial applications.

References

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