Rapid flux phenotyping to accelerate metabolic engineering of cyanobacteria

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Project Goals: The objective of this project is to develop a systematic approach to identify bottlenecks within metabolic pathways that limit carbon flux towards enhanced lysine (LYS) and free fatty acid (FFA) production in genetically engineered cyanobacteria. We are developing methods based on desorption electrospray ionization-ion mobility-mass spectrometry imaging (DESI-IM-MSI) and stable isotope (^{13}C) based nonstationary metabolic flux analysis (INST-MFA) to rapidly quantify metabolite abundance and metabolic flux in bacterial cultures.

We have developed DESI-IM-MSI methods to rapidly sample and annotate biosynthetically produced molecules from microorganisms[1]. DESI-IM-MSI methods to sample microbial metabolites are of particular interest for screening purposes due to rapid sample acquisition and minimal sample preparation associated with ambient sampling. Using the imaging capabilities of this technology, we have associated the composition and amounts of biosynthetic products to different mutations, samples, or colonies within a MSI experiment. Our current work in the development of this DESI-IM-MSI platform has shown success in sampling FFAs from engineered *E. coli* grown on surfaces, LYS from modified cyanobacteria under solid phase growth, and various excreted metabolites within cellular media.

We also developed and successfully utilized INST-MFA to delineate the photoautotrophic metabolism of cyanobacteria[2,3,4]. This work describes our current efforts at refactoring previously engineered lysine-producing *Synechococcus* PCC 7002 strains[5] to identify flux bottlenecks that can be targeted to enhance strain performance. We compared the flux differences between high (TK.032) and low (AM.319) LYS producing strains with a wild-type (WT) control PCC7002 strain. Fluxes through enolase (ENO), pyruvate kinase (PK), and pyruvate dehydrogenase (PDH) were higher, while fluxes through malic enzyme (ME) and phosphoenolpyruvate carboxylase (PEPC) were lower in TK.032 as compared to AM.319 and WT strains. We hypothesize that by perturbing the expression of enzymes around these network nodes, we can remove potential flux bottlenecks and enhance LYS productivity.

Our studies demonstrate the promise of DESI-IM-MSI and INST-MFA to provide rapid analytical readouts on metabolic production and efficiency, which has broad implications in the field of synthetic biology. Further, the union of DESI-IM-MSI and INST-MFA presents a technology capable of rapid annotation of metabolism for optimizing cyanobacterial and algal strains for biotechnological/industrial applications.

References

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