

52. Isotopically nonstationary ^{13}C flux analysis of isobutyraldehyde production in *Synechococcus elongatus*

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Project Goals: This project aims to develop strains of cyanobacteria that are optimized for producing advanced biofuels. This will be done by applying isotopically nonstationary ^{13}C metabolic flux analysis (INST-MFA) to quantify photoautotrophic metabolism in engineered cyanobacterial strains and then redirecting carbon flux toward biofuel production using rational pathway manipulations identified by ^{13}C MFA.

Recent studies have demonstrated the feasibility of converting energy from sunlight and carbon from CO_2 directly into biofuels using photosynthetic microorganisms. Despite the advances made in cyanobacterial biofuels production, the productivities achieved are currently too low for industrial feasibility and few tools are available that specifically address the challenges of determining and redirecting metabolic flux in photosynthetic microbes. Our group is developing novel approaches that use ^{13}C metabolic flux analysis (MFA) to quantitatively assess in vivo metabolic phenotypes of photoautotrophs. Previously, we mapped carbon fluxes in the cyanobacterium *Synechocystis* sp. PCC 6803 by applying isotopically nonstationary MFA (INST-MFA), which involves model-based regression of transient ^{13}C -labeling patterns of intracellular metabolites. The flux analysis revealed unanticipated photosynthetic inefficiencies tied to oxidative metabolic pathways, despite minimal photorespiration.

We have now applied a similar modeling approach to mapping photoautotrophic metabolism in another strain of cyanobacteria, *Synechococcus elongatus* PCC 7942. Metabolism was quantified in the wild-type strain of *S. elongatus*, as well as a strain engineered to produce isobutyraldehyde (IBA, a direct precursor of isobutanol). GC-MS profiling of isotopically labeled intracellular metabolites, growth, and IBA production were used to create flux maps of central carbon metabolism in the WT and IBA-producing strains. The flux analysis results quantitatively describe increased flux through phosphoenolpyruvate carboxylase (PEPC) rather than pyruvate kinase (PK) in both studied strains, with an increased overall PEPC flux in the IBA-producing strain. This pointed to an alternative carbon route to pyruvate formation, a major precursor to IBA (phosphoenolpyruvate \rightarrow oxaloacetate \rightarrow malate \rightarrow pyruvate \rightarrow IBA).

Based on these results, we generated three cyanobacterial single gene overexpression strains in the IBA-producing background for reactions involved in the pyruvate formation bypass: IBA/PEPC^{ox}, IBA/MDH^{ox}, and IBA/ME^{ox}. We found that the IBA/ME^{ox} strain showed a significant improvement in IBA production, while maintaining a growth rate comparable to its IBA-producing parental strain. We also generated an overexpressed pyruvate kinase in the IBA-producing background strain, but found that IBA production did not increase and the growth rate actually decreased.

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