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 (^{13}C-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.

Photosynthetic microorganisms are promising systems for converting energy from sunlight and carbon from CO_2 directly into renewable fuels and chemicals. Despite recent advances in cyanobacterial biofuels production, the productivities achieved are yet to be economicaly feasible 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-MFA to quantitatively assess *in vivo* metabolic phenotypes of photoautotrophs. Previously, we used this approach to map carbon fluxes in wild-type *Synechococcus elongatus* PCC7942 (WT) and a mutant (SA590) engineered to convert pyruvate to isobutyraldehyde (IBA) [1]. Compared to WT, ^{13}C-MFA revealed an increased flux through a pyruvate kinase (PK) bypass pathway in SA590. As a result, we generated SA590 mutants that singly overexpress each gene in the PK bypass pathway: PEP carboxylase (PEPC), malate dehydrogenase (MDH), and malic enzyme (ME). These mutants showed significant improvements in IBA production while maintaining comparable growth rates to SA590.

We recently examined the intracellular metabolite pool sizes of our mutants to elucidate the effects of the singly overexpressed PK bypass genes. Compared to SA590, a large increase in malate pool size was observed in strain SA590-MDH. We hypothesize that the flux to malate could be redirected toward pyruvate (and hence toward increasing IBA production) by tandem overexpression of ME and PEPC. Hence, two additional mutants were generated to test this hypothesis: SA590-MDH-ME and SA590-MDH-ME-PEPC. This presentation summarizes our efforts to date and demonstrates the utility of ^{13}C-MFA in guiding rational pathway engineering of photosynthetic microorganisms for biofuel production.

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[1] S. Atsumi, W. Higashide, J.C. Liao, Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde, Nature Biotechnology, 27 (2009) 1177-U1142.