IS 3.2 - Novel stable isotope approaches to identify flux bottlenecks in photosynthetic microbes

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Engineering host cell metabolism to promote high yield and specific productivity is a major goal of the biotech industry. ^{13}C metabolic flux analysis (MFA) provides a rigorous approach to quantify host metabolic phenotypes by applying isotope tracers to map the flow of carbon through intracellular biochemical pathways. In particular, transient measurements of isotope incorporation following a step change from unlabeled to labeled CO_2 can be used to estimate photosynthetic carbon fluxes by applying isotopically nonstationary MFA (INST-MFA) [1]. We have previously developed a package of MATLAB routines called INCA [2] that automates the computational workflow of INST-MFA. INCA was the first publicly available software package capable of applying INST-MFA to biologically relevant metabolic networks. We have recently applied INCA to model the photoautotrophic metabolism of *Synechococcus* 7942 strains that have been engineered to produce the industrial biochemical isobutyraldehyde (IBA) [3]. The flux analysis identified a potential bottleneck at the pyruvate kinase (PK) reaction and indicated significant flux through an alternative three-step route from PEP to pyruvate involving PEP carboxylase (PEPC), malate dehydrogenase (MDH), and malic enzyme (ME). Based on these results, we overexpressed PK or combinations of PEPC, MDH, and/or ME to engineer strains with significant improvements in IBA production. ^{13}C flux analysis of the resulting strains identified further targets for debottlenecking IBA production in *Synechococcus* 7942, some of which are currently under investigation. These studies have established ^{13}C INST-MFA and the INCA software package as a comprehensive platform to map carbon fluxes in cyanobacteria and other photosynthetic microbes. (Supported by DOE award DE-SC008118.)

1. Young, J.D., et al., *Mapping photoautotrophic metabolism with isotopically nonstationary ^{13}C flux analysis.* Metab Eng, 2011. **13**(6): p. 656-65.
2. Young, J.D., *INCA: a computational platform for isotopically non-stationary metabolic flux analysis.* Bioinformatics, 2014. **30**(9): p. 1333-5.
3. Jazmin, L.J., et al., *Isotopically nonstationary 13C flux analysis of cyanobacterial isobutyraldehyde production.* Metab Eng, 2017. **42**: p. 9-18.