Genome-scale fluxome of the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973

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Project Goals: The goal of this project is to analyze the genome-scale fluxome of *Synechococcus elongatus* UTEX 2973 (here after *Synechococcus* 2973) as part of a system level study of this fast-growing cyanobacterial strain. This would enable us to identify if there is any specific pathway utilization pattern associated with fast growth rate. The knowledge base generated from this study would help in the development of *Synechococcus* 2973 as a versatile host for a sustainable solar-based bioprocess.

Photosynthesis produces a wide variety of biochemicals from cheap and renewable raw materials such as water, sunlight and CO_2. The oxygenic phototrophs, cyanobacteria, are more pliable for genetic modification than plants and micro-algae. This makes them an ideal host for the photosynthesis-based production of industrially relevant biochemicals and biofuels. However, the slow growth rates of well-studied model strains prevented industrial application of these organisms as solar-based production platforms. The fast-growing cyanobacterium, *Synechococcus* 2973, has a short doubling of 2.1h which is comparable with the widely used industrial production host yeast (Yu et al., 2015). This makes it a potential candidate host that could sustain a solar-based bioprocess at an industrial scale. Systems level analysis of such fast-growing photoautotrophs will accelerate their development into a successful production host. Besides, such analysis would help us unlock factors that influence the growth rate of photosynthetic organisms (Mueller et al., 2017). The knowledge base, generated from such analysis, could help us further increase the growth rates of photosynthetic organisms making them more suitable for industrial production processes.

In this meta-analysis, the genome scale fluxome of *Synechococcus* 2973 was elucidated using isotopic non-stationary ^{13}C-metabolic flux analysis with experimentally measured labeling dynamics of 13 central carbon metabolites obtained from a previously reported study (Hendry et al., 2018). To begin with, we created a genome scale carbon mapping model, *i*mSyu593, using the existing mapping model for *Synechocystis* sp PCC 6803, *i*mSyn617, as the starting point. The mapping model, *i*mSyu593, traced the flow of carbons through 593 reactions encompassing central carbon metabolism, amino acid metabolism and other peripheral pathways. Flux distribution revealed that almost all (>96%) of the assimilated carbons were directed towards biomass formation. This high carbon conversion is the result of reincorporation of oxidized carbons and preferential usage of non-decarboxylating reactions such as phosphoketolase. The reincorporation of oxidized carbon compensated for the carbon loss associated with the observed higher flux through the photorespiratory C2 cycle. This allowed the organism to use the photorespiratory C2

cycle for the synthesis of glycine and serine without a significant decrease in the carbon efficiency. Unlike in other cyanobacteria, the malic enzyme flux was found to be dispensable since Pyruvate Kinase was the major source of pyruvate. Acetyl CoA was synthesized using the carbon efficient phosphoketolase pathway instead of Pyruvate dehydrogenase. These findings suggest the existence of a carbon efficient metabolism in *Synechococcus* 2973 alongside its faster growth kinetics.

References:

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