Contrasting the metabolic capabilities of *Cyanothece* 51142 and *Synechocystis* 6803

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Project Goals: The current project uses systems biology to define the metabolic networks involved in the photobiological production of advanced biofuels and/or their chemical precursors by cyanobacteria. By encasing transcriptomics, metabolic profiling, mutagenesis, physiological analysis and genome-scale metabolic modeling in a systems biology framework, our goals are: i) to develop genome-scale models of two widely used cyanobacterial species, *Cyanothece* 51142 and *Synechocystis* 6803, ii) to refine these models based on systems level information obtained from experimental studies (i.e., transcriptome, metabolome, etc.), and iii) to genetically modify these strains based on model predictions, experimentally evaluate their performance and subsequently enhance the production yield of desired products.

Cyanobacteria are an important group of photoautotrophic organisms that can synthesize valuable bio-products by harnessing solar energy. Cyanobacteria contribute significantly to biological carbon sequestration, O_2 production and the nitrogen cycle. They exhibit robust growth under diverse environmental conditions and have minimal nutritional requirements. They are also endowed with high photosynthetic efficiencies and diverse metabolic capabilities that confer the ability to convert solar energy into a variety of biofuels and their precursors. However, less well studied are the similarities and differences that exist between the metabolic capabilities of different species of cyanobacteria that may contribute toward their niche biological functions and their suitability as microbial production systems. Towards goal (i) above, here we introduce and compare genome-scale models for two phylogenetically related cyanobacterial species, namely *Cyanothece 51142 i*RS764 and *Synechocystis 6803 i*RS706. Model *i*RS764 is comprised of 764 genes, 1,910 reactions and 1,862 metabolites, whereas model *i*RS706 spans 706 genes, 1,844 reactions and 1,894 metabolites. As many as 1,628 reactions and 1,702 metabolites are common in these two models. All reactions are elementally and charge balanced and localized in three different intracellular compartments (i.e., cytoplasm, carboxysome and thylakoid lumen). GPR (gene-protein-reaction) associations are also established based on the functional annotation information and homology prediction. We contrast flux balance analysis results under different

physiological conditions, (i.e., phototrophic, chemotrophic and mixotrophic) and explore their impact on biomass formation, nitrogen fixation and hydrogen yield of these two cyanobacterial species.

Additionally, towards goal (ii) above, we have begun characterizing genetic elements active at different phases of cyanobacterial culture, including during exponential growth and stationary phase. We have identified putative promoters that are highly active at stationary phase and have constructed a library of expression vectors that target genes to areas of the genome that are highly transcribed during stationary phase.

Table 1: *Synechocystis* 6803 *iRS*706 and *Cyanothece* 51142 *i*RS764 model statistics

	***Synechocystis 6803 iRS*706 model**	***Cyanothece 51142 iRS*764 model**
Included genes	706	764
Proteins	604	660
Single functional proteins	258	247
Multifunctional proteins	192	210
Protein complexes	3	4
Isozymes	27	30
Multimeric proteins	107	149
Others[a]	17	20
Reactions	1,844	1,910
Metabolic reactions	1,834	1,900
Transport reactions	5	5
GPR associations		
Gene associated (metabolic/transport)	1,798	1,836
Spontaneous[b]	18	19
Nongene associated (metabolic/transport)	18	40
Exchange reactions	5	5
Metabolites	1,894	1,862
Cytoplasmic	1,889	1,857
Extracellular	5	5

[a]Others include proteins involve in complex relationships, e.g. multiple proteins act as protein complex which is one of the isozymes for any specific reaction.

[b]Spontaneous reactions are those without any enzyme as well as gene association.