153. Systems Biology of Autotrophic-Heterotrophic Symbionts for Bioenergy

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Project Goals: The goal of this proposal is to combine autotrophs and heterotrophs as a novel sustainable symbiotic platform for the production of biofuels. Genome-scale reconstructions of autotrophic-heterotrophic co-cultures will be deployed to decipher and predict metabolic interactions and furthermore to optimize system performance for production of desired biofuels products.

Reconstructions are biochemically, genetically and genomically structured knowledge-bases that contain information of reaction stoichiometry, reaction reversibility, and the association between genes, proteins and reactions. Currently genome-scale metabolic reconstructions are available for a number of heterotrophs appropriate for biofuels. In this project, a reconstruction for the autotroph, Chlorella vulgaris has been generated in this framework to include the DOOR operon database, genome

The first step in the genome-scale metabolic network reconstruction process involves the generation of a draft reconstruction and the genome annotation. Different approaches to annotate the transcripts were used; BLASTp against SwissProt enzyme protein sequences, InterProScan and PRIAM, the 85% of the Chlorella's predicted genes were annotated.

The draft reconstruction was done based on homology to Chlamydomonas rheinhardtii. The draft reconstruction accounts for 621 genes associated with 1,108 reactions and 1,249 metabolites distributed in six compartments: cytoplasm, extracellular space, chloroplast, mitochondria, thylakoid, and glyoxysome. In the second step the draft reconstruction is manually refined following a protocol for generating a high-quality genome-scale metabolic reconstruction, during the manual curation the biomass function (objective function) is defined, it contains the cell composition, minerals and energetic requirements to generate biomass; for this step literature, annotation of other models and databases were used in order to improve and complete the reconstruction.

The current reconstruction for C. vulgaris contains over 900 genes for more than 1750 reactions, almost 1600 metabolites, and includes 250 transport reactions. Lipid metabolism represents 35% of the model; other main pathways such as photosynthetic pigments and carbohydrate storage were also included. Over 200 references have been added to the model. Finally, using the COBRA Toolbox the manually curated reconstruction is analyzed for connectivity, mass and charge balance, and converted into a functional mathematical model. The functions in the automated reconstruction will be evaluated using physico-chemical constraints and tested against well-known metabolic capabilities of C. vulgaris such as growth rate, by-products and secretion.

The final C. vulgaris reconstruction is currently validated using experimental data such as cellular biochemical composition obtained under different growth conditions. Given the importance of a comprehensive organization of the available data and information to be used during the model curation, a specific bibliome database for C. vulgaris has been created.

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