Elucidating Principles of Bacterial-Fungal Interactions

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Project Goals: The goals of this new project are to develop hybrid machine learning/simulation models of *Pseudomonas fluorescens/ Laccaria bicolor interactions and dynamics*. These hybrid data-analytic/simulation models will be used to carry out virtual experiments and develop fundamental understanding of the interactions between *Pseudomonas fluorescens* and *Laccaria bicolor.* At the same time, we will carry out experiments aimed at developing and testing quantitative assays to measure the same interactions, and whose data will inform the virtual experiments. We will:

- Evaluate the impacts of (1) thiamine and phenazines and (2) trehalose, produced respectively by *P. fluorescens* and *Laccaria*, on the metabolisms of each other. Metabolic exchange is an emerging theme in bacterial-fungal and bacterial-bacterial interactions.
- Characterize *Laccaria*-stimulated chemotaxis of *P. fluorescens* by coupling trehalose signaling and metabolism to chemotaxis *P. fluorescens*.
- Experimentally investigate (1) *Pseudomonas fluorescens* chemotaxis and metabolism of *Laccaria* produced metabolites, and metabolism of *P. fluorescens* produced metabolites in *Laccaria*.

Abstract. In comparison to bacterial-bacterial interactions, there is very little known about bacterial-fungal interactions even though these interactions are thought to be fundamentally important to DOE missions in sustainability, crop biofuel development and biosystem design. In biofuel crops, many crop root systems live in mutualistic symbiosis with fungi and bacteria. Mycorrhiza helper bacteria (MHB) increase host root colonization by mycorrhizal fungi, which in turn act as a micro-root system to provide the plant with soil nutrients. Recent work on the *Populus* root microbiome has determined that the interactions between the mycorrhizal fungus *Laccaria bicolor* and the bacterium, *Pseudomonas fluorescens* are key to fitness of the plant. These organisms, *Laccaria* and *P. fluorescens*, are the focus of this project to understand fundamental principles of interactions between fungi and bacteria from the perspective of material exchange and energetics, and how material and energetics are linked in inter- and intra-microbial subsystems.

The first task of this project is to develop a *hybrid simulation-machine learning model of Laccaria*. We are constructing a hybrid simulation-reinforcement learning model for *Laccaria* that combines metabolic control analysis, physics-based mass action kinetics with experimental data to predict experimentally faithful dynamics of the organism. The model will include all of central metabolism and secondary metabolism including protein production and the production

of biosynthetic enzymes. Under this task, we are using the *Laccaria* model to understand the growth energetics of *Laccaria* under various conditions pertaining to the questions of interest. We will evaluate the material and energy flow in *Laccaria* when sub- inhibitory levels of phenazines are present, with the naïve hypothesis is that material flow will be redirected away from respiration, possibly towards filament growth or branching. We will evaluate the impact of thiamine uptake from the environment on metabolism, with the naïve hypothesis that filament growth and branching will increase. We will predict conditions in which trehalose synthesis and export are favorable, and conditions in which water generated from metabolism is exported out of the cell. Currently the model includes central metabolism, cell wall synthesis and trehalose synthesis, as shown in the figure.

The second task is to construct a similar hybrid simulation-machine learning model of *P. fluorescens*, but to also couple the metabolism of this model with chemotaxis. We will use the *P. fluorescens* model to evaluate the change in growth and thermodynamic cost of thiamine production, and to understand the mechanism of trehalose stimulated chemotaxis of *P. fluorescens.*

The thiamine biosynthesis pathway is regulated by a thiamine riboswitch in *P. fluorescens*. When thiamine taken up from the environment, the riboswitch turns off the production of the enzymes needed to synthesize thiamine. The thermodynamic cost of thiamine production can be determined by understanding the changes in enzyme expression/activity, which the model will predict, when thiamine is present or not. The cost of enzyme synthesis can be estimated from either average cost of protein synthesis, or specifically from the amino acid sequences of the specific enzymes.

In order to understand the interplay and feedback between metabolism and chemotaxis, we are coupling metabolism to chemotaxis in *P. fluorescens.* Stimulation of the two-component chemoreceptor system alone is not sufficient to drive chemotaxis. Energy must also be redirected from other cellular processes to drive the rotation of flagella. Virtual experiments are helping us to develop hypotheses on how metabolism might be redirected for this purpose.

The third task is to experimentally investigate (1) *P. fluorescens* chemotaxis and metabolism of *Laccaria* produced metabolites and (2) biofilm formation between *P. fluorescens* and *Laccaria*.

We are initially focusing on several *Pseudomonas* strains that have differential responses on *Laccaria* (e.g. GM41, GM18, GM17). Bacteria utilize a chemoreceptor-phosphorelay system to sense and respond to chemical gradients. The binding of attractants to membrane bound methyl-accepting chemotaxis proteins (MCPs) initiates this process which leads to effects on motility behavior.

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