Integrated Genome-Scale Modeling of Syntrophic Consortia reveals Microbial Community Dynamics and Mechanisms of Electron Transfer

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Project Goals: The long-term goal of our project, which is entitled "Systems Level Analysis of the Function and Adaptive Responses of Methanogenic Consortia", is to develop genome-scale metabolic models of microbial communities that play an important role in the global carbon cycle that can be coupled with the appropriate physical-chemical models to predict how the microbial communities will respond to environmental perturbations, such as climate change. The short-term objective of the current research is to determine if the syntrophic associations that are central to the functioning of methanogenic terrestrial wetlands can be predictively modeled with genome-scale metabolic models.

The previously described[1] syntrophic co-culture of *Geobacter metallireducens* and *Geobacter sulfurreducens* serves as a genetically tractable model for the process of direct interspecies electron transfer hypothesized to be important in terrestrial methanogenic environments. Therefore, this co-culture is serving as an initial test case to determine if it is possible to use genome-scale metabolic models to predict the physiological responses of microorganisms engaging in direct interspecies electron transfer in complex communities and to characterize the relative efficiencies of direct electron exchange versus other alternatives, such as interspecies hydrogen or formate transfer.

In order to model different modes of electron transfer, it is important to accurately account for the energetics involved in extracellular electron transport. Hence, the existing genome-scale reconstructions of *G. sulfurreducens* and *G. metallireducens* were expanded to reflect the most updated annotations, a distinct periplasm compartment, and detailed biosynthetic pathways. Importantly, both these models now include detailed representation of the energy metabolism involved in extracellular electron transfer. The models now account for all the possible routes of electron transfer in and out of the cell through the various electron carriers such as cytochromes, ferredoxin, quinones, NAD, and FAD. In addition to the stoichiometry associated with the respective redox reactions, these pathways also account for thermodynamic consistency, appropriate gene association and cellular localization of the different electron carriers. The *G. sulfurreducens* model now consists of 829 genes and 1079 reactions, while the *G. metallireducens* model consists of 974 genes and 1173 reactions.

We further developed a modeling framework to integrate these two genome-scale models into a combined model to study the metabolic interactions. This framework includes a shared metabolite pool to account for the metabolic exchanges between the constituents

of the consortia. Computational simulations revealed that the optimal ratio of the constituents contained 21% *G. metallireducens.* This prediction is in accordance with experimental observations of a composition of 15% *G. metallireducens*.

Flux balance analysis simulations indicated that direct interspecies electron transfer is more efficient than interspecies hydrogen transfer for the growth of the consortia. This prediction is consistent with the previous observation[1] that selective pressure for rapid syntrophic growth selected for a mutation that promoted direct electron transfer. Acetate secreted by *G. metallireducens* was used as a carbon source as well as an additional source of electrons for *G. sulfurreducens*.

We also modeled a newly developed co-culture in which the *G. sulfurreducens* strain was incapable of acetate oxidation because the citrate synthase gene was deleted. This eliminated additional grow of *G. sulfurreducens* with acetate as the electron donor. The optimal ratio of the constituents in this co-culture was predicted to be 45% *G. metallireducens*, which compared well with the experimental observation of 50%. Again, direct electron transfer was predicted to be preferred over interspecies hydrogen transfer.

We further performed high-throughput transcriptomic profiling (RNA-seq) and physiological screens of both the wild type and mutant aggregates and analyzed them in the context of the metabolic model to gain insights into the mechanisms of electron transfer. Differential expression analysis revealed that 481 genes had significant changes in expression levels between wild-type and citrate synthase mutants. The global effects of these gene expression changes on the dynamics of the microbial community are being investigated with the aid of the integrated community model. We have also performed genome re-sequencing of the aggregates and identified SNPs that have accumulated as a result of the selective pressure of syntrophy.

In summary, this study represents an integrated multi-omic approach to elucidate the electron transfer mechanism and to characterize the metabolic phenotype of a laboratory evolved syntrophic consortium. The models being developed in these studies will be important for understanding the functioning of anaerobic terrestrial microbial communities and predicting the influence of environmental changes on methane emissions and other aspects of carbon cycling.

References:

1. Summers, Z. M., H. E. Fogarty, C. Leang, A. E. Franks, N. S. Malvankar, and D. R. Lovley. 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science 330:1413-1415

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