Exobiology Through the Lens of Constraint-Based Metabolic Modeling

Jared T. Broddrick[1], Mary N. Parenteau[1]
[1]Exobiology Branch, NASA Ames Research Center, Moffett Field, CA 94035
jtb@ucsd.edu

Introduction: A focus of many astrobiology questions resides on the interplay between biochemistry and geochemistry. This biotic-abiotic interface is intimately connected to habitability. Computational systems biology provides a paradigm to characterize this interface. Here we describe the application of constraint-based metabolic modeling to characterize photosynthetic microorganisms inhabiting a modern analog to the Earth's primordial ocean. Biochemistry is captured in a metabolic network that is converted to a mathematical model. Geochemistry results in constraints that limit the capabilities of the metabolic network. The combination of the two within a single framework can elucidate the co-evolution of life and the environment.

Methodology: Constraint-based reconstruction and analysis is a systems biology technique for characterizing and analyzing metabolic networks. The methodology is composed of three components: the network, the constraints and the objective.

The network represents the chemical reactions occurring in the system. These reactions can be biotic or abiotic. When the biochemical reactions in the network are derived from the whole-genome sequence of an organism, or community of organisms, the model is considered a genome-scale metabolic model.

Constraints are quantitative limitations placed on the rate of chemical reactions. The constraints reduce the number of paths through the network to the subset that is feasible under a given set of conditions.

The objective selects a feasible route through the network that is optimal for a selected cellular objective. For simple laboratory conditions, maximization of growth-rate is a common objective. The cellular objective in environments relevant to astrobiology are likely different.

Exobiology case study- a primordial ocean analog: We sampled phototrophic mats from Chocolate Pots hot spring (CP) in Yellowstone National Park (YNP), USA, an analog for Archean ferruginous oceans. The goal is to use a genome-scale metabolic model to determine whether the cyanobacteria are using Fe(II) as a donor for photosynthesis (Pierson et al., 1999). The mat samples were sequenced using the Oxford Nanopore MinION sequencing platform. Whole-genome assemblies will serve as the basis for genome-scale metabolic models. The geochemical data from the Fe(II)-rich vent water serves as the system constraints. By combining the biochemical and geochemical data in a consolidated, quantitative framework, we plan to attribute the abiotic and biotic contributions to observed biosignatures, such as iron oxidation.

Results:

Accuracy of the MinION assemblies: First, we assessed the capacity of the MinION sequencer to generate sufficiently accurate assemblies for genome-scale modeling. We created a known heterogeneous sample of three bacterial species and sequenced the mixture. The resulting assembly resulted in three closed genomes that matched the starting species. Models derived from these assemblies were comparable to models built from hybrid assemblies using MinION and Illumina reads.

Isolation of dominant phototrophs in the CP mats: Mat samples were cultured in a variety of artificial cell culture media compositions. Highly enriched cultures were obtained for a *Synechococcus*/*Chloroflexi* co-culture and for and *Cyanothece* species. Pigment-specific excitation coupled to fluorescence microscopy was used to evaluate culture purity. These enriched cultures will enable focused biochemical characterization of the metabolic potential of each species.

Sequencing and model building: Additionally, these cultures, along with native CP mat samples, were sequenced using the MinION system. The resulting reads were base-called using the Guppy program (Oxford Nanopre version 2.3.5) using both the traditional and flip-flop algorithms. Model construction is currently on-going.

References:

Pierson, B. K., et al. (1999) *Appl. Environ. Microbiol.* **65**, 5474-5483.