

Quantifying Differential Expression and Identifying Bottlenecks in Methanogenic Pathways

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Abstract: To facilitate our BER funded research to develop models of acetotrophic methanogenic microbes that predictively capture changes in cell behavior with single cell resolution due to dynamic environments and constraints imposed by communities, we request sequencing experiments from JGI and single cell microscopy and mass spectrometry analyses from EMSL. The model archaea of interest include *Methanosarcina acetivorans* and *M. barkeri*, two versatile methanogens integral to the global carbon cycle, are capable of growing on over eight different carbon sources, various salt conditions and are known to fix atmospheric nitrogen. Furthermore, they have been identified in syntrophic relationships with sulfur and iron reducing bacteria, and there is potential that they could be combined with other microbes to facilitate the final degradation of biomass waste into usable and storable bioenergy in the form of methane.

Seventy-two RNA sequencing and 30 noncoding/small RNA sequencing experiments under varied carbon source, nitrogen availability, salt conditions are requested as they will provide data with which to identify the network of interactions of transcription factors and small regulatory RNA make with genes across the metabolic map. Combining these constraints with flux balance analysis (FBA) models of the methanogens' metabolism we can extend the models to accurately model changes in metabolic pathway usage. With these models, bottlenecks for growth can be identified, guiding genetic and metabolic engineering efforts.

Additionally, we request the unique capabilities of the EMSL in single molecule fluorescence imaging to help quantify average protein number of key proteins, as well as single cell variability that arise due to intrinsic and extrinsic noise. These values can be used with our recently developed population FBA to simulate the state of many individual cells to examine how variability affects pathway usage and causes emergence of different behavior among an isogenic population. To further constrain the metabolic model and our recently developed kinetic model of methanogenesis, we propose to leverage the vast mass spectrometry capabilities at EMSL. Colonies of the organisms will be grown in ¹³C labeled carbon sources, which with the rate of ¹³C incorporation into proteins will be used along with the fluorescence protein counts to identify average protein count and production rate across the genome.

Finally, the wealth of data will be integrated into our combined spatially-resolved/FBA models of cell colonies. These methods will allow us single cell resolution of genetic/metabolic state that arises due to the neighboring environment. This will allow us to identify factors that contribute to resource competition, differential cell fates, origins of syntrophic interactions and growth behaviors of colonies. The models and data will vastly expand our knowledge of methanogens. Particularly, they may be used to identify strategies to maximize biofuel production. More generally, models will provide means to understand how cells grow and differentiate in the context of a world where competition and cooperation are two sides of the same coin.