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Publication Date

2009-02-08

Development and Analysis of Multispecies Consortia to Study Microbial Community Stress and Survival

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Acknowledgements

This work was part of the Virtual Institute for Microbial Stress and Survival (<http://VIMSS.lbl.gov>) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

Abstract:

Cultivation of individual microbial species has been at the core of experimental microbiology for more than a century but offers only a glimpse into the metabolism and ecophysiological potential of most microorganisms. Microbial communities, not individual species, control process rates and drive key biogeochemical cycles, including those that determine the transformation of environmental pollutants of concern to the DOE. Thus controlled studies of model consortia comprised of multiple species that mediate such processes are essential for advancing DOE objectives in bioremediation and other applications.

As part of phase two of the Environmental Stress Pathway Project (ESPP) team, the goals of this project are: (i) to develop a stable syntrophic microbial consortia in continuous flow systems which can be used for physiological and functional genomic studies, (ii) to study how organisms change their activity on a molecular level in response to other community members in the co-culture by analyzing the gene transcription, metabolite flux and growth, and (iii) to study how these changes in activity and community organization affect the resistance and resilience of microbial communities in response to stress, invasion and other perturbations on a molecular level.

The project workflow has two major and interrelated components: microbial cultivation and stress experiments and development and application of analytical methods to characterize the consortia. *Clostridium cellulolyticum* was chosen as the basal organism for initial the three & four member syntrophic assemblies as it can ferment cellobiose, producing acetate, lactate, ethanol and hydrogen. The secondary stage in the syntrophic chain is represented by *Desulfovibrio vulgaris* and by *Geobacter sulfurreducens*, which utilize the *C. cellulolyticum*-produced metabolites. *D. vulgaris* and *G. sulfurreducens* are provided with sulfate and fumarate respectively as electron acceptors. Additional studies with a methanogens (*Methanococcus maripaludis* and other species) are also being pursued. Methods for tracking population dynamics such as quantitative PCR and species specific fluorescently labeled antibodies have been developed and have shown that stable assemblages comprised of these species can be achieved. The chemostat instrumentation operates multiple fermenters that are fed from the same medium source. The multiple fermentation setup can be used for providing biological replicates as well as for conducting stress experiments. Methods for quantitative metabolite analysis via HPLC, GC/MS have been successfully developed and the data obtained from

analytical methods are consistent with the expected growth and metabolite uptake and depletion patterns for the consortium.

Our initial metabolic analyses of the trimember consortia indicates that growth of *C. cellulolyticum* is carbon limited by the cellobiose concentration. However, its fermentative product acetate remains in abundance despite serving as a carbon source for *D. vulgaris* and a carbon and electron source for *G. sulfurreducens*. *D. vulgaris* likely relies on hydrogen for an electron source and the small amount of lactate from *C. cellulolyticum* and is thereby growth limited by electron donor potential. Growth of *G. sulfurreducens* is limited by abundance of its electron acceptor, fumarate. In experiments with the methanogen (*M. maripaludis*) added to the consortia its populations are inherently less stable and hover at levels nearing the detection limit of the QPCR assay, although methane can often be detected at low levels suggesting biological activity of these organisms. After achieving this stable configuration, we have recently developed a four species microarray for tracking transcriptional activity of these species and comparisons with parallel mass spectrometry-based proteomics. We have begun analyzing data from growth experiment designed to compare stable consortia conditions contrasted with individual species grown alone, and are planning near term experiments to understand resistance and resilience of these communities under various perturbation scenarios on a molecular level.