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Title
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ESPP Mission

DOE oversees 350 cleanup projects involving soil contaminated with imported/radionuclides. The life-cycle cost of these projects is at least $220 billion over 70 years, without breakthroughs. A thorough understanding of the biogeochemistry, especially stress responses in metal/radionuclide bacteria, enables prediction of natural attenuation and new strategies for remediation saving DOE billions in cleanup, risk assessment, and environmental stewardship. This application is representative of an array of environmental, ecological, and bioenergy stewardship challenges that rest on developing a detailed understanding of environmental microbial physiology, community interactions, population genetics and functional evolution. The diversity of knowledge/technology necessary to accomplish these goals necessitates a team science approach, building a sophisticated experimental and computational infrastructure.

The Virtual Institute of Microbial Stress and Survival (http://vimss.lbl.gov) was established in 2002 through DOE Genomics:GTL funding of the Environmental Stress Pathway Project (ESPP). Using stress-response and metal reduction as target processes, and the environmentally important sulfate reducing bacteria, specifically *Desulfovibrio vulgaris* Hildenborough, as an organismal focus, ESPP is developing this system in two main thrusts: 1. Environmental characterization of biogeochemistry and microbial processes at metal/radionuclide contaminated sites, and 2. Laboratory and computational characterization of environmental stress pathways in microbes. Phase 2 of this project is increasing focus on molecular determinants of community activity, stability and ecology. This research is managed by three core teams: Applied Environmental Microbiology Core, Functional Genomics and Imaging Core, and Computational and Systems Biology Core.

The diversity, complexity and interoperability of the capabilities implemented by our core facilities take resources beyond the capabilities of any one or two labs and have enabled us to develop protocols for bringing environmental organisms to model organism status relatively quickly and to learn a great deal about the stress response, regulatory and metabolic pathways of *Dv*. The figure above is a collage of results from various studies employing all the techniques of VIMSS in comparative genomics, functional genomics, and environmental genomics.

We have also been able to leverage our resources to create a new set of technologies to help expand our efforts on single organism and community comparative functional genomics and function. We are expanding our characterization tools to include high throughput genetic techniques, new metabolomics methods for mixed cultures, new profiling technologies for understanding population and gene expression changes (functional metagenomics) over time in complex field studies, as well as the computational tools with which to manage, analyze and interrelate these data sets.

http://vimss.lbl.gov

Brief Summary of Accomplishments

In the first phase of this project, we have succeeded in bringing a member of an important environmental class of microbe, the sulfate reducing bacteria (SRB), to nearly model organism status. In the last five years, we have been able to gain great insight into the stress response and metabolism of *Desulfovibrio vulgaris* Hildenborough (*Dv*). To do so we have developed an efficient genetic system; created a general purpose microbial systems biology pipeline; measured a wide range of genome-scale physiological responses to perturbations found in DOE contaminated environments; inferred regulatory systems and their evolution and conservation across metal-reducing SRB; and have just begun to probe *Dv* interactions with community members at DOE contaminated sites. The ESP team has gelled as a mature working group of scientists able to balance the scale and coordination necessary for environmental systems biology with the special individual expertise to pursue detailed follow up projects. This phase of the project has produced over 104 publications while team members have collectively made several hundred presentations of this work worldwide. The initial project has resulted in the development of a number of new technologies for functional genomics, data analysis, and physiological control of culture conditions. We have developed two community computational resources for comparative functional analysis of microbial genomes, MicrobesOnline (http://microbesonline.org) and RegTransBase (http://regtransbase.lbl.gov). We have 27 posters at this meeting outlining a number of the particular successes of this project as well as some of the work in preparation for phase two.

In phase two we will use this well-developed foundation to launch an expedition to discover the molecular mechanisms by which microbial community structure, function, and stability affects stress response and activity in the lab and in the field. We are now able to link field experiments strongly to controlled experiments with constructed consortia in the laboratory. We will continue to enrich our knowledge of the cellular networks of *Dv* and its relatives and expand our study to include its association with the methanogens with which it forms syntrophic associations. In stable co-cultures of *Desulfovibrio*-like organisms (Dvo) and methanogens, we will discover the molecular basis of their metabolic coupling and other factors that lead to their unique environment, active association leading to population growth, metal-reducing activity and resource utilization. We will construct more complex consortia to study the role of functional diversity, redundancy, and stress response in creating more or less stable microecologies via interactions at the community level. Finally, we will observe related microbes at DOE metal contaminated sites before, during, and after stimulation/stress to determine the differences and similarities to our laboratory experiments. There is little understanding of the molecular basis of microbial community formation, stability, resilience, specificity, activity and ecology, and we will be carrying out one of the first large-scale systems biology effort to explore these ubiquitous and important phenomena.

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