ENIGMA Environmental Microbiology: Metagenomics-Enabled Understanding Of Microbial Communities At DOE Contaminated Sites

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Project Goals: Although high throughput sequencing and associated (meta)genomic technologies provide an avenue to determine genetic and organismal diversity of an ecosystem, linking the genetic/population diversity to phenotypic diversity across different organizational levels (e.g., molecular, cellular, populations, communities and ecosystems) is extremely challenging. As a part of the overall ENIGMA goal, the ultimate aim of this project is to utilize (meta)genomic technologies to better understand the mechanistic connections between molecular-level interactions/processes and community-level processes/functions. The following three specific objectives have been pursued: (i) To determine adaptation and molecular mechanisms of *Desulfovibrio vulgaris* Hildenborough (DvH) in response to multiple environmental stresses; (ii) To understand microbial community functional diversity at U/Cr-contaminated sites and develop high-throughput functional gene arrays (FGAs) for microbial community analysis; and (iii) To determine the responses, interactions, mechanisms and dynamics of groundwater/sediment microbial communities to U/Cr contamination and bioremediation treatments.

Long-term experimental evolution of DvH. To better understand the mechanistic connections between molecular-level functions and community-level processes, experimental evolution has been carried out to determine molecular mechanisms of DvH in response to high salinity. Significantly increased salt resistance was observed in evolved DvH (eDvH) with increased biomass, higher growth rate and shorter lag phases. Whole genome sequencing of eDvH at 1200 generations revealed specific point mutations and deletions. Their contribution to increased salt resistance has been proven by mutagenesis and phenotype analyses. Glu and Ala significantly increased in eDvH. After 5000 generations, the final biomass and growth rate of eDvH in the medium with high salinity was similar to that in the medium without extra salt. To further investigate the dynamics of evolution, repeatability of the evolution, whole genome sequencing, fitness assay and site-directed mutagenesis are in progress.

GeoChip-based metagenomic technology development. We have developed the GeoChip 4.0 series (4.0-4.2) for characterizing microbial communities. The GeoChip 4.0 series are manufactured based on the NimbleGen microarray format. For example, GeoChip 4.0 contains 120,054 distinct probes, and covers 200,393 coding sequences for 539 gene families in different microbial functional processes. The StressChip subset contains 22,855 probes covering 79,628 gene sequences for 46 genes involved in microbial responses to environmental stresses (e.g., temperature, osmolarity, oxidative status, nutrient limitation). The specificity, sensitivity and quantification of the developed GeoChip 4.0 series were evaluated computationally and

experimentally. High specificity was observed for both synthesized oligonucleotides and genomic DNA from pure strains; the sensitivity was estimated to be 0.5 µg of DNA; the log(signal intensity) vs. log(DNA concentration) was highly correlated (R = 0.925). All the results showed that the GeoChip 4.0 series are specific, sensitive, and quantitative tools for characterizing microbial communities.

GeoChip applications. GeoChips have been used to study groundwater microbial communities to examine sustained reduction of contaminants using slow-degrading/slow-hydrolysis e-donors. At the Oak Ridge site, a one-time injection of emulsified vegetable oil (EVO) was used to examine U(VI) bioreduction and immobilization. Samples collected from the control and treatment wells (W1-7) were analyzed using GeoChip 3.0. Acetate, from EVO biodegradation, stimulated NO_3^-, Mn(IV), Fe(III), SO_4^{2-}, and U(VI) bioreduction in W1-7 and increased functional gene diversity. After EVO depletion, functional gene diversity declined. Fe(III)- and sulfate-reducing bacteria could play key roles in U(VI) reduction, whereas acetogens, denitrifiers and methanotrophs could be important for e-donor production and maintaining favorable reducing conditions. At the Hanford site, a one-time injection of poly-lactate was used to test Cr(VI) bioreduction. The groundwater microbial communities were monitored for 390 days using GeoChip 4.0. Cr(VI) was effectively reduced and functional gene diversity increased. Fe(III)- and sulfate-reducing bacteria could play key roles in Cr(VI) reduction, whereas denitrifiers could be important for maintaining reducing conditions.

Metagenomic sequencing. We have sequenced or resequenced metagenomes and dominant isolates from Oak Ridge FRC wells FW106 (contaminated with uranium, nitric acid, organics, and mercury) and FW301 (pristine). The FW106 metagenome was previously sequenced, and the results suggest extensive lateral transfer of metal resistance and organic compound metabolism genes. To extend these analyses, the metagenomes of FW106 and FW301, and genomes of multiple isolates of the dominant *Rhodanobacter* strain found in FW106 were sequenced or resequenced using Illumina sequencing technology. We are currently conducting comparative analyses of FW106 to FW301 to identify ecological trends observed between pristine and highly stressed groundwater communities. Furthermore, we are comparing the FW106 metagenome to *Rhodanobacter* isolates to confirm predicted lateral transfer events.

Molecular ecological network analysis. A novel random matrix theory-based approach has been developed to construct molecular ecological networks (MENs) based on GeoChip hybridization or high-throughput sequence data. Various mathematic and statistical tools and methods have been integrated into a comprehensive MEN analysis pipeline (MENAP). We have applied this approach to construct and analyze MENs from the Oak Ridge EVO experiment described above. Functional MENs were constructed from three GeoChip datasets: (i) Early EVO injection (≤31 days), (ii) Days 80 to 140, and (iii) Control well (7 time points) and pre-injection (0 day). All three constructed functional MENs posed general network characteristics (scale-free, small world and modularity), and the topology of these functional MENs was distinctly different, suggesting that the interactions among different microbial functional groups/populations in each community were dynamically altered during uranium bioremediation. Additionally, the changes in network structure were significantly correlated with environmental geochemical dynamics and EVO concentrations.

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