

Project Title: Deduction and Analysis of the Interacting Stress Response Pathways of Metal/Radionuclide-reducing Bacteria

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Summary

Three major objectives have been pursued in the Zhou group at the University of Oklahoma (OU): (i) understanding of gene function, regulation, network and evolution of *Desulfovibrio vulgaris* Hildenborough in response to environmental stresses, (ii) development of metagenomics technologies for microbial community analysis, and (iii) functional characterization of microbial communities with metagenomic approaches. In the past a few years, we characterized four CRP/FNR regulators, sequenced ancestor and evolved *D. vulgaris* strains, and functionally analyzed those mutated genes identified in salt-adapted strains. Also, a new version of GeoChip 4.0 has been developed, which also includes stress response genes (StressChip), and a random matrix theory-based conceptual framework for identifying functional molecular ecological networks has been developed with the high throughput functional gene array hybridization data as well as pyrosequencing data from 16S rRNA genes. In addition, GeoChip and sequencing technologies as well as network analysis approaches have been used to analyze microbial communities from different habitats. Those studies provide a comprehensive understanding of gene function, regulation, network, and evolution in *D. vulgaris*, and microbial community diversity, composition and structure as well as their linkages with environmental factors and ecosystem functioning, which has resulted in more than 60 publications.

(i) Understanding of gene function, regulation, network and evolution of *D. vulgaris* in response to environmental stresses. Salt stress is found to co-exist in many environments like DOE-contaminated sites. A long-term experimental evolution was established to select salt-adapted strains. Control and salt-stressed lines (six lines each) have been experimentally evolved under standard (LS4D defined medium) and salt stress (LS4D + 100 mM NaCl) conditions for 4000 generations. *D. vulgaris* lines quickly adapted to salt stress at about 100 generations and the salt resistance keeps improving as generations increase. The dynamics of the improvement on salt resistance suggested that *D. vulgaris* lines genetically adapted to the salt stress, and that such an adaptation reached a relatively stable stage at approximately 1000 generations. The Illumina sequencing analysis of the isolated clones from evolved lines (1200 generations) and the ancestor revealed the mutations accumulated during evolution. A total of nine SNPs and two deletions in evolved salt-adapted line, 14 SNPs and one deletion in evolved control line, and five SNPs, 14 insertions and four deletions in the ancestor were identified and confirmed by Sanger sequencing. Gene transcriptional profiling of the isolated salt-evolved clone demonstrated the increased expression of the genes involved in exclusion of Na⁺, influx of osmoprotectants, energy metabolism, iron uptake and decreased expression of flagella assembling systems and phosphate

transport compared to the ancestor strain. Consistent with the gene transcriptional profiling data, Glu and Ala were found accumulated and lysine was less abundant in the isolated salt-evolved clone. The most abundant Glu and Ala appeared to be the major osmoprotectants for salt stress. Further analysis of deletion mutant of *DVU0597* (*lysS*, SNP found in evolved clone) confirmed the contribution of this gene for increased salt resistance. Also, four annotated *crp/fnr* genes were functionally characterized with mutagenesis, transcription profiling, physiology and competition assay, and the results suggest that these four regulators have distinct functions in response to different stresses.

(ii) Development of metagenomics technologies for microbial community analysis. We have developed various metagenomics technologies for characterizing microbial community structure. First, based on previous GeoChips, we have developed GeoChip 4.0, a more comprehensive GeoChip to facilitate our analysis of microbial communities from a variety of habitats. GeoChip 4.0 contains 120,054 distinct probes, covering 200,393 genes involved in different functional processes important to biogeochemistry, ecology, environmental sciences and human health. Among them, a total of 21,560 probes covering 55,425 gene sequences for 45 functional genes involved in responses to environmental stresses, such as temperature, osmolarity, oxygen, and nutrients, which is termed as StressChip, and StressChip has been applied to analyze many sets of environmental samples. As a new version, GeoChip 4.0 is developed on a NimbleGen 12x135K platform that each chip contains 12 arrays. Both computational and experimental evaluations show that GeoChip 4.0 is a highly specific, sensitive and quantitative tool for microbial community analysis. Also, a random matrix theory-based conceptual framework for identifying functional molecular ecological networks is developed with the high throughput functional gene array hybridization data. Our results indicated that RMT is powerful in identifying functional molecular ecological networks in microbial communities. Elucidating network interactions in microbial communities and their responses to environmental changes is fundamentally important for research in microbial ecology, systems microbiology, and global change. In addition, amplicon sequencing approaches have been widely used in microbial ecology, but we found that its reproducibility and quantitative capability were quite low, mainly due to random sampling. Various approaches have been developed to predict and minimize the artifacts associated with random sampling processes.

(iii) Functional characterization of microbial communities with metagenomic approaches. Metagenomic technologies, especially GeoChip and metagenome sequencing have been used to understand the functional diversity, composition, structure, function, and dynamics of microbial communities from a variety of habitats. Here we present two examples of studies at DOE relevant sites with GeoChip or metagenome sequencing. One is to examine effects of emulsified vegetable oil (EVO) on microbial communities from a uranium contaminated aquifer at the DOE Oak Ridge (Oak Ridge, TN) site using GeoChip 3.0. EVO was injected one-time into a U(VI) contaminated aquifer as a slow-released alternative electron donor for biostimulation of U(VI) reduction. Groundwater samples were collected from one upgradient control (W8) and seven downgradient wells (W1-7) to monitor subsurface geochemistry and temporal dynamics of microbial communities over a 9-month period. Acetate was detected as one of the intermediates from biodegradation of EVO. During this period, while the microbial community function and structure in W8 remained unchanged, significant enrichments of genes involved in the biodegradation reactions of EVO and reduction of the electron acceptors via denitrification,

dissimilatory and assimilatory nitrate reduction, and reduction of Mn(IV), iron (III), sulfate and U(VI) were observed in W1-7, corresponding to the acetate production and electron acceptor (e-acceptor) reduction. The other is metagenome sequencing of two microbial communities, FW106 (contaminated well) and FW301 (background well) at DOE Oak Ridge sites. Our analysis of FW106 sequencing data showed that exposure to high concentrations of heavy metals, nitric acid and organic solvents has resulted in a massive decrease in species and allelic diversity as well as a significant loss of metabolic diversity. β - and γ -Proteobacterial populations and key functional genes for stress responses, metal resistance and organic contaminant degradation were significantly enriched. An initial comparison of the two metagenomes indicates that species, strain and metabolic diversity are severely reduced in the FW106 stressed community. In addition, GeoChip has been used to analyze microbial communities from a variety of habitats, such as soil samples from a uranium mill tailings remedial action (UMTRA) site (Rifle, CO), sediment samples from heavy metal contaminated Lake DePue, water samples from acid mine drainage, and soil samples from arsenic contaminated sites.

Publications (P: partially supported by this project)

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