Final Report

Metagenomics-enabled understanding of the functions and activities of microbial communities at ERSP field research

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The focus of our work is to better understand the bioremediation of uranium in the subsurface. To evaluate the natural occurring uranium-immobilizing bacterial populations, we have anaerobically enriched uranium contaminated soil sediments (FW107, FW102-2, and FW102-3) collected from ORNL iFRC site (S3 area). Electron acceptors, NO_3^-, Fe^{3+}, and U^{6+}, were supplemented in the enrichments as selection factors. We previously reported the shifts of bacterial community profiles in enrichments. After two serial transfers, bacterial 16S rRNA gene surveys revealed that Fe^{3+} and U^{6+} supplemented enrichments had similar community structures. Genus *Pelosinus* was found dominant in Fe^{3+} and U^{6+} supplemented FW107 microcosms and present but substantially less abundant in FW102-2 and FW102-3 enrichments. Unclassified *Clostridiales* and *Geobacter* groups were most abundant in Fe^{3+} and U^{6+} supplemented FW102-2 microcosms, respectively and *Geobacter* dominated the FW102-3 community after it was enriched with Fe^{3+} and U^{6+}. Non-metric multidimensional scaling analysis of the original soil sediment and enriched bacterial community sequences based on the complete linkage clustering revealed that the shifts were substantial. Significant decrease of several unclassified bacterial groups, including unclassified *Proteobacteria*, unclassified *Deltaproteobacteria* and unclassified *Desulfobulbacease* due to the enrichment process were observed ($P \leq 0.05$). Genera *Pantoea*, *Trichococcus*, and *Sulfurospirillum* increased significantly ($P \leq 0.05$) in nitrate-supplemented microcosms. No increase of bacterial groups in iron and uranium enrichments could be concluded as statistically significant. Consistent with the phylogenetic results, GeoChip analysis performed on these samples showed significant decrease ($P \leq 0.01$) in abundance of the chlorobenzoate dioxygenase gene, that was previously identified in several *Proteobacteria*, after the enriching process. Several genes related to metal resistance, such as ferrochelatase, antioxidant and lead resistance, also decreased significantly in enrichments ($P \leq 0.01$). In contrast, several genes related to electron transportation (e.g., NADH quinone, cytochromes) increased significantly ($P \leq 0.01$), particularly in nitrate suppplemented microcosms. Interestingly, the abundance of ABC antibiotic transporter genes increased significantly ($P \leq 0.01$) in iron and uranium enriched consortia but not in the nitrate ones.

Towards our goal of identifying previously unknown uranium immobilizers, we have isolated 54 bacterial isolates from Fe^{3+} and U^{6+} supplemented enrichments. The growth rates of these isolates at 25°C vary from 6 hours to 22 hours per generation. All isolates belonged to the phylum Firmicutes and can be categorized into four groups, *Clostridium* XI, *Clostridium* XIVa, *Clostridium sensu stricto,* and *Pelosinus,* based on their 16S rRNA gene sequences. The majority of the sequences in *Clostridium* XIVa group were identical to the 16S rRNA gene sequence of *Desulfotomaculum guttoideum,* a sulfate-reducing bacterium. At 95% to 97% similarity, sequences in *Clostridium sensu stricto* group were closely related to *Clostridium acetobutylicum,*

Clostridium *butyricum,* and *Clostridium puniceum.* While *Clostridium acetobutylicum* was recently reported as a uranium reducing bacterium, the other two have not been linked to uranium reduction. The *Pelosinus* isolates we have identified were closely related to *Pelosinus sp.* strain UFO1, recently reported as a uranium immobilizing strain. Out of 54 isolates, only one was identified as *Clostridium venationis* in group *Clostridium* XI. Metal reduction has not been reported for this species. We are in the process of evaluating the uranium immobilization rate by these isolates and preliminary studies have shown that these isolates are resistant to 250μM of U^{6+}. We have used REP-PCR to de-replicate the isolates into about 35 groups in preparation for a systematic analysis of U^{6+} immobilization by synthetically reconstructed communities.

We also used a comprehensive functional gene array (GeoChip) on field samples from the iFRC to detect and monitor microbial communities involved in U reduction on site. Groundwater microbial communities within a pilot-scale test system established for the biostimulation of U(VI) reduction in the subsurface by injection of ethanol were examined during various operational periods using GeoChip 3.0. Functional community dynamics were examined during periods of active U(VI) reduction, maintenance of reduced U(IV), starvation, reoxidation by O_2, and nitrate exposure. During the active reduction phase, both Fe- and sulfate-reducing bacterial populations reached their highest levels and the U concentrations in the groundwater were significantly correlated with the total abundance of c-type cytochrome and *dsrAB* genes. Detrended correspondence analysis (DCA) showed a shift towards a different community structure after ethanol injections resumed compared to the periods of starvation and exposure to DO. Overall, results indicated that ethanol was the main factor affecting community structure, although some changes could be attributed to DO. After exposure to nitrate the diversity and richness of the microbial community increased several fold but quickly returned to pre-nitrate levels. DCA indicated a shift in the overall community structure after nitrate exposure but the community began to return to pre-exposure structure once nitrate was removed. The relative abundance of several nitrogen cycling genes showed an increase immediately after nitrate exposure, including ammonification, denitrification, and nitrogen fixation genes indicating a stimulation of these communities. Sediment microbial communities within in this system, from two different treatment zones, were also examined. The results showed that different microbial communities were established in different wells and high gene overlap was observed from wells within the same treatment zone. Higher microbial functional gene number, diversity and abundance were observed within the active bioremediation zone. The microbial community structure was highly correlated with the hydraulic flow rate and geochemical conditions of the treatment zone, especially pH, manganese concentration and electron donor level.

Samples were also collected from groundwater monitoring wells along a contamination gradient at the iFRC and were analyzed using GeoChip 2.0. Results revealed less overlap between wells with different levels of U and NO_3^- contamination. While diversity of nitrate-fixation genes decreased in NO_3^--contaminated wells, the diversity of metal reduction and resistance genes did not correlate with metal concentrations. Signal intensity did, however, increase in heavily contaminated wells, indicating a larger percentage of organisms with metal- related genes. Sulfate-reduction genes had greater diversity and greater signal intensity in more contaminated wells. Individual principle component analyses (PCA) of the gene diversity and geochemistry of the wells separated them in similar ways. Canonical correspondence analysis indicated that pH

was an important variable that correlated with gene diversity in the lowest-contamination well, while NO_3^- and U correlated with the most highly contaminated well. Overall, contaminant level appears to have significant effects on the functional gene diversity along the contaminant plume at the FRC.

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