Radiochemically-Supported Microbial Communities: A Potential Mechanism for Biocolloid Production of Importance to Actinide Transport

University-Led Research

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This work focuses on the hypothesis that radiogenic substrates such as H_2 at DOE sites may support the growth of microorganisms, and thus indirectly influence the mobility of redox-sensitive radionuclides. Consistent with this hypothesis are early project results indicating the presence of H_2 and SSU rRNA genes, closely related (up to 99% sequence identity) to candidatus *Desulforudis audaxviator*, in fluids from underground nuclear test cavities (U12N.10 tunnel and ER-EC-11) at the Nevada National Security Site (NNSS). In deep South African mines, *D. audaxviator* is believed to utilize H_2 and SO_4^{2-} from radiochemical reactions; and until recently, this region was thought to define the geographic limit of the genus. Thus, the detection of *D. audaxviator* in radioactive subsurface water at the NNSS supports a radiochemical lifestyle for *D. audaxviator*. The current project builds upon these observations with the sampling of new sites, detailed microbial community and chemistry assessments, and radiochemical modeling.

In 2011, groundwater was obtained from six new wells from the NNSS and Nevada Test and Training Range: ER-20-5 #1 and #3 (4/26/11), ER-20-8 (6/27/11), ER-20-4 (9/21/11), U12N vent hole #2 (10/5/2011), and ER-EC-12 (11/28/11). These samples are currently being analyzed for dissolved ions, gases, and radiologic components. All samples contained sulfate (3 mg/L to ~140 mg/L) but only the U12N sample contained measurable H_2S (~6 µM). Four of six sulfate samples have been analyzed for sulfur isotopes, with $\delta^{34}S$ values ranging from +4.43‰ to +17.44‰. H_2S fractionation values are pending. Corresponding DNA extracts are currently undergoing molecular community assessments including T-RFLP, SSU rRNA gene libraries and 454 pyrotag analysis. Various physiotypes were also cultivated using defined media based on NNSS groundwater. Aerobic heterotrophs were present at all sites analyzed, with densities ranging from 10^4-10^6 cells mL^{-1}. Sulfate-reducing bacteria were also present (~10^3-10^4 cells mL^{-1}) at sites ER-EC-11 and U12N. Enrichments from U12N grew equally well in the presence or absence of ^{239}Pu, suggesting a community adapted to a high radiation environment.

To better constrain the abundance of *D. audaxviator* in the U12N.10 tunnel and ER-EC-11 samples, DNA extracts were subjected to pyrotag analysis (~20,000 reads, M. Sogin Lab, MBL). Sequences from the Actinobacteria, Deinococcus-Thermus, Firmicutes, and Proteobacteria phyla were detected in both ER-EC-11 and U12N.10; the U12N.10 tunnel sample also contained Bacteroidetes, Chlorobi, and Nitrospirae. Over 40 genera of *Clostridia* were present, with *Symbiobacterium* and *Dethiobacter* dominating the ER-EC-11 and U12N.10 libraries, respectively. *Clostridia* comprised 25% of the U12N.10 library, and this sample also produced many sequences affiliated with *D. audaxviator* (93-99% similar). Flow cytometric cell sorting was performed (six 384 well plates, Bigelow Lab. R. Stepanauskas) on a cellular retentate washed free of radioactivity from U12N, with subsequent multiple displacement amplification (MDA) yielding ~60 useable sequences. Full genome sequencing will follow if a single amplified genome (SAG) from a North American *D. audaxviator* is obtained.