Cultural activity may adversely affect the performance of a nuclear waste repository by enhancing radionuclide solubility and migration. Examples of this are: radionuclide complexation with carbon dioxide generated from the degradation of waste organics and bioclastic transport. Organisms may be introduced during mining and repository operations or with the emplaced waste. Emplaced organisms may have been studied because of the difficulty in acquiring actual samples. In the case of the Waste Isolation Pilot Plant (WIPP), located in a salt cavern, high salt concentrations may effectively limit the activity of these organisms. Unprecedented access to two aged and disintegrated drums of nuclear waste (~500-1100 mCi radioactivity per gram waste; mostly americium and plutonium) yielded samples for both molecular and culture work. No DNA could be extracted from the drum with higher activity. Bacterial clone libraries were constructed from the DNA of the low-activity drum and yielded sequences exclusively from the phylum Actinobacteria (majority Dietizia). Cultures yielded both Actinobacteria (Arthrobacter and Brachybacterium) and Firmicutes (Bacillus sp.). Illumina HiSeq data support an Actinobacteria-dominated community, with a potential contribution from β-Proteobacteria. Neither archaeal nor eukaryotic DNA was detected.

**METHODS**

**Sample description**
- Waste originally generated by Department of Energy sites involved in production, testing, and cleanup of nuclear weapons during and after World War II
- Drum burial in underground pits for ~50 years

**Sample handling and analyses**
- Waste samples processed in ambient air-filled glove box designed for radioactive sample manipulation
- Aliquots were removed and processed for ICP-MS and scintillation counting analyses
- DNA purification and analysis
- Phylogenetic analysis of isolated clones

**DNA Extraction**
- No significant reduction in radioactivity of final extract using EDTA pretreatment
- No DNA from higher-radioactivity drum (drum 9)

**Results: DNA Analyses**

**Phylogenetic Affiliation of clones**
- Exclusively Actinobacteria; majority Dietizia

**RESULTS: CULTURE ANALYSES**

**DNA Extraction**
- Five isolates obtained: Three Bacillus sp., Arthrobacter, and Brachybacterium
- No growth at NaCl > 1.2 M; too potential for activity in brine
- Growth of Arthrobacter sp. in presence of [U] as high as 22 M
- Survival of Arthrobacter sp. up to 5 weeks in high-Mg brine (2.8 M NaCl + 0.95 M Mg) and 4 weeks in high NaCl brine (4.25 M) 
- Biosolifluid potential

**DISCUSSION**

The successful performance of a nuclear waste repository is measured by its ability to prevent the release of radionuclides into the surrounding environment or to limit that release to levels deemed acceptable by the appropriate regulatory agencies and public. Access to waste drum contents has allowed us to investigate the potential impact that emplaced organisms may have on repository performance.

The influence of emplaced organisms may have begun as soon as the waste was packaged decades ago. Waste components—such as cathodic, sulfates, or low-molecular-weight organics—may have served as substrates for these organisms, with all other conditions for survival and growth were met. Over time, the lack of moisture and level of radioactivity may have selected for certain organisms, such as the Actinobacteria. This has been shown in radioactive waste-contaminated soils and biofilms exposed to Chernobyl radiation fallout. Spore-forming bacilli may have remained dormant until favorable conditions were imposed. The salt tolerance tests indicate that none of the waste organisms is likely to be active in the high ionic strength brine expected in the WIPP (2 – 5 M), suggesting that any role in waste degradation will be limited to the confines of the waste container prior to contact with brine. However, given their potential to adsorb radionuclides and survive up to 6 weeks in WIPP brine, they may play a role in biosolifluid transport.

We acknowledge that a sample size of 2 is hardly representative of the >90,000 m3 of waste that have already been emplaced at the WIPP. However, it is possible that sample access will never be granted again, and this study provides the first accounting of a microbial community in actual transuranic drum waste.

**REFERENCES**


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