Culture-Dependent and Independent Analyses of Low- to Intermediate-Level Radioactive Waste: Assessment of Two Disinterred, Aged Waste Drums

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BACKGROUND

Microbial 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 biocolloid transport.

Organisms may be introduced during mining and repository operations or with the emplaced waste. Emplaced organisms have never been studied because of the difficulty in acquiring actual samples. In the case of the Waste Isolation Pilot Plant (WIPP), located in a subterranean salt formation, high salt concentrations may effectively limit the activity of these organisms.

Unprecedented access to two aged and disinterred drums of nuclear waste (~500-1100 nCi 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 *Dietzia*). Cultures yielded both *Actinobacteria* (*Arthrobacter* and *Brachybacterium*) and *Firmicutes* (*Bacillus* spp.). Illumina HiSeq data support an *Actinobacteria*-dominated community, with a possible 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 clean-up of nuclear weapons during and after World War II
- Drum burial in underground pits for ~ 50 years

Constituent	Drum 6	Drum 9
Organic sludge (kg)	120	106
Graphite (kg)	0.50	2.00
Fe-based Metal (kg)	Trace	Trace
Rubber	Trace	0
Transuranic alpha (nCi/g)	481	1085
Am-241 (mCi)	55.3	72.9
Pu-239 (mCi)	7.64	16.1
Pu-240 (mCi)	1.75	3.67

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

- MoBio's PowerSoil DNA Purification kit, ± EDTA pre-treatment for removal of radionuclides
- Clone libraries of the 16S ribosomal RNA gene
- Illumina HiSeq sequencing on a pooled sample

Culture-based analyses

- Plating onto various solid media
- Putative identification of distinct colonies by 16S rRNA gene sequences
- Salt-tolerance tests in R2B + varying salt concentrations
- Uranium tolerance tests in R2B + U(VI)-carbonate and U(VI)-citrate

RESULTS: DNA ANALYSES

DNA Extraction

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

Phylogenetic Affiliation of clones:

- **Exclusively *Actinobacteria*; majority *Dietzia***

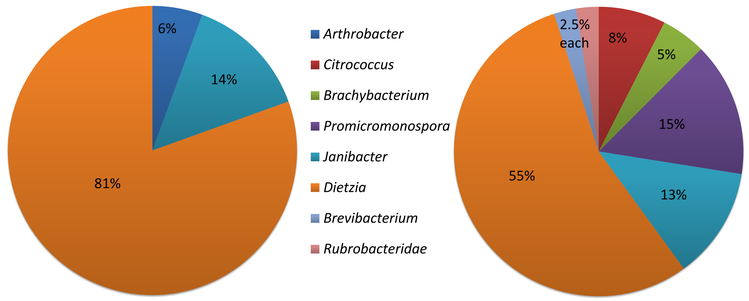

Figure 1. Phylogenetic distribution of clone libraries for DNA-positive drum, with and without EDTA pre-treatment.

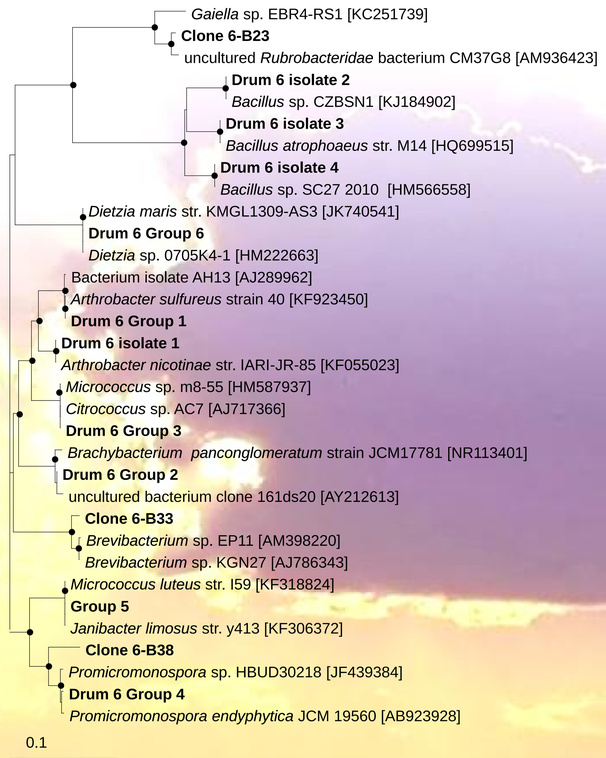

Figure 2. Phylogenetic tree of clone and isolate 16S rRNA gene sequences

RESULTS: CULTURE ANALYSES

- Five isolates obtained: three *Bacillus* spp., *Arthrobacter*, and *Brachybacterium*
- No growth at [NaCl] > 1.2 M → **low potential for activity in brine**
- Growth of *Arthrobacter* sp. in presence of [U] as high as 2 mM
- Survival of *Arthrobacter* sp. up to 5 weeks in high-Mg brine (2.8 7 M NaCl + 0.95 M Mg) and 4 weeks in high NaCl brine (4.25 M) → **biocolloid potential**

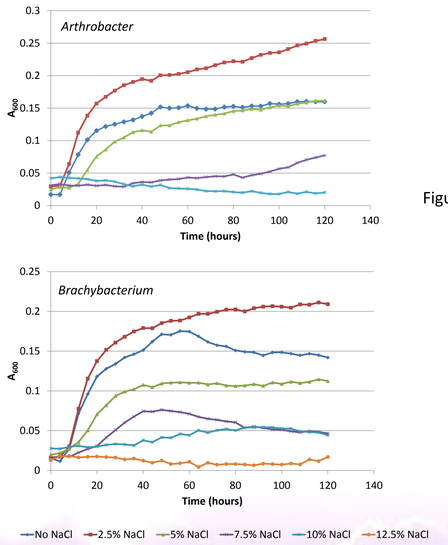

Figure 3. Salt tolerance growth assays

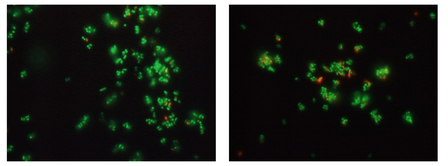

Figure 4. Live/Dead staining of *Arthrobacter* at week 4 in WIPP brines

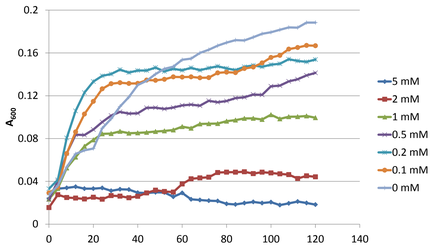

Figure 5. Growth of *Arthrobacter* sp. in presence of U(VI)-citrate

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 cellulosics, solvents, or low-molecular weight organics—may have served as substrates for these organisms, if 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[1-3]. 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 (I = 5-6 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 biocolloid transport.

We acknowledge that a sample size of 2 is hardly representative of the >90,000 m^3 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.

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[3]Ragon M, Restoux G, Moreira D, Møller AP, López-García P. Sunlight Exposed Biofilm Microbial Communities are Naturally Resistant to Chernobyl Ionizing-Radiation Levels, PLoS ONE 6.

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