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Geomicrobiology of High-Level Nuclear Waste-Contaminated Vadose Sediments at the Hanford Site, Washington State

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Sediments from a high-level nuclear waste plume were collected as part of investigations to evaluate the potential fate and migration of contaminants in the subsurface. The plume originated from a leak that occurred in 1962 from a waste tank consisting of high concentrations of alkali, nitrate, aluminate, Cr(VI), ^{137}Cs, and ^{99}Tc. Investigations were initiated to determine the distribution of viable microorganisms in the vadose sediment samples, probe the phylogeny of cultivated and uncultivated members, and evaluate the ability of the cultivated organisms to survive acute doses of ionizing radiation. The populations of viable aerobic heterotrophic bacteria were generally low, from below detection to ~10^4 CFU g^{-1}, but viable microorganisms were recovered from 11 of 16 samples, including several of the most radioactive ones (e.g., >10 μCi of ^{137}Cs/g). The isolates from the contaminated sediments and clone libraries from sediment DNA extracts were dominated by members related to known gram-positive bacteria. Gram-positive bacteria most closely related to *Arthrobacter* species were the most common isolates among all samples, but other phyla high in G+C content were also represented, including *Rhodococcus* and *Nocardia*. Two isolates from the second-most radioactive sample (>20 μCi of ^{137}Cs g^{-1}) were closely related to *Deinococcus radiodurans* and were able to survive acute doses of ionizing radiation approaching 20 kGy. Many of the gram-positive isolates were resistant to lower levels of gamma radiation. These results demonstrate that gram-positive bacteria, predominantly from phyla high in G+C content, are indigenous to Hanford vadose sediments and that some are effective at surviving the extreme physical and chemical stress associated with radioactive waste.

As a result of World War II and the subsequent Cold War, a large nuclear complex was developed in the United States, including large land tracts in Nevada, Idaho, and Washington state. Over a 40-year period, approximately 104 metric tons of plutonium was extracted from irradiated uranium at various sites within this complex. The result of the fuel chemical reprocessing at the Hanford Site, near Richland, Washington, and the Savannah River Site, near Aiken, South Carolina, was an accumulation of approximately 90 million gallons of high-level radioactive waste (HLW). Most of the waste was stored in tanks of various sizes and designs at Hanford and Savannah River, with lesser amounts at other sites across the United States.

At Hanford alone, approximately 107,000 tons of nuclear fuel was irradiated in nine reactors. Pu was extracted from the irradiated fuel by three different reprocessing schemes: reduction-oxidation process, bismuth-phosphate, and plutonium-uranium extraction process (27). Much of the waste from irradiated fuel processing was stored in 177 single-shell and double-shell underground storage tanks that now contain approximately 55 million gallons of poorly characterized but highly radioactive waste. The tanks are below ground and are covered with approximately 3 m of soil and gravel. The earliest tanks, used since 1944, had a design life of 10 to 20 years; leaks were first suspected in 1956 and were confirmed in 1959. The amount and distribution of waste leakage from the Hanford tanks is unknown, but present estimates range from 0.6 to 1.5 million gallons. This waste contains approximately 1 million Ci of radiation, primarily from ^{137}Cs, but the HLW soon after reprocessing contained high levels of short-lived radionuclides, including ^{106}Ru, ^{144}Ce, ^{147}Pm, and others (28). The wastes leaked from these tanks have been in contact with surrounding soils and vadose sediments for decades and have undergone significant geochemical and radiological transformations. Wastes also contained an estimated 870 tons of chemicals.

Microorganisms in terrestrial subsurface environments play a major role in the cycling of elements as well as weathering of rocks and sediments and can affect the geochemical properties of groundwater (25) by modifying the fate and transport of organic and inorganic contaminants. While the vadose region of the subsurface generally does not support robust microbial populations, particularly in arid regions, there have been numerous reports of viable microorganisms associated with unsaturated zone soils and sediments (15, 21, 31, 33), including at the Hanford Site (9, 24, 30). Water potentials in the vadose zone generally do not directly restrict microbial activity, because many microorganisms are relatively tolerant to the matric water potentials typical of vadose sediments (30). Rather, it is relatively thin, discontinuous water films that retard the diffusion of solutes, including nutrients and metabolic waste products that restrict microbial metabolism (41).

During the summer of 2000, a slant borehole was drilled beneath tank SX-108 at Hanford's S-SX tank farm that intercepted a vadose zone contaminant plume of high-level nuclear waste. The purpose of this sampling effort was to assess the distribution of contaminants and to obtain scientific informa-

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tion regarding processes that may influence the fate and transport of the contaminants. The plume was characterized by high concentrations of radionuclide and chemical contaminants, elevated temperature, and low moisture content. Some samples exhibited the highest levels of radioactivity (>50 μCi g^{-1}) of any soils or sediments yet collected at Hanford. As part of this effort, core samples were analyzed for viable microbial populations, and DNA from the isolates and sediments was subjected to phylogenetic analysis to identify the microorganisms. The main objectives of this research were to analyze the microbiological properties of SX-108 sediment samples in relation to sediment properties and contaminant distributions and to assess potential biogeochemical effects on contaminant fate and transport.

MATERIALS AND METHODS

Sampling location and procedures. During late July and early August of 2000, core samples were collected from the vadose zone beneath the SX-108 tank located within waste management area S-SX on the U.S. Department of Energy's (DOE's) Hanford Site. Tank SX-108 first received waste from Hanford Site nuclear fuel reprocessing operations in 1955, and the first leaks were believed to have occurred around 1962. The leaked wastes contained high solute concentrations as a result of self boiling and evaporation in the tank induced by the decay of short-lived radioisotopes. The geology at this location has been described elsewhere (45).

Percussion (cable tool) drilling was used to advance the borehole, and core samples were collected by using split-spoon techniques (42). The borehole was drilled at a 30° angle to intercept the subsurface at locations directly below leaked tank SX-108 (Fig. 1). Subsurface vadose samples were collected by procedures that do not use circulating drilling fluids that can promote core contamination (26). Due to regulatory requirements to accurately define contaminant distributions without artifacts, considerable care was taken to prevent cross-contamination of core samples.

In an effort to assess the effect of HLW contamination on the native vadose microbial population, two core samples from an adjacent uncontaminated borehole (299-W22-48) were obtained. These samples, designated RG1 and RG4, were collected from the same stratigraphic position as the SX-108 slant borehole cores. RG1 was from 25 m and RG4 from 27 m beneath the surface.

Sediment treatments. Sediment was aseptically removed from the inner portion of core liners and was placed in sterile Whirlpak bags. Viable aerobic heterotrophic bacteria in untreated sediment were enumerated by dilution plate count methods (see below). Sediment was also used to directly inoculate liquid enrichment cultures. In addition, uncontaminated sediment (50 g) was irradiated at doses of 5 and 10 kGy with a ^{60}Co source (MDS Nordion Inc., Kanata, Ontario, Canada) immediately prior to analysis by dilution plate count on peptone-tryptone-yeast extract-glucose (PTYG) agar medium (22). Sediment (50 g) was also placed inside an airtight vessel with desiccant (Drierite) to determine the effects of desiccation on the population of viable organisms. Moisture content (wt/wt) for both sediment samples decreased from 4.7% (RG1) and 9.0% (RG4) to 0.2% after 28 days, at which point the populations of viable aerobic heterotrophic bacteria were enumerated.

Culturing. Untreated and treated vadose sediments were subjected to a variety of microbiological cultivation methods to determine the size and diversity of viable microbial populations. Based on the results of previous research involving vadose samples from the Hanford Site (5, 9, 24, 30), we focused our cultivation efforts on aerobic chemoheterotrophic bacteria but included enrichments for select physiological groups of anaerobic bacteria because of their potential for influencing contaminant chemical behavior. To this end, several types of agar and broth media were inoculated with each of 16 sediment samples obtained from the SX-108 borehole. Targeted microbial functional groups included aerobic heterotrophic bacteria, ammonia- and nitrite-oxidizing autotrophic bacteria, denitrifying bacteria, fermentative bacteria, Fe(III)-reducing bacteria, and sulfate-reducing bacteria. Details of these cultivation methods have been reported elsewhere (22, 38). Briefly, both dilution plate count and broth enrichment approaches were used. Broth media were inoculated directly with ~1 g of sediment each. For dilution plates, sediment was suspended in the sterile pyrophosphate buffer, mixed vigorously, diluted, and spread on agar plates (22). Agar plates and enrichment broth were incubated at room temperature in the dark unless otherwise noted.

Agar plates were examined over a period of several months, but the number of bacterial colonies was determined at 14 days. Distinct colony types based on color, size, and morphology were noted, picked, and streaked onto fresh medium for isolation. For some core samples, bacterial colonies failed to develop on agar plates but growth was evident in broth enrichments. In these situations, a small volume of enrichment broth was transferred to fresh medium, including agar plates, in an attempt to isolate additional microorganisms. The cultures were preserved by freezing in 40% glycerol at −80°C. Culture stocks are maintained at Pacific Northwest National Laboratory and were also deposited with the DOE Subsurface Microbial Culture Collection at Florida State University (3).

Isolate 16S rRNA gene (rDNA) restriction fragment length polymorphism and phylogenetic analyses. Bacterial cultures (isolates) were subjected to phylogenetic analysis by sequencing the 16S rRNA gene. The phylogenetic positions were analyzed by using distance matrix, maximum likelihood, and parsimony methods. Distance matrix analysis was performed with the PHYLIP group of computer programs (19). Distances were calculated with the method of Jukes and Cantor (29), and phylogenies were estimated with the FITCH option, which uses the Fitch-Margoliash criterion (20), and some related least-squares criteria. Maximum likelihood analysis was performed with the fastDNAml program (40). Parsimony analysis was carried out with the PAUP software package (PAUP* 4.0, beta version 4c) (47). A heuristic search was done first (using the standard program defaults), after which a bootstrap analysis (19) was used to assess the branch points of the resulting phylogenetic trees. A consensus tree was generated by bootstrapping at the greater-than-50% confidence limit, with 1,000 replications.

Community 16S rDNA analysis. DNA was purified from sediment samples 3a, 5a, 6a, 8a, 12a, and 17a (Table 1). Ten 0.5-g aliquots of each sediment sample were processed using the FastDNA Spin kit for soil (Qbiogene), and the 10 50-μl eluants were pooled. PCR mixtures (50 μl) contained 1 μl of template, 1× PCR buffer, 1.5 mM $MgCl_2$, 250 μM each deoxynucleoside triphosphate, 500 nM each primer, and 0.25 μl (1.25 U) of HotStar Taq (QIAGEN). Template (1 μl) was added to separate reaction mixtures at full strength and at 1:5, 1:15, 1:50, and 1:150 dilutions. rDNAs were amplified with universal primers 8f (5′-AGAGTTTGATCCTGGCTCAG-3′; 34) and 1390r (5′-ACGGGCGGTGTGTRCAA-3′; 50) and *Archaea* primers 21f (5′-TTCCGGTTGATCCYGCCGGA-3′) and 958r (5′-YCCGGCGTTGAMTCCAATT-3′) (18). Reaction mixtures were incubated in a Quadra thermal cycler (MJ Research) at 95°C for 10 min, followed by 35 cycles at 94°C for 1 min, 53°C for 45 s, and 72°C for 2 min and then a final extension of 10 min at 72°C. In some cases, 1 μl of amplified product was used as template in a seminested PCR with 518f (5′-CCAGCAGCCGCGGTAAT-3′) and 1390r primers. PCR products were verified by agarose gel electrophoresis, purified by using the QIAquick kit (QIAGEN), and ligated into pCR4-TOPO (Invitrogen). Ligations were shipped to the DOE Production Genomics Facility, where transformants were prepared and inserts were sequenced using standard protocols (http://www.jgi.doe.gov/Internal/prots_index.html). Sequence reads were analyzed against the Ribosomal Database Project database by blastN.

Ionizing radiation resistance. Select isolates were analyzed for resistance to ionizing radiation from a ^{60}Co source (MDS Nordion Inc.).

Cultures (50 ml) were grown in a medium of isolation, typically PTYG medium (22), to about mid-log to early stationary phase, and 10 ml was dispensed into triplicate 15-ml conical polypropylene tubes. Duplicate cultures were exposed to 2.5, 5, or 20 kGy while a single unexposed culture was used as a control. Cultures were kept on ice during irradiation to minimize growth. After exposure, 1-ml aliquots were removed from the tubes, diluted in sterile phosphate-buffered saline, and plated on agar medium. Agar plates were incubated at 30°C and examined for growth daily for up to 7 days. Percent survival was calculated as the population of cells surviving a given exposure relative to the unexposed control.

RESULTS

Vadose sediment physical and chemical properties. The chemical and physical properties of the cored sediments (Table 1) reflect the complex effects of waste leakage from Hanford tank SX-108, subsequent migration of the tank liquor through the vadose zone, and geochemical reaction with vadose sediments. The slant borehole successfully traversed and allowed sampling of sediments beneath tank 108 that were contaminated with ^{137}Cs and other chemical and radiological contaminants. Leaked wastes were very hot due to radioactive decay of short-lived isotopes during waste storage in the 1950s and

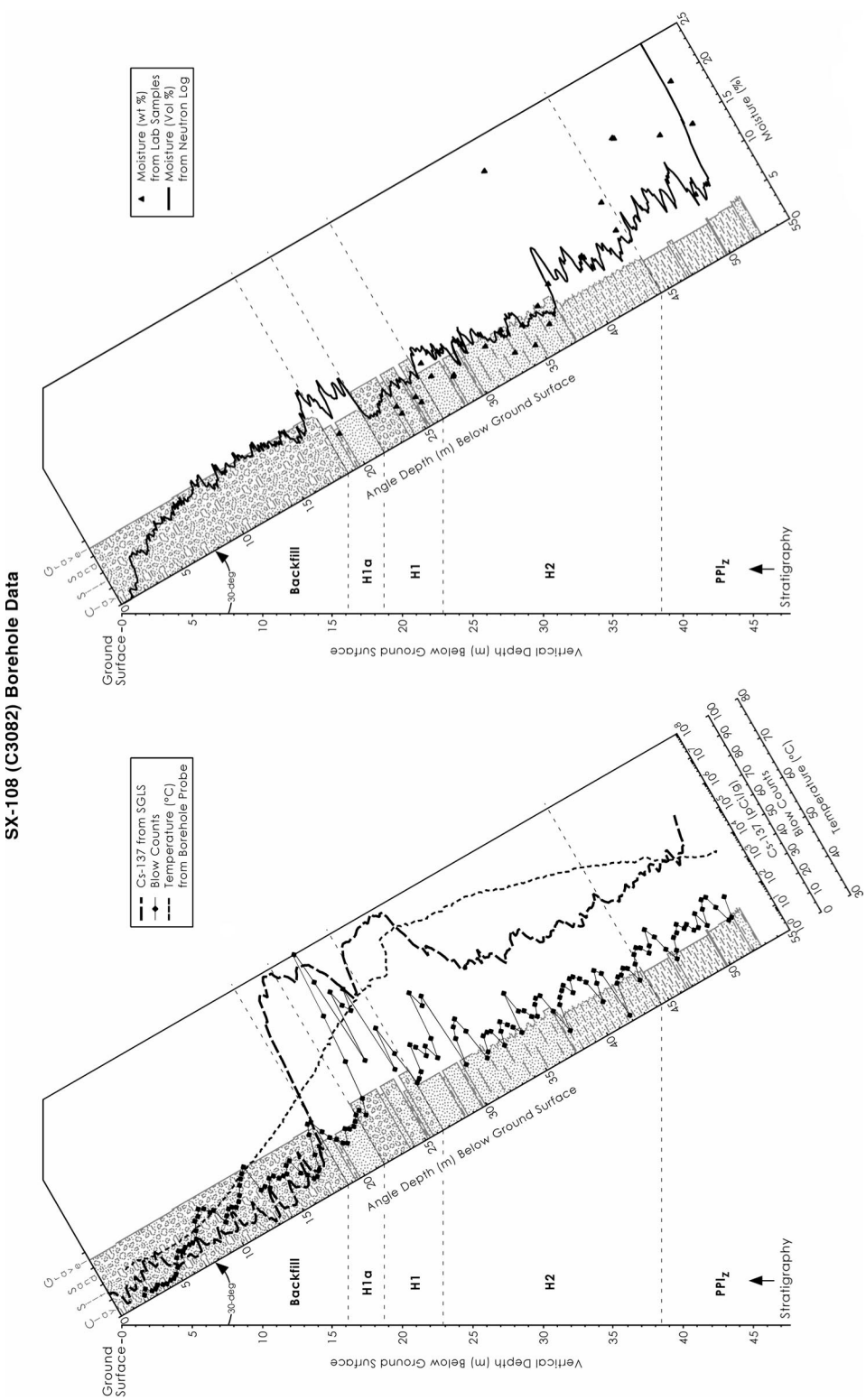

FIG. 1. Field results from the SX-108 core, including stratigraphy, counts of ^{137}Cs from down-hole spectral gamma logging, in situ temperature, and moisture content from down-hole neutron logging and laboratory measurements. High counts of ^{137}Cs were observed immediately after samples were passed through the backfill surrounding the tank (see Fig. 2). Blow counts refers to the number of hammer strikes required to advance the borehole a given distance by cable tool drilling. SGLS, spectral gamma logging system. (Reprinted from reference 46 with permission of the publisher.)

TABLE 1. Chemical and physical characteristics of vadose samples from beneath Hanford waste tank SX-108[c]

Sample	Vertical depth (m)	Water content (%)	pH	Conductivity (mS cm^{-1})	Detected amt of:			
					^{137}Cs (nCi g^{-1})	Cr[a] (μg g^{-1})	NO_3^-[a] (mg $liter^{-1}$)	NO_2^-[a] (mg $liter^{-1}$)
1a	16.6	4.3	9.2	0.40	3.06×10^3	0.02	7.0	BD[b]
3a	20.5	2.8	9.6	0.70	1.95×10^4	0.98	29.1	0.4
4a	21.8	2.8	9.5	0.58	1.38×10^3	0.86	23.5	0.3
5a	23.1	4.7	9.8	0.88	6.52×10^3	3.64	92.8	0.3
6a	24.4	3.7	8.0	16.71	5.31×10^4	483.83	11,740	BD
7a	25.6	6.2	9.6	54.62	2.14×10^4	309.73	46,640	BD
8a	26.9	6.0	7.9	49.01	5.55×10^2	829.76	39,710	87.5
9a	28.2	2.4	7.9	31.76	0.17	512.62	22,850	57.1
10a	29.5	1.9	8.2	25.56	0.45	398.13	18,990	59.0
11a	30.8	3.2	8.4	13.93	0.91	0.90	9,520	<10
12a	32.0	21.4	8.0	2.36	0.34	0.29	1,530	<1
13a	34.5	7.6	8.0	29.78	0.52	430.95	22,200	72.5
14a	37.0	12.0	7.8	30.24	0.84	297.83	21,500	46.3
15a	39.5	17.4	7.5	40.01	0.59	336.50	34,600	34.4
16a	41.9	7.5	7.2	5.80	0.01	0.11	4,190	<10
17a	43.9	19.7	7.2	3.74	0.18	0.09	2,390	<1

[a] Concentration in 1:1 water extract.
[b] BD, below detection (0.1 mg $liter^{-1}$).
[c] Reprinted from reference 46 with permission of the publisher.

1960s and high concentrations of ^{137}Cs associated with the HLW. Heating of the vadose sediments altered water seepage patterns in the subsurface and resulted in large-scale moisture redistributions. Thermal modeling of the SX tank farm and the SX-108 subsurface (43, 48) indicated that the temperature may have exceeded 100°C as deep as 24 m beneath the tanks at the time of the SX-108 leak (ca. 1962). At the time the samples were collected (2000), the temperatures had cooled from the estimated maximum (100°C) and ranged from near ambient (~37°C) to 75°C (Fig. 1). The maximum subsurface temperature occurred near the lower depth of ^{137}Cs penetration (e.g., ~19 m). The effects of the thermal load were evident in the moisture contents of the various sediment samples as sediments were desiccated to depths of >20 m beneath the tanks (Table 1 and Fig. 1).

The pH of the sediments varied from 7.2 near the base of the borehole to >9 for several of the sediment samples collected from the upper region of the profile (Table 1). The moderately alkaline pH indicated that significant waste-sediment reaction had occurred that neutralized the high pH (>14) of the original waste from the reduction-oxidation process. The samples that were higher in the profile also contained the greatest concentrations of ^{137}Cs, with sample 6a exceeding 50 μCi g^{-1} (Table 1 and Fig. 1 and 2). These high ^{137}Cs concentrations resulted from the sorptive concentration of Cs^+ by the abundant micaceous fraction of the sediment. These samples rep-

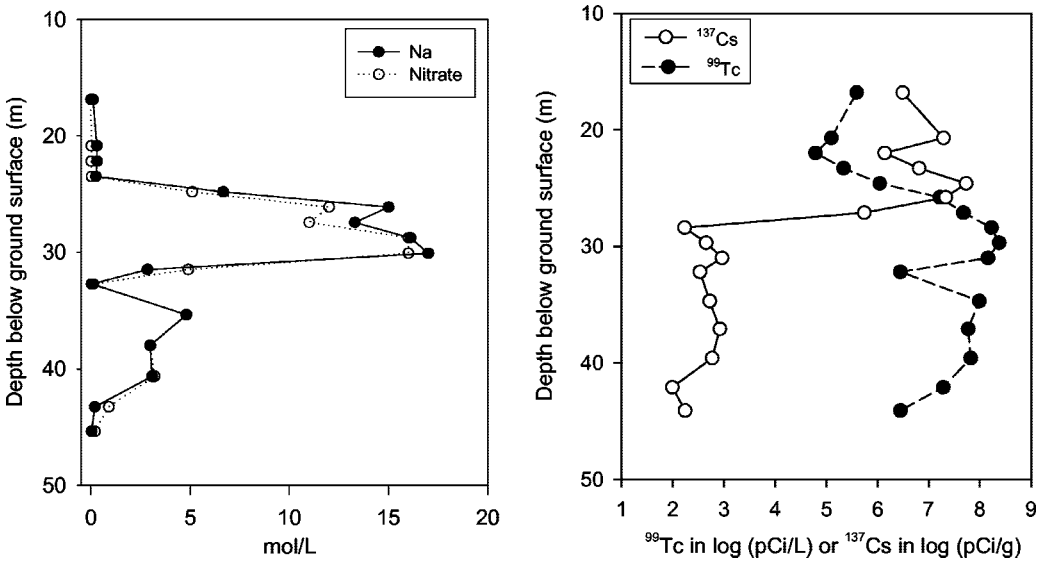

FIG. 2. Porewater concentrations of Na, NO_3, and ^{99}Tc [$Tc(VII)O_4^-$] in the borehole samples determined by water extraction (46) and laboratory water content measurements. Also shown for reference is the sorbed concentration of ^{137}Cs determined by high-resolution gamma energy analysis. (Reprinted from reference 46 with permission of the publisher.)

TABLE 2. Viable aerobic heterotrophic bacteria in vadose samples from beneath Hanford waste tank SX-108

Sample	Vertical depth (m)	Viable plate counts (log CFU g^{-1})[a]			Growth in broth enrichments (PTYG/R2A) at[c]:			
		PTYG	R2A	Actino	pH 7, 21°C	pH 10, 21°C	pH 7, 50°C	pH 10, 50°C
1a	16.6	4.0	4.0	4.0	++/++	++/++	±/++	−/++
3a	20.5	BD[b]	BD	BD	±/±	±/±	−/−	−/−
4a	21.8	3.7	BD	2.9	++/++	++/++	±/±	±/±
5a	23.1	BD	BD	BD	±/±	±/±	±/±	++/±
6a	24.4	BD	BD	BD	±/±	−/±	−/−	−/−
7a	25.6	3.2	3.1	3.2	±/−	±/±	−/−	±/−
8a	26.9	BD	BD	BD	±/±	±/±	±/−	±/−
9a	28.2	2.6	BD	BD	++/−	−/−	−/++	++/±
10a	29.5	BD	1.8	BD	−/−	−/−	±/−	±/±
11a	30.8	BD	BD	BD	−/−	−/−	±/−	±/−
12a	32.0	2.7	2.7	2.7	++/++	++/++	±/++	±/++
13a	34.5	BD	BD	BD	−/−	++/±	±/−	++/−
14a	37.0	BD	BD	BD	±/−	±/−	±/−	±/−
15a	39.5	BD	1.8	BD	−/−	++/−	±/−	±/−
16a	41.9	3.3	1.5	BD	++/−	−/−	−/−	±/−
17a	43.9	>4.3	>4.3	>4.3	++/++	++/++	±/−	±/−

[a] Actino, growth on actinomycete isolation agar (DIFCO).
[b] BD, below detection or <1.8 log CFU/g.
[c] ++, growth in original enrichment and transfer; ±, growth in original enrichment but not transfer; −, no growth. The backslashes separate results from PTYG and R2A enrichments.

resent some of the most highly radioactive sediment samples yet collected at the Hanford Site. The highest concentrations of water-extractable Cr and nitrate are coincident and generally occur deeper in the profile than Cs, except in the cases of samples 6a to 8a. These differences result from the relative mobility of Cs^+ and the negatively charged chromate and nitrate ions (for examples see references 36 and 49). The nitrate concentration in many of the samples was strikingly high, exceeding 10 g $liter^{-1}$ in 1:1 water extracts in 50% of the samples. Computed pore water concentrations of NO_3^- based on the measured water contents of the sediments ranged between 5 and 15 mol $liter^{-1}$ in the core of the plume (e.g., 24.4 to 29.5 m and 34.5 to 39.5 m; Fig. 2). Nitrite concentrations were substantially lower than those of nitrate but nonetheless exceeded 30 mg $liter^{-1}$ in 1:1 water extracts in 6 out of 16 samples.

Technetium-99, the other major radiologic contaminant in the SX-108 vadose zone plume, existed deeper in the profile than ^{137}Cs (Fig. 2). ^{99}Tc is a long-lived mobile radionuclide ($t_{1/2} = 2.13 \times 10^5$ years) that decays by beta emission in the form of the pertechnetate anion [$Tc(VII)O_4^-$]. The distribution of ^{99}Tc was nearly identical to that of NO_3^- and defined the extent of the HLW vadose zone plume. The sorption status of ^{137}Cs and ^{99}Tc was distinct. ^{137}Cs was strongly adsorbed as a high-affinity exchange complex on micaceous minerals that resist desorption except in saline electrolytes (35). In contrast, ^{99}Tc was not adsorbed and existed as a solute in pore waters and as salt in air-filled pores.

Viable microbial populations. In general, the populations of aerobic heterotrophic bacteria as determined by dilution plate counts were low, ranging from below detection to $>10^4$ CFU g^{-1} in the deepest sediment collected (17a) (Table 2). Of the three different agar media used in this study, PTYG yielded the highest populations of aerobic heterotrophic bacteria while R2A yielded fewer or no colonies for three samples; however, it provided for growth of a few colonies on two samples (10a and 15a) where PTYG agar did not.

Based on previous investigations, we anticipated relatively low population densities of aerobic heterotrophic bacteria in the contaminated vadose sediments. Therefore, liquid enrichments were included in the microbiological analyses. For a number of sediment samples, including highly radioactive sediments 3a, 5a, 6a, and 8a, positive broth enrichments were obtained where populations were below detection by dilution plate count techniques. Although most transfer attempts from the enrichments into fresh broth medium were unsuccessful, a number of isolates from the original enrichments were obtained by streak plate purification on agar medium, including several from the highly radioactive sediments. Many of the sediments that yielded successful enrichments at pH 7 also exhibited growth in the same medium where the pH was initially adjusted to 10. It is not possible from these analyses to establish whether the organisms that grew in the pH 10 enrichments were similar or distinct from those that grew at pH 7. Regardless, these results indicate the presence of organisms in the contaminated vadose sediments that were able to grow at alkaline pH values.

Because we anticipated elevated temperatures of the sediments beneath SX-108, replicate PTYG and R2A broth enrichments were also incubated at 50°C. Similar to the pH 10 enrichments, growth was common in many of the original enrichments but only a few of the cultures were successfully transferred (Table 2). Interestingly, the cultures that successfully transferred originated from some of the same samples for which the 21°C enrichment cultures also were successfully transferred; these included samples 1a, 9a, and 12a. A temperature of 50°C was selected for incubation of enrichment cultures, because it was estimated (e.g., Fig. 1) that this would approximate the in situ temperature for most of the sampled depths, although for some of the samples the temperatures were found to be higher (Fig. 1).

Because NO_3^- was a common tank waste constituent and the concentrations were remarkably high in a majority of the sediments examined, we initiated enrichments for denitrifying bacteria. Cores 12a and 17a were the only samples where the

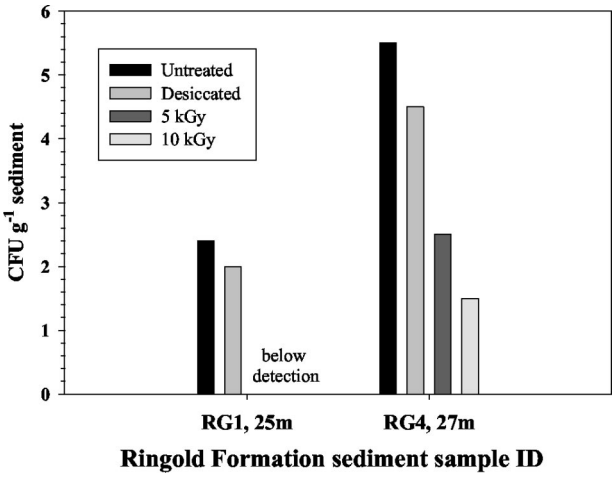

FIG. 3. Influence of acute doses of ionizing radiation (^{60}Co) on populations of viable aerobic heterotrophic bacteria in uncontaminated Hanford vadose zone sediments as determined by dilution plate counts on PTYG agar medium.

presence of viable denitrifying bacteria was confirmed. No sulfate-reducing or fermentative bacteria were cultured from any of the samples that were analyzed.

Uncontaminated vadose sediment microbial populations. Two vadose samples were obtained from uncontaminated sediments from a borehole adjacent to the SX-108 slant borehole for comparison. These samples, designated RG1 and RG4, were from the same depths as the SX-108 samples that had the highest concentrations of ^{137}Cs and, therefore, were stratigraphically similar. The population of viable aerobic heterotrophic bacteria in sample RG1 (Fig. 3) was low (2.4 log CFU g^{-1}) but was comparable to the population size associated with sample 7a (Table 2) from the SX-108 borehole obtained from approximately the same depth. In contrast, the population from untreated RG4 sediment was relatively high at 5.5 log CFU g^{-1}. This result is in considerable contrast to that for the sediment from SX-108 collected at approximately the same depth (8a), which exhibited no growth on PTYG agar even at the lowest dilution.

In order to assess the potential effects of drying and ionizing radiation on the population of viable vadose zone bacteria, uncontaminated sediments were subjected to desiccation or exposure to gamma radiation. The results from these experiments revealed that desiccation decreased the population sizes of aerobic heterotrophic bacteria in RG1 and RG4 by 2.5- and 10-fold, respectively (Fig. 3). Exposure to ionizing radiation had a much greater effect on the population size of viable aerobic bacteria, eliminating growth from the RG1 sample at both doses and decreasing the population size in RG4 by 3 and 4 orders of magnitude for acute exposures of 5 and 10 kGy, respectively.

Phylogeny and radiation resistance of isolates. More than 110 cultures of aerobic heterotrophic bacteria were isolated and purified from the various enrichments and dilution plates (Table 3). To obtain insights into the genetic diversity and phylogeny of the isolates, the cultures were subjected to 16S rDNA gene sequencing.

The genera represented among the isolates from the SX-108 samples included gram-positive bacteria high in G+C content that are typical inhabitants of soil and vadose sediments. Isolates whose closest match was a member of the genus *Arthrobacter* were the most common for cultures from both SX-108 and 299-W22-48 boreholes (Table 3). Other gram-positive genera commonly represented among the isolates included *Staphylococcus* and *Nocardia* in addition to relatives to several unclassified bacteria high in G+C content. Gram-negative genera were less common, but representatives included *Pseudomonas* and *Sphingomonas* as well as close relatives to a number of unclassified α-, β-, and γ-Proteobacteria. Interestingly, several isolates from sample 7a, one of the most radioactive samples collected, were closely related to *Deinococcus radiodurans*, a bacterium that can withstand acute doses of ionizing radiation to 15 kGy without lethality (17).

There are several interesting observations regarding the phylogenetic distributions of the isolates in Table 3. Only gram-positive and/or organisms high in G+C content were cultured from the most highly radioactive sediments, 1a to 7a (Table 1). In contrast, below sample 7a organisms related to gram-negative bacteria were relatively common, representing ~45% of the isolates. Many of the same genera in the SX-108 vadose sediments were also present in the uncontaminated vadose sediments from borehole 299-W22-48.

Nineteen of the 20 radiation-resistant isolates were gram-positive bacteria high in G+C content, 13 of which were phylogenetically related to members of *Arthrobacter* and its close relative *Micrococcus*. Only one (10c-1) of the 13 isolates related to gram-negative bacteria exhibited any resistance to 2.5 kGy of gamma radiation. Three of the four isolates with some resistance to 5 kGy were most closely related to an uncultured *Micrococcus luteus*-like bacterium identified in a clone library obtained from a sludge sample from a recirculating two-stage bioreactor (14). The two isolates (7b-1 and 7c-1) that exhibited the highest levels of radiation resistance, with >0.2% of the population of 7b-1 cells surviving exposure to 20 kGy, were most closely related to *D. radiodurans*, one of the most radiation-resistant organisms known. The source of these strains was sample 7a, which had the second highest concentration of ^{137}Cs at 21.4 μCi g^{-1}.

Community 16S rDNA analysis. The direct extraction of nucleic acids from vadose sediments followed by PCR amplification, cloning, and sequencing allowed for a cultivation-independent analysis of microbial phylogeny to complement the characterization of sediment isolates. With the bacterial primers, the 1:5-diluted DNA template produced the strongest bands on agarose gels for samples 12a and 17a, with very weak bands present with template at full strength and no bands present at 1:50 and 1:150 dilutions. For samples 3a, 5a, 6a, and 8a, no PCR products were observed on gels regardless of template level. Use of a seminested PCR produced visible products in these samples, with the exception of 8a. The archaeal primers failed to produce a PCR product in any of the sample extracts, regardless of template concentration. Competitive PCR containing 1:5 dilutions of indigenous template spiked with various amounts of *Escherichia coli* genomic DNA showed (with the exception of sample 8) between 300 and 900 copies of indigenous 16S target in the reaction, equivalent to 150,000 to 450,000 copies on a per-gram-of-sediment basis (data not shown). The extent to which PCR was able to sample

TABLE 3. Phylogenetic and gamma radiation resistance characteristics of isolates from contaminated (SX-108 slant borehole) and uncontaminated (299-W22-48) Hanford vadose sediments

Core identity and sediment	Isolate identity	Nearest GenBank relative	SimRank	Accession no.	Dose[a] (kGy)	% Irradiation survival
SX-108						
1a	1b-1	*Arthrobacter globiformis*	0.982	AY561524	2.5	0.24
	1c-1	*Arthrobacter* sp. strain CF-46	0.970	AY561525	2.5	2.4×10^{-4}
	1c-2	*A. globiformis*	0.967	AY561526	2.5	9.4×10^{-3}
4a	4a-1	*Rhodococcus fascians*	0.988	AY561527		
	4a-2	*Clavibacter michiganense*	0.816	AY561528		
	4a-3	*Microbacterium oxydans*	0.946	AY561529	2.5	0
	4a-4	*Nocardia corynebacteroides*	0.991	AY561530	2.5	0
	4b-1	*N. corynebacteroides*	0.983	AY561531	2.5	0
	4b-2	*Staphylococcus warneri*	0.988	AY561532		
	4b-3	*Nocardioides plantarum*	0.907	AY561533		
	4c-1	*N. plantarum*	0.913	AY561534		
5a	5L-1	*Arthrobacter agilis*	0.967	AY561535	2.5	2.5×10^{-4}
	5L-2	*Agrococcus jenensis*	0.939	AY561536	2.5	1.1×10^{-3}
	5L-3	*Bacillus licheniformis*	0.977	AY561537	2.5	0
7a	7b-1	*D. radiodurans*	0.980	AY561538	20	0.21
	7c-1	*D. radiodurans*	0.978	AY561539	20	2.4×10^{-2}
	7L-1	*M. luteus*	0.942	AY561540	2.5	0.86
8a	8c-1	Unnamed β-Proteobacterium	0.871	AY561542	2.5	0
	8b-1	*Sphingomonas asaccharolytica*	0.968	AY561541		
9a	9c-3	Unnamed α-Proteobacterium	0.966	AY561544	2.5	0
	9c-2	*Arthrobacter* sp. strain CF-46	0.991	AY561543		
	9c-4	*Dermabacter hominis*	0.966	AY561545		
	9c-5	*Verrucosispora gifhornensis*	0.904	AY561546		
10a	10c-1	γ-Proteobacterial clone G21	0.929	AY561547	2.5	1.7×10^{-3}
	10c-2	Unnamed α-Proteobacterium	0.877	AY561548	2.5	0
12a	12a-1	*P. stutzeri*	0.997	AY561549		
	12b-1	*P. stutzeri*	0.994	AY561550	2.5	0
15a	15a-1	*Terrabacter tumescens*	0.948	AY561551		
	15c-1	*Azospirillum lipoferum*	0.855	AY561552	2.5	0
16a	16b-1	*Staphylococcus pasteuri*	0.986	AY561553	2.5	4.1×10^{-2}
	16b-2	*S. warneri*	0.981	AY561554		
	16b-4	*A. globiformis*	0.969	AY561555		
	16b-5A	*S. warneri*	0.999	AY561556		
	16b-5B	*S. warneri*	0.989	AY561557		
	16c-1a	*A. globiformis*	0.973	AY561558		
17a	17a-1	*Arthrobacter nicotinovorans*	0.960	AY561559	2.5	5.6×10^{-3}
	17a-2	*Arthrobacter* sp. strain CF-46	0.959	AY561560	2.5	3.5×10^{-2}
	17a-3	*P. stutzeri*	0.994	AY561561	2.5	0
	17a-4	*P. stutzeri*	0.990	AY561562		
	17a-5	*Streptomyces sampsonii*	0.932	AY561563		
	17b-1	*Arthrobacter* sp. strain CF-46	0.909	AY561564	2.5	6.7×10^{-4}
	17b-2	*Pseudomonas* sp. strain BRW1	0.998	AY561565		
	17c-1	*Arthrobacter* sp. strain CF-46	0.939	AY561566	2.5	0
	17c-2	*Pseudomonas* sp. strain BRW1	0.998	AY561567		
299-W22-48						
RG1	RG-1	*Arthrobacter* sp. strain CF-46	0.970	AY561568		
	RG-2	*Janibacter limosus*	0.858	AY561569		
	RG-3	*Variovorax* sp. strain WFF52	0.927	AY561570		
	RG-4	*Variovorax* sp. strain WFF52	0.931	AY561571		
	RG-5	*Arthrobacter* sp. strain S2215	0.947	AY561572		
	RG-6	*Mycobacterium hodleri*	0.909	AY561573		
	RG-7	*M. hodleri*	0.908	AY561574	5	8.8×10^{-4}
	RG-9	*Terrabacter* sp. strain DPO 1361	0.840	AY561575		
	RG-59	*A. globiformis*	0.928	AY561618		
	RG-60	*Alcaligenes* sp. strain 05–51	0.803	AY561619		
	RG-61	*A. globiformis*	0.936	AY561620		
RG4	RG-10	*Streptomyces* sp. strain 254	0.936	AY561576		
	RG-11	γ-Proteobacterial clone JAP412	0.973	AY561577		
	RG-12	*Arthrobacter* sp. strain AC-48	0.905	AY561578		
	RG-13	*Arthrobacter* sp. strain S22215	0.951	AY561579		
	RG-14	*R. fascians*	0.965	AY561580		
	RG-15	*A. agilis*	0.952	AY561581		
	RG-16	Unnamed β-Proteobacterium	0.865	AY561582		
	RG-17	*Bradyrhizobium* sp. strain BDV 5840	0.874	AY561583		
	RG-18	Unnamed β-Proteobacterium	0.865	AY561584		
	RG-19	Unnamed β-Proteobacterium	0.867	AY561585		
	RG-20	*Detolaasinbacter tsukamotoae*	0.798	AY561586	2.5	0
	RG-21	γ-Proteobacterial clone JAP412	0.964	AY561587		

Continued on facing page

TABLE 3—*Continued*

Core identity and sediment	Isolate identity	Nearest GenBank relative	SimRank	Accession no.	Dose[a] (kGy)	% Irradiation survival
	RG-22	*Arthrobacter* sp. strain S22215	0.958	AY561588		
	RG-23	*Arthrobacter oxydans*	0.948	AY561589		
	RG-24	*Arthrobacter* sp. strain S22215	0.958	AY561590		
	RG-67	*Stenotrophomonas maltophilia*	0.964	AY561626	2.5	0
	RG-68	*S. maltophilia*	0.965	AY561627	2.5	0
RG1-10kGy[b]	RG-25	*Staphylococcus epidermidis*	0.985	AY561591	2.5	2.6×10^{-2}
	RG-26	*Brevibacillus agri*	0.965	AY561592	2.5	0
	RG-64	*M. luteus*	0.960	AY561623	5	0.44
	RG-65	*M. luteus*	0.896	AY561624	5	0.51
	RG-66	*Arthrobacter ramosus*	0.955	AY561625		
RG1-des	RG-62	*A. ramosus*	0.918	AY561621		
	RG-63	*Microbacterium oxydans*	0.939	AY561622		
	RG-69	*Pseudomonas migulae*	0.958	AY561628	2.5	0
	RG-70	*P. migulae*	0.957	AY561629	2.5	0
RG4-10kGy[b]	RG-29	*M. luteus*	0.955	AY561593	5	0.46
	RG-30	*Brevibacillus agri*	0.955	AY561594	2.5	0
	RG-71	*M. oxydans*	0.940	AY561630		
	RG-72	*Arthrobacter* sp. strain CF-46	0.919	AY561631		
	RG-72A	*Arthrobacter* sp. strain S21004	0.932	AY561632		
	RG-73	*Arthrobacter* sp. strain CF-46	0.922	AY561633		
RG4-des	RG-33	*A. oxydans*	0.958	AY561595		
	RG-34	*Streptomyces griseus*	0.936	AY561596		
	RG-35	*Arthrobacter* sp. strain CF-46	0.933	AY561597		
	RG-36	*Streptomyces setonii*	0.888	AY561598		
	RG-37	*Pseudomonas migulae*	0.943	AY561599	2.5	0
	RG-38	*Arthrobacter* sp. strain CF-46	0.941	AY561600		
	RG-39	*Arthrobacter globiformis*	0.987	AY561601		
	RG-40	*C. michiganense*	0.778	AY561602		
	RG-43	*Arthrobacter* sp. strain 19B	0.953	AY561603	2.5	1.9×10^{-4}
	RG-45	*Arthrobacter* sp. strain S21004	0.947	AY561604	2.5	3.1×10^{-2}
	RG-46	Unnamed β-Proteobacterium	0.863	AY561605		
	RG-47	*Streptomyces* sp. strain 254	0.944	AY561606		
	RG-48	*Rhodococcus* sp.	0.922	AY561607		
	RG-49	*A. agilis*	0.975	AY561608		
	RG-50	*Rhodococcus* sp.	0.939	AY561609	2.5	0
	RG-51	*Streptomyces* sp. strain 254	0.920	AY561610		
	RG-52	Unnamed β-Proteobacterium	0.912	AY561611		
	RG-53	*Arthrobacter* sp. strain 19B	0.948	AY561612		
	RG-54	*A. agilis*	0.921	AY561613		
	RG-55	*Arthrobacter* sp. strain CF-46	0.985	AY561614		
	RG-56	*Arthrobacter* sp. strain 19B	0.968	AY561615		
	RG-57	*Pseudomonas migulae*	0.973	AY561616	2.5	0
	RG-58	*Arthrobacter* sp. strain 19B	0.946	AY561617		

[a] Dose to cultures provided via ^{60}Co irradiator.
[b] Before plating, sediment was subjected to 10 kGy of gamma radiation from a ^{60}Co source.

these low-biomass communities was poor because the detection level, determined to be 80,000 copies by spiking the 1:5 dilutions of indigenous template with known amounts of non-indigenous 16S target into PCRs, was only two- to sixfold lower than the indigenous template concentrations (data not shown). Nevertheless, blastN analysis of sequences revealed between 2 and 11 genera per sample and 22 genera across all samples.

There was relatively good agreement, at the genus level, between the bacterial phylogenies obtained by the cultivation-independent cloning and sequencing approach and the samples from which isolates were obtained and characterized. Gram-positive bacteria high in G+C content, including members of *Arthrobacter*, *Bacillus*, *Streptomyces*, and *Nocardioides*, were among the most common genera represented among the cloned sequences (Table 4) and were also represented among the isolates (Table 3), especially *Arthrobacter*. Among the gram-negative genera represented in the clone libraries, *Sphingomonas* and *Pseudomonas* were also present, including a sequence closely related to *Pseudomonas stutzeri* from sample 12a (Table 4), the same sample from which an isolate closely related to *P. stutzeri* was obtained (Table 3). A *P. stutzeri*-like sequence was also obtained from the 17a clone library that was phylogenetically similar to three of the nine isolates from this sample.

DISCUSSION

In spite of harsh chemical and physical conditions imposed on vadose sediments by wastes leaked from tank SX-108 (Tables 1 and 2), viable aerobic heterotrophic bacteria were recovered from 11 of the 16 sediment samples. Due to low population densities it is difficult to discern trends in either population size or presence of aerobic heterotrophic bacteria in relation to sediment properties such as pH, water content, and contaminant concentration (Table 1). Several sediment samples, 1a, 4a, and 7a, with relatively low water contents and high radioactivity also contained moderate populations of heterotrophic bacteria. The highest viable populations were associated with samples that had not been subjected to heating and drying or severe contaminant exposure: 17a (>4.3 log CFU

TABLE 4. Phylogenetic association of clones from vadose sediments recovered from the SX-108 slant borehole

Core identity	Clone	Nearest GenBank relative	Identity[a] (%)	Accession no.
3a	RAY457.x1	*Bacillus* sp. strain YY	719/723 (99)	AY579781
	RAY473.x1	Unidentified eubacterium clone BSV05 from anoxic soil (likely *Bacillus*)	720/726 (99)	AY579782
	RAY479.x1	Uncultured soil bacterium clone 432-1 (likely *Bacillus*)	735/753 (97)	AY579783
	RAY516.x1	*Achromobacter xylosoxidans* strain 2002-55549	729/729 (100)	AY579784
	RAY554.x1	Uncultured bacterium clone 623-1 (likely *Arthrobacter*)	756/760 (99)	AY579785
	RAY592.x1	*Arthrobacter* sp. strain SMCC G968	593/600 (98)	AY579787
	RAY651.x1	Bacterium strain LMG 18435 (likely *Bacillus*)	736/741 (99)	AY579788
	RAY690.x1	Bacterium K2-24 (likely *Bacillus*)	543/553 (98)	AY579789
5a	RAZ387.x1	*Methylobacterium extorquens* ATCC14718	693/693 (100)	AY579790
	RAZ409.y1	*Nocardioides plantarum* DSM 11054T	612/621 (98)	AY579791
6a	RBA441.y1	*Taxeobacter* sp. strain SAFR-033	632/675 (93)	AY579792
	RBA464.x1	*Achromobacter xylosoxidans* CIP 7132t	652/654 (99)	AY579793
	RBA468.y1	β-Proteobacterium A0647	663/703 (94)	AY579794
	RBA471.y1	*Sphingomonas phyllosphaerae* FA2	740/741 (99)	AY579795
	RBA480.y1	*Methylobacterium extorquens* ATCC14718	563/563 (100)	AY579796
	RBA484.y1	Uncultured *Alcaligenes* sp. clone ON5 or *Bordetella hinzii*	620/625 (99)	AY579797
	RBA486.x1	Bacterium strain 86356 (likely *Sphingomonas*)	620/627 (98)	AY579798
	RBA505.x1	Uncultured β-Proteobacterium clone pA42B412	638/638 (100)	AY579799
	RBA669.x1	Uncultured bacterium clone cvf122070 (CFB group)	578/582 (99)	AY579800
	RBA761.y1	*Sphingomonas paucimobilis* ATCC 29837	431/440 (97)	AY579801
12a	RBB389.x1	*Achromobacter xylosoxidans* strain 2002-55549	710/713 (99)	AY579802
	RBB392.y1	*Streptomyces* sp. strain KN-1220	598/603 (99)	AY579803
	RBB399.y1	*Streptomyces* sp. strain VTT E-99-1326 (A4)	711/711 (100)	AY579804
	RBB431.x1	*Saccharothrix tangerinus* strain MK27-91F2	623/625 (99)	AY579805
	RBB518.x1	*P. stutzeri* strain ASK-1	657/657 (100)	AY579806
	RBB541.y1	*A. globiformis* JCM 1332	361/362 (99)	AY579807
	RBB579.x1	*S. paucimobilis* strain ATCC 29837	652/753 (99)	AY579808
	RBB612.x1	*A. agilis* strain WED2.2	705/705 (100)	AY579809
	RBB674.y1	*Arthrobacter* sp. strain Fa21	538/549 (97)	AY579810
	RBB697.x1	*Nocardiodes* sp. strain NCFB3005 or *Aeromicrobium* sp. strain GWS-BW-H252	604/614 (98)	AY579811
	RBB732.x1	*Stenotrophomonas maltophilia* strain 6B2-1	621/624 (99)	AY579812
17a	RBC394.x1	*Arthrobacter* sp. strain pfB10	547/554 (98)	AY579813
	RBC400.x1	*Arthrobacter* sp. strain 19503	717/717 (100)	AY579814
	RBC407.x1	*Geodermatophilus* sp. strain 4S	594/607 (97)	AY579815
	RBC412.y1	*Kocuria erythromyxa* ATCC 187T	565/568 (99)	AY579816
	RBC413.y1	*Achromobacter xylosoxidans* strain 2002-55549	676/676 (100)	AY579817
	RBC423.y1	Uncultured earthworm cast bacterium clone c276 (likely *Amycolatopsis*)	661/681 (97)	AY579818
	RBC435.x1	Uncultured actinobacterium clone APe4_57 (likely *Arthrobacter*)	663/666 (99)	AY579819
	RBC437.x1	Uncultured actinobacterium clone APe4_57 (likely *Actinobispora*)	597/606 (98)	AY579820
	RBC439.x1	*Nocardioides* sp. strain NCFB3007	646/651 (99)	AY579821
	RBC450.x1	*Arthrobacter crystallopoietes* DSM 20117	555/564 (98)	AY579822
	RBC489.x1	*P. stutzeri* strain ASK-1	675/676 (99)	AY579823
	RBC620.x1	*Blastococcus aggregatus* strain DSM 4725T	562/566 (99)	AY579824
	RBC630.x1	*Arthrobacter* sp. strain An5	632/632 (100)	AY579825
	RBC645.x1	*A. agilis* strain WED2.2	666/666 (100)	AY579826
	RBC716.x1	*Streptococcus sanguis* ATCC 10556	611/614 (99)	AY579827
	RBC738.x1	Phenanthrene-degrading bacterium 70-2 (likely *Janthinobacterium*)	661/673 (98)	AY579828
	RBC759.x1	*Arthrobacter aurescens*	685/688 (99)	AY579829

[a] Nucleotides identical to nearest Genbank relative/total nucleotides of clone sequence.

g^{-1}) from SX-108 and RG4 (5.5 log CFU g^{-1}) from the 299-W22-48 uncontaminated borehole. RG4 was from an uncontaminated region of the vadose zone, and 17a was among the least contaminated samples from SX-108. Because no attempts were made to measure total microbial biomass in these samples, it was not possible to draw any conclusions regarding relationships between total microbial biomass and sediment properties.

One of the caveats that must be recognized with a study of this type is the limitation associated with using cultivation-based methods exclusively for microbiological characterization. In some environments, the population size of the cultured prokaryotic community can be as much as 2 to 4 orders of magnitude below the population size determined by direct microscopic counting (2). In spite of their limitations, cultivation methods have previously been successfully applied to characterizing subsurface microbial populations in saturated (4, 23) and unsaturated (9, 24) nonradioactive subsurface sediments. The use of cultivation-based methods over sequence-based methods has the advantage that cultures can be used for physiologic and metabolic analyses (1). In this study, we applied both methods to investigate the phylogenetic composition of the microbial populations associated with contaminated subsurface sediments from the Hanford Site. We found the results (Tables 3 and 4) of both methods to be in reasonably good agreement, and they were consistent with previous find-

ings (24, 30), supporting the idea that viable populations in Hanford vadose sediments are sparse but are typically higher in regions where the moisture contents are elevated.

Isolates related to members of the gram-positive bacteria high in G+C content dominated the cultures obtained from both the contaminated and uncontaminated vadose sediments, and they exclusively represented organisms isolated from either highly radioactive SX-108 samples or irradiated uncontaminated sediments (Table 3). The same group also dominated the phylogeny of cloned sequences obtained from sediment DNA extracts (Table 4). In contrast to the highly radioactive and gamma-irradiated samples, nearly half of the isolates from sediment samples 17a and RG4 that had little or no contamination and relatively high water contents were gram-negative Proteobacteria. Although the results are not quantitative, the phylogenetic diversity and the dominance of gram-positive bacteria high in G+C content was greater in the sequenced sediment DNA clones from samples 12a and 17a (Table 4) than was represented among the isolates from these same samples (Table 3). Desiccation alone did not eliminate the isolation of gram-negative bacteria from RG1 or RG4, as did gamma irradiation (Table 3), suggesting that ionizing radiation, perhaps in combination with other contaminants, may have had a significant effect on the phylogenetic composition of the vadose microbial population.

Previous studies have indicated that, in general, gram-positive bacteria such as *Arthrobacter* spp. are more drought tolerant than gram-negative organisms like *Pseudomonas* spp. (13, 32, 44). In fact, *Arthrobacter* members appear to be well adapted to life in arid soils (12), and some members are adept at surviving for extended periods of desiccation (8). Members of the genus *Arthrobacter* also appear to be well adapted to vadose sediments of the Hanford Site, as approximately one-third of the total isolates and a significant number of cloned sequences (11 out of 48) from this study were related to members of this genus. This is about the same proportion of total viable aerobic chemoheterotrophic bacteria as was isolated from pristine Ringold Formation sediments obtained from another location on the Hanford Site (6). *Arthrobacter* spp. were also common isolates in a third study of vadose zone sediments at the Hanford Site (10). The phylogeny of the Ringold Formation *Arthrobacter* strains has been investigated in detail, and many of the isolates appear to represent novel species within the genus (16). Additional genera represented among the vadose zone cultures and sediment DNA-cloned sequences from this study that were also found in previous analyses of uncontaminated subsurface sediments from the Hanford Site (6, 10) include *Rhodococcus*, *Staphylococcus*, *Streptomyces*, *Nocardioides*, *Bacillus*, and *Sphingomonas*.

One of the more intriguing results from this study was the isolation of two cultures from core 7a (25.6 m) that were resistant to extreme (20 kGy) laboratory doses of gamma radiation. This sample was obtained from the highest ^{137}Cs concentration region of the plume. Both of these isolates were closely related to *D. radiodurans*, a bacterium that is well recognized for its remarkable ability to withstand high levels of ionizing radiation. To our knowledge, this is the first time that *D. radiodurans*-like strains have been isolated from a radionuclide-contaminated environment. It is possible that *Deinococcus* is indigenous to Hanford soils and vadose zone sediments and that the harsh environment of the SX-108 contaminant plume led to conditions that selected for this highly stress-resistant organism. The ecological habitat of deinococci is poorly defined, but they do appear to be widely distributed in soils (11, 39). Additional studies are presently under way to determine if *Deinococcus* is a cosmopolitan inhabitant of Hanford Site soils. Mattimore and Battista (37) have shown that in *D. radiodurans* some genes that are necessary to survive irradiation are also necessary for desiccation resistance. However, a recent report (7) has shown the existence of genes in *D. radiodurans* that affect desiccation resistance but not radiation resistance, indicating that resistance to these conditions may involve different mechanisms.

Although all the factors influencing the microbiological characteristics of the SX-108 vadose sediments are unclear at this time, finding viable aerobic heterotrophic bacteria in radioactive sediments beneath the SX-108 tank may have important implications for the fate and transport of waste-associated contaminants. Microorganisms, in general, have the capacity for a wide range of biogeochemical transformations, including various reactions with waste constituents. For example, microorganisms are capable of degrading a wide range of organic compounds, oxidizing and reducing multivalent metals and radionuclides, such as Cr, U, and Tc, oxidizing ammonium to nitrite and nitrate, reducing nitrate or nitrite to ammonium or N_2, and for sorption and/or assimilation of a range of cations, including Cs and Sr. An important consideration for microbial-driven biogeochemical processes in the vadose sediments, including interactions with contaminants, is the availability of water. Assuming that the water contents measured on the core sediment samples accurately reflect in situ water distributions, it is clear that microbial processes in the upper 31 m are presently of little consequence to contaminant fate and transport because diffusion of solutes would be extremely limited and microbial cells are sparse and will likely be inactive or dormant. However, any future increases in moisture content due to either episodic natural or artificial (24) recharge or alteration in regional climate patterns could result in significant increases in the size and activity of microbial populations in vadose sediments. Indeed, moisture calculations for the S-SX tank farm indicate that subsurface water contents are increasing as the system slowly re-equilibrates from the extreme thermal loads imposed through HLW waste boiling. This high thermal load decreased in the early 1970s as the decay of short-lived radionuclides declined.

We have confirmed the presence of viable bacteria in vadose zone sediments contaminated with high-level radioactive waste beneath waste tank SX-108 on DOE's Hanford Site. The site has experienced extreme geochemical, thermal, and radiological conditions in the past and still represents a harsh chemical and radiological environment. The culturable microbiota was comprised predominantly of aerobic chemoheterotrophic bacteria, mainly gram-positive organisms, including several highly radiation-resistant isolates related to *D. radiodurans*. Although these organisms are likely inactive or dormant under present environmental conditions, the ability of these organisms to survive under extreme conditions for extended periods in vadose sediments indicates that they could influence contaminant fate and transport should moisture regimes be altered in the future.

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REFERENCES

1. **Achenbach, L. A., and J. D. Coates.** 2000. Disparity between bacterial phylogeny and physiology—comparing 16S rRNA sequences to assess relationships can be a powerful tool, but its limitations need to be considered. ASM News **66:**714–715.
2. **Amann, R., W. Ludwig, and K.-H. Schleifer.** 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59:**143–169.
3. **Balkwill, D.** 1993. DOE makes subsurface cultures available. ASM News **59:**504–506.
4. **Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas.** 1989. Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep Southeast Coastal Plain subsurface sediments. Appl. Environ. Microbiol. **55:**1058–1065.
5. **Balkwill, D. L., E. M. Murphy, D. M. Fair, D. B. Ringelberg, and D. C. White.** 1998. Microbial communities in high and low recharge environments: implications for microbial transport in the vadose zone. Microb. Ecol. **35:**156–171.
6. **Balkwill, D. L., R. H. Reeves, G. R. Drake, J. Y. Reeves, F. H. Crocker, M. Baldwin-King, and D. R. Boone.** 1997. Phylogenetic characterization of bacteria in the subsurface microbial culture collection. FEMS Microbiol. Rev. **20:**201–216.
7. **Battista, J. R., M. J. Park, and A. E. McLemore.** 2001. Inactivation of two homologues of proteins presumed to be involved in the desiccation tolerance of plants sensitizes *Deinococcus radiodurans* R1 to desiccation. Cryobiology **43:**133–139.
8. **Boylen, C. W.** 1973. Survival of *Arthrobacter crystallopoietes* during prolonged periods of desiccation. J. Bacteriol. **113:**33–57.
9. **Brockman, F. J., T. L. Kieft, J. K. Fredrickson, B. N. Bjornstad, S. W. Li, W. Spangenburg, and P. E. Long.** 1992. Microbiology of vadose zone paleosols in south-central Washington state. Microb. Ecol. **23:**279–301.
10. **Brockman, F. J., C. J. Murray, E. M. Murphy, B. N. Bjornstad, D. Balkwill, D. B. Ringelberg, S. Pfiffner, and R. Griffiths.** 1997. Microbial life in the unsaturated subsurface under conditions of extremely low recharge: an extreme environment, p. 388–394. *In* R. B. Hoover (ed.), Instruments, methods, and missions for the investigation of extraterrestrial microorganisms. Proceedings of SPIE, vol. 3111. Society of Photo-optical Instrumentation Engineers, Bellingham, Wash.
11. **Brooks, B. W., and R. G. E. Murray.** 1981. Nomenclature for "*Micrococcus radiodurans*" and other radiation-resistant cocci: *Deinococcaceae* fam. nov. and *Deinococcus* gen. nov., including five species. Int. J. Syst. Bacteriol. **31:**353–360.
12. **Cacciarai, I., and D. Lippi.** 1987. Arthrobacters: successful arid soil bacteria. Arid Soil Res. Rehab. **1:**1–30.
13. **Chen, M., and M. Alexander.** 1973. Survival of soil bacteria during prolonged desiccation. Soil Biol. Biochem. **5:**213–221.
14. **Christensson, M., L. L. Blackall, and T. Welander.** 1998. Metabolic transformations and characterisation of the sludge community in an enhanced biological phosphorous removal system. Appl. Microbiol. Biotechnol. **49:** 226–234.
15. **Colwell, F. S.** 1989. Microbiological comparison of surface soil and unsaturated subsurface soil from a semiarid high desert. Appl. Environ. Microbiol. **55:**2420–2423.
16. **Crocker, F. H., J. K. Fredrickson, D. C. White, D. B. Ringelberg, and D. L. Balkwill.** 2000. Phylogenetic and physiological diversity of *Arthrobacter* strains isolated from unconsolidated subsurface sediments. Microbiology **146:**1295–1310.
17. **Daly, M. J., O. Y. Ling, and K. W. Minton.** 1994. Interplasmidic recombination following irradiation of the radioresistant bacterium *Deinococcus radiodurans.* J. Bacteriol. **176:**7506–7515.
18. **DeLong, E. F.** 1992. *Archaea* in coastal marine environments. Proc. Natl. Acad. Sci. USA **89:**5685–5689.
19. **Felsenstein, J.** 1996. Inferring phylogenies from protein sequences by parsimony, distance, and likelihood methods. Methods Enzymol. **266:**418–427.
20. **Fitch, W. M., and E. Margoliash.** 1967. Construction of phylogenetic trees. Science **155:**279–284.
21. **Franzmann, P. D., L. R. Zappia, B. M. Patterson, J. L. Rayner, and G. B. Davis.** 1998. Mineralisation of low concentrations of organic compounds and microbial biomass in surface and vadose zone soils from the Swan Coastal Plain, Western Australia. Aust. J. Soil Res. **36:**921–939.
22. **Fredrickson, J. K., and D. L. Balkwill.** 1998. Sampling and enumeration techniques, p. 239–254. *In* R. S. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler (ed.), Techniques in microbial ecology. Oxford University Press, New York, N.Y.
23. **Fredrickson, J. K., D. L. Balkwill, J. M. Zachara, S. W. Li, F. J. Brockman, and M. A. Simmons.** 1991. Physiological diversity and distributions of heterotrophic bacteria in deep Cretaceous sediments of the Atlantic Coastal Plain. Appl. Environ. Microbiol. **57:**402–411.
24. **Fredrickson, J. K., F. J. Brockman, B. N. Bjornstad, P. E. Long, S. W. Li, J. P. McKinley, J. V. Wright, J. L. Conca, T. L. Kieft, and D. L. Balkwill.** 1993. Microbiological characteristics of pristine and contaminated deep vadose sediments from an arid region. Geomicrobiol. J. **11:**95–107.
25. **Fredrickson, J. K., and T. C. Onstott.** 2001. Biogeochemical and geological significance of subsurface microbiology, p. 3–38. *In* J. K. Fredrickson and M. Fletcher (ed.), Subsurface microbiology and biogeochemistry. Wiley-LISS, Inc., New York, N.Y.
26. **Fredrickson, J. K., and T. J. Phelps.** 1996. Subsurface drilling and sampling, p. 526–540. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. American Society for Microbiology, Washington, D.C.
27. **Gephart, R. E., and R. E. Lundgren.** 1996. Hanford tank cleanup: a guide to understanding the technical issues. PNNL-10773 Pacific Northwest National Laboratory, Richland, Wash.
28. **Jones, T. E., R. A. Watrous, and G. T. Maclean.** 2000. Inventory estimates for single-shell tank leaks in S and SX tank farms RPP-6285. CH2M HILL Hanford Group, Inc., Richland, Wash.
29. **Jukes, T. H., and C. R. Cantor.** 1963. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism. Academic Press, New York, N.Y.
30. **Kieft, T. L., P. S. Amy, F. J. Brockman, J. K. Fredrickson, B. N. Bjornstad, and L. L. Rosacker.** 1993. Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites. Microb. Ecol. **26:**59–78.
31. **Kieft, T. L., E. M. Murphy, D. L. Haldeman, P. S. Amy, B. N. Bjornstad, E. V. McDonald, D. B. Ringelberg, D. C. White, J. Stair, R. P. Griffiths, T. C. Gsell, W. E. Holben, and D. R. Boone.** 1998. Microbial transport, survival, and succession in a sequence of buried sediments. Microb. Ecol. **36:**336–348.
32. **Kieft, T. L., D. B. Ringelberg, and D. C. White.** 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microbiol. **60:**3292–3299.
33. **Konopka, A., and R. Turco.** 1991. Biodegradation of organic compounds in vadose-zone and aquifer sediments. Appl. Environ. Microbiol. **57:**2260–2268.
34. **Lane, D. J.** 1991. 16S/23S rRNA Sequencing, p. 115–147. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons Ltd., New York, N.Y.
35. **Liu, C., J. M. Zachara, S. C. Smith, J. P. McKinley, and C. C. Ainsworth.** 2003. Desorption kinetics of radiocesium from subsurface sediment at the Hanford Site, USA. Geochim. Cosmochim. Acta **67:**2893–2912.
36. **Liu, C. X., J. M. Zachara, O. Qafoku, and S. C. Smith.** 2003. Effect of temperature on Cs+ sorption and desorption in subsurface sediments at the Hanford Site, USA. Environ. Sci. Technol. **37:**2640–2645.
37. **Mattimore, V., and J. R. Battista.** 1996. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. **178:**633–637.
38. **McKinley, J. P., T. O. Stevens, J. K. Fredrickson, J. M. Zachara, F. S. Colwell, K. B. Wagnon, S. A. Rawson, and B. N. Bjornstad.** 1997. The biogeochemistry of anaerobic lacustrine and paleosol sediments within an aerobic unconfined aquifer. Geomicrobiol. J. **14:**23–39.
39. **Murray, R. G. E.** 1992. The family *Deinococcaceae*, p. 3732–3744. *In* A. Ballows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, vol. 4. Springer-Verlag, New York, N.Y.
40. **Olsen, G. J., H. Matsuda, R. Hagstrom, and R. Overbeek.** 1994. fastDNAmL: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput. Appl. Biosci. **10:**41–48.
41. **Papendick, R. I., and G. S. Campbell.** 1981. Theory and measurement of water potential, p. 1–22. *In* J. F. Parr, W. R. Gardner, and L. F. Elliott (ed.), Water potential relations in soil microbiology. Soil Science Society of America, Madison, Wis.
42. **Phelps, T. J., and J. K. Fredrickson.** 2002. Drilling, coring, and sampling subsurface environments, p. 679–695. *In* M. J. McInerney (ed.), Manual of environmental microbiology, 2nd ed. ASM Press, Washington, D.C.
43. **Pruess, K., S. B. Yabusaki, C. I. Steefel, and P. C. Lichtner.** 2002. Fluid flow, heat transfer, and solute transport at nuclear waste storage tanks in the Hanford vadose zone. Vadose Zone J. **1:**68–88.
44. **Robinson, J. B., P. O. Solonius, and F. E. Chase.** 1965. A note on the differential response of *Arthrobacter* spp. and *Pseudomonas* sp. to drying in soil. Can. J. Microbiol. **11:**746–748.
45. **Serne, R. J., G. V. Last, G. W. Gee, H. T. Schaef, D. C. Lanigan, C. W. Lindenmeir, R. E. Clayton, V. L. LeGorre, R. D. Orr, B. C. F., I. V. Kutnyakov, T. C. Wilson, and D. A. Myers.** 2001. Geologic and geochemical data

collected from vadose sediments from borehole SX 41–09–39 in the S/SX waste management area and preliminary interpretations. Pacific Northwest National Laboratory, Richland, Wash.

46. **Serne, R. J., H. T. Schaef, G. V. Last, D. C. Lanigan, C. W. Lindenmeir, R. E. Clayton, V. L. LeGore, M. J. O'Hara, C. F. Brown, R. D. Orr, I. V. Kutnyakov, T. C. Wilson, D. B. Burke, B. A. Williams, and B. N. Bjornstad.** 2001. Geologic and geochemical data collected from vadose zone sediments from the slant borehole under SX-108 in the S/SX waste management area and preliminary interpretations. PNNL-2001–4. Pacific Northwest National Laboratory, Richland, Wash.
47. **Swofford, D. L.** 2000. PAUP: phylogenetic analysis using parsimony, 4.0, beta version 4a ed. Sinauer Associates, Sunderland, Md.
48. **White, M. D., S. B. Yabusaki, and K. Pruess.** 2001. Nonisothermal multiphase fluid flow and transport: multitank modeling in the SX tank farm, p. D277–D305. *In* A. J. Knepp (ed.), Appendix D: digest of S&T program evaluations. CH2M HILL Hanford Group, Inc., Richland, Wash.
49. **Zachara, J. M., C. C. Ainsworth, G. E. Brown, Jr., J. G. Catalano, J. P. McKinley, O. Qafoku, S. C. Smith, J. E. Szecsody, S. J. Traina, and J. A. Warner.** 2004. Chromium speciation and mobility in a high level waste vadose zone plume. Geochim. Cosmochim. Acta **68:**13–30.
50. **Zheng, D., E. W. Alm, D. A. Stahl, and L. Raskin.** 1996. Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies. Appl. Environ. Microbiol. **62:**4504–4513.