# Subsurface Bacterial Community Dynamics in the Presence of Plutonium and Uranium

*Cheryl R. Kuske (PI)[1], Mary Neu (Co-PI)[2], Elizabeth Cain[1], Susan Barns[1], Gary Icopin[2], and Sean Reilly[2]*

*[1]Bioscience Division, [2]Chemistry Division, Los Alamos National Laboratory, Los Alamos, NM*

The goals of this project are to (1) determine the effects of different forms and concentrations of Pu and U on the abundance and composition of bacterial communities in soils, and (2) to identify bacterial species that are active in the presence of actinides, and that may be responsible for the reduction of actinides in anaerobic subsurface environments. We are conducting replicated laboratory time course experiments where soil is exposed to different forms of Pu(V/VI) or U(VI) under aerobic or anaerobic conditions. Using a suite of DNA- and RNA-based methods we are monitoring changes in the bacterial community in response to actinides at different concentrations. In preliminary experiments using T-RFLP community fingerprints and clone/sequence libraries, we observed major shifts in the soil bacterial community under anaerobic conditions, in response to U(VI) and Pu (VI) exposure as well as to the $NaNO_3$ and NaCl controls. The major bacterial genera detected in clone libraries after Pu or U exposure were completely different than the major species in the parent soil. Ongoing experiments on a LANL soil include more in-depth comparisons of community changes in the presence of Pu(VI), U(VI), and the Pu(IV)EDTA complex, under anaerobic and aerobic conditions. Nucleic acids have been extracted from 28 different treatments (4 reps/trt) and DNA- and RNA-based comparisons are underway. These include documenting community shifts under the different conditions using 16S rRNA T-RFLP fingerprinting, clone/sequence libraries, and comparisons of DNA- and RNA-based measurements. Measurement of bacterial abundance of Fe(III)- and sulfate-reducing bacterial groups known to reduce U(VI) will be conducted using real-time quantitative PCR assays, followed by analysis of species composition in those groups found to be active and/or correlated with actinide presence. Experiments planned for February 2005 will incorporate additional soils/sediments from the Savannah River site. This study will contribute to the NABIR program by providing information on the dynamics of natural soil and sediment communities in the presence of Pu(V/VI) and U(VI) that complements ongoing pure culture and field studies.