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Division Group III, General Microbiology- Division I- I04- Microbial Responses to Stress and Environmental Stimuli

Title: Analysis of Organisms Surviving in Highly Contaminated Environments

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Background: One of the main goals of the DOE is to clean up metal and radionuclide contamination of soil and groundwater at DOE sites. One possible solution to this problem is bioremediation. In order to understand bioremediation in contaminated environments, it is first necessary to examine the organisms present in these environments and analyze their responses to stress conditions on a genetic level.

Methods: We extracted high molecular weight DNA from organisms present in contaminated soil sediment samples using a method which preserves the integrity of the DNA. Because the number of organisms in these samples was low, the genomic DNA was amplified using a phage polymerase amplification system. 16S rRNA analysis was then used to examine the microbial diversity of the samples. The amplified DNA was also used in the construction of large and small insert DNA libraries. These libraries were then screened for the presence of histidine kinase genes with homology to a subfamily of *Desulfovibrio vulgaris* histidine kinases.

Results: Genomic DNA has been extracted and amplified from nine different sites at the NABIR field research center. 16S rRNA analysis revealed the presence of distinct bacterial phyla, including proteobacteria, acidobacteria, and planctomycetes. Small and large insert libraries were constructed for all samples and examined for clonal diversity. Plaque hybridization of these libraries to histidine kinase homologous probes resulted in multiple positive clones. These clones will be compared and used to develop a better understanding of cellular responses to different environmental factors.

Conclusion: These experiments have furthered the understanding of how the biological organisms in a contaminated system are organized, regulated and linked. This will ultimately lead to an understanding of the ability of these organisms to attenuate metal and radionuclide contamination, and help to provide a method for maximizing this attenuation.