

## Geomicrobiological Investigations of Microbial Communities in Ferromanganese Deposits in Lechuguilla Cave, New Mexico, U.S.A.

Low-density ferromanganese deposits coat the walls and ceilings of Lechuguilla Cave, an ancient, deep, and oligotrophic subterranean environment. To investigate our hypothesis that these deposits may be formed by microbial processes, we performed a small subunit ribosomal RNA (SSU rRNA) sequence-based study of extracted DNA from both environmental samples and cells from enrichment cultures. DNA was extracted from samples from three sites (approximately 300, 231, and 228 m below the surface); rRNA genes were amplified by PCR, cloned, and sequenced. To expand our knowledge of possible manganese- and iron-oxidizing bacteria, additional rRNA gene studies were carried out on manganese and iron enrichment cultures containing minimal or no organic carbon and inoculated with the ferromanganese deposits from Lechuguilla Cave. Sequence analysis of clones from community DNA and enrichment culture clones showed the presence of putative iron- and manganese-oxidizing bacteria whose closest relatives are known to oxidize or reduce iron or manganese, including *Hyphomicrobium*, *Pedomicrobium*, *Bacillus*, *Leptospirillum ferrooxidans*, *Stenotrophomonas*, *Pantoea*, and *Devosia riboflavina* (Hyphomicrobium group). Manganese enrichment cultures were examined at intervals of several months using scanning electron microscopy and x-ray diffraction (XRD) to characterize crystals formed in the cultures. We observed a progression of crystallization from amorphous to poorly crystalline to highly crystalline materials over eight months of culture growth. XRD revealed development in the cultures of buserite and vernadite, two manganese-oxide minerals. No crystal formation was observed in media not inoculated with bacteria. The growth of manganese minerals in cultures inoculated from natural ferromanganese deposits, and the SSU rRNA study provide support for our hypothesis that microorganisms may contribute to the dissolution of limestone walls and the formation of manganese and iron oxide-rich materials. The rRNA analysis has revealed a rich and diverse microbial community in these unusual secondary mineral formations.