On the Nature of Bacterial Communities from Four Windows Cave, El Malpais National Monument, New Mexico, USA

Northup, Diana E., Biology Department, University of New Mexico; Connolly, Cynthia A., Socorro High School; Trent, Amanda, Biology Department, University of New Mexico; Boston, Penelope J., Earth and Environmental Science Department, New Mexico Tech; Peck, Vickie, Sandia National Laboratory; Donald O. Natvig, Biology Department, University of New Mexico

One of the striking features of some lava tubes is the extensive bacterial mats that cover the walls. Yet, despite their prominence in lava tubes, little is known about the nature of these bacterial communities. To rectify this situation we have investigated the bacterial mats that cover the walls of Four Windows Cave, a lava tube in El Malpais National Monument, New Mexico (see Figure 1). These bacterial mats, which occur in the twilight zone adjacent to algal mats, and in dark zone of the lava tube, cover from 25-75% of the wall. Their macroscopic and microscopic visual appearance suggests that these bacterial mats are composed of actinomycetes, bacteria that commonly inhabit caves. Actinomycetes are a group of Gram-positive bacteria that break down complex organic matter and thrive in environments where nutrients are sparse and conditions extreme. With a temperature of 0-2°C and seeping organic matter for nourishment, Four Windows provides an excellent habitat for these bacteria. Some types of a are medicinally and agriculturally significant because they excrete antibiotic products to repel invaders. Cave bacterial mats may have such antibiotic properties. Vacuuming of the bacterial mats and the adjacent algae, demonstrated the presence of collembola and mites on the algae and no invertebrates on the bacterial mats.

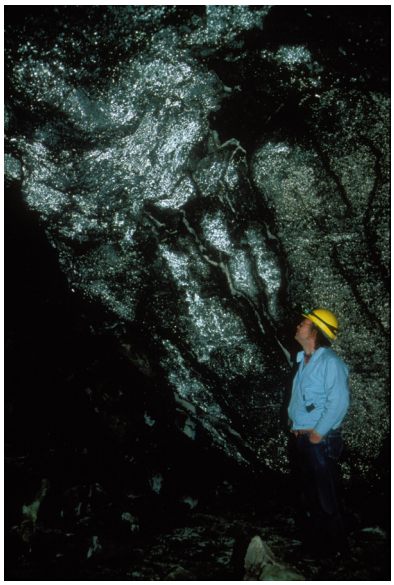
In an effort to phylogenetically characterize bacterial colony members, we extracted DNA from wall rock communities, using a soil DNA extraction technique developed at Los Alamos National Laboratories. The DNA was purified, the 16S rRNA gene was amplified using PCR, amplification products were cloned, and thirty clones were sequenced in their entirety. A restriction fragment length polymorphism (RFLP) analysis of 11 clones exhibited unique banding, an indicator of genetic diversity. Comparison of our sequences with those in the Ribosomal Database II revealed that the Four Windows bacterial sequences are most closely related to actinomycetes, as suspected. Some clones also showed similarities to environmental soil strains. Other clones are related to genera such as *Nocardia* and *Frankia*, although not closely. These results reveal a diverse community of bacteria and the presence of several novel bacterial species.

To investigate the degree to which the actinomycetes had adapted to the lava tube environment, we also investigated the ability of bacteria cultured from these mats to withstand the effects of ultraviolet (UV) radiation. Bacteria from the mats and from the surface rocks above the lava tube were cultured on R2A medium on-site in Four Windows Cave, were allowed to grow for 24-hours in the cave environment, and were then transported to the laboratory where they were grown at 2°C in an incubator. We subjected twelve isolates from the lava tube to one dose (100 seconds) and a half dose (50 seconds) of UV radiation. For controls, we subjected six isolates from the cave surface to the same radiation

treatments and also allowed replicates of all the isolates to grow without any radiation. The results showed a general trend in which microbes isolated from the lava tube were much more UV sensitive than the microbes isolated from the surface. However, all of the microbes tested displayed at least slight sensitivity to UV radiation. Based on the results, the bacterial colonies currently inhabiting the Four-Windows lava tube appear to be at least somewhat cave-adapted.

Our studies of the actinomycete communities in Four Windows Cave reveal a diverse community of bacteria that may produce secondary compounds that make them unpalatable to invertebrates. These bacteria appear to have become at least somewhat cave-adapted as evidenced by their loss of UV resistance.

Figure 1: Mats of actinomycete bacterial spread across walls in Four Windows Cave,



El Malpais National Monument. During some seasons the bacterial mats are hydrophobic and the walls appear "silvered" when light is shone upon them.