CAVE MICROBIAL COMMUNITIES: IS PROTECTION NECESSARY AND POSSIBLE?

DIANA E. NORTHUP
Department of Biology, MSC03 2020, University of New Mexico, Albuquerque, NM 87131, USA

Microbial communities in caves vary from the striking microbial mats observed in many lava tubes worldwide, to occasional colonies on the wall, to invisible biofilms on rock walls and ceilings of caves, to microbial end products, such as iron oxides. The investigations of the last decade, using culture-independent techniques in which we extract DNA from environmental samples and sequence clones to identify organisms present, have revealed a wealth of microbial species never described. Some of those organisms have been implicated in the dissolution and precipitation of cave rock and secondary minerals. Some may be critical in cycling nitrogen and carbon from the surface to the subsurface. Others have been shown to have the ability to kill cancer cells or produce antibiotics that might replace some of those antibiotics that are failing due to antibiotic resistance. Thus, we know that microorganisms found in caves are valuable, both for their intrinsic nature, and because they may prove useful to humans. Are they valuable enough to us to consider protecting them from we humans that explore their native cave habitat? To what degree do we, as cave visitors, impact these communities when we visit caves (Northup and Welbourn, 1995)?

The degree to which we impact cave microbial communities depends on the nature of the cave. Mammoth Cave in Kentucky, USA, and other caves like it, have rivers or streams running through major portions of the cave. Such caves are likely impacted by human visitation much less than arid-land caves, such as Lechuguilla Cave in New Mexico, USA. These drier and warmer caves contain significant microbial communities that can fall prey to a variety of impacts. When we explore caves, we leave behind pieces of ourselves: skin cells, bacteria and fungi from our hair and skin, hair, and occasionally worse things such as feces, urine, or mud and dirt from other caves, which carry their own microbial passengers. One of the major impacts that we can have on low-nutrient caves is the enrichment of the organic carbon present in those caves. We also compact any soil present and might leave other pollutants that affect microbial communities. How can we protect these microbial communities from such threats?

Several strategies can lower the impact that we have on cave microbial communities. Cleaning our gear, clothes, boots, and bodies between cave trips can limit the amount of cross-contamination that occurs among caves and lower the amount of organic carbon enrichment that occurs. Establish trails and camp areas (if camping is an issue) to confine human impact to a limited area of the cave. Encourage everyone to eat over bags to catch crumbs. A much more controversial strategy is the establishment of "microbial preserves" to preserve areas of unusually promising microbial potential by limiting the amount of human impact on the area.

Cave microbial communities can represent an extremely valuable resource that is worth protecting by modifying our behavior in visiting caves. The payoff may be an antibiotic that saves your life someday!

1. Introduction

Microorganisms in caves range from completely invisible to highly colorful microbial mats (Fig. 1) that line the walls of lava tubes to microbial waste products, such as iron oxides. It is hard to appreciate and value something that you cannot even see, but there are many compelling reasons to protect and conserve cave microorganisms and their habitat.

In the last two decades we have seen a major increase in research into the role that microorganisms play in the dissolution of bedrock and other surfaces and the precipitation of secondary mineral deposits (BARTON AND NORTHUP, 2007; NORTHUP AND LAVOIE, 2001). New discoveries of sulfur oxidizing bacteria in caves are revealing microbial roles in cycling sulfur in caves and enlargement of cave passages through sulfuric acid

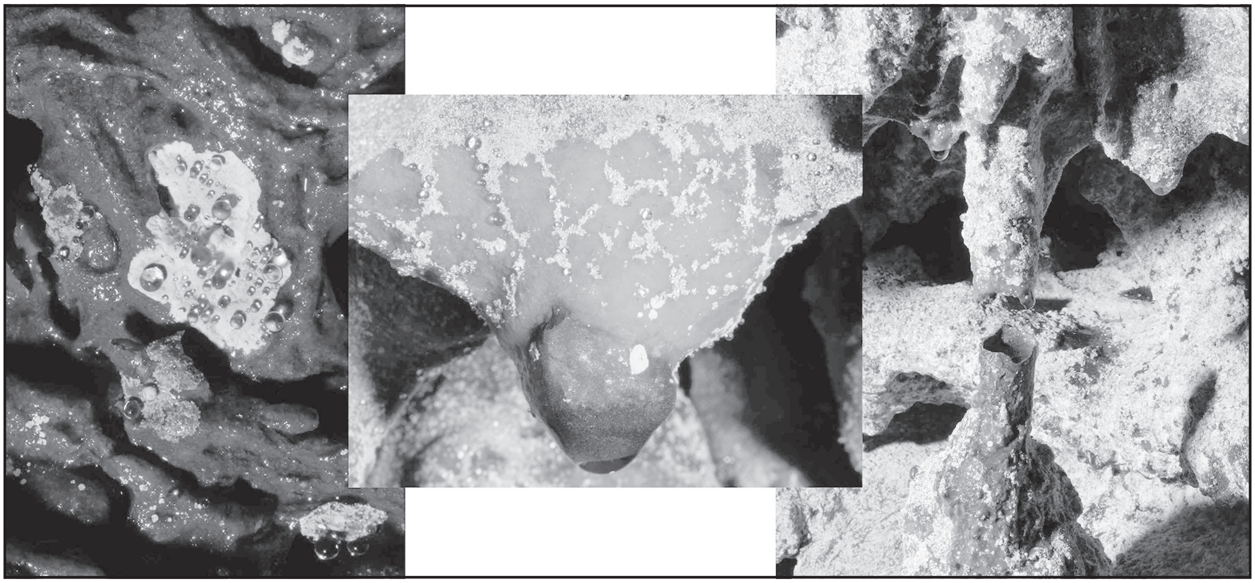

Figure 1: White, yellow, gold, and, pink microbial mats commonly line the walls and ceilings of moist lava tubes worldwide. Photos copyright 2008 Kenneth Ingham.

dissolution, as well as a possible role in sulfuric acid driven speleogenesis (ENGEL et al., 2004; HOSE et al., 2000). SPILDE et al. (2005) have shown a microbial role in the production of ferromanganese deposits in arid-land caves. These and other studies are showing key geomicrobiological roles for microorganisms in caves. Although much remains to be learned about the microbial role in energy transfer and elemental cycling, some evidence suggests that microorganisms facilitate the transfer of energy between cave life and organic carbon and serve as food for the cave life (SIMON et al., 2007). Perhaps most exciting is the amount of novel biodiversity that culture-independent molecular studies are revealing in caves (e.g. BARTON et al., 2004; GONZALEZ et al., 2006; NORTHUP et al., 2003). Some of these novel (and not so novel) species may produce chemical compounds that are very useful to humans, such as new antibiotics to replace those to which bacteria are now resistant (Dapkevicius, Terrazas and Northup, unpub. data). The geomicrobiological studies and those of novel microbial biodiversity also serve to aid our understanding of how to detect life on other planets, such as Mars, where life is likely to shelter from harsh surface conditions in the subsurface (BOSTON et al., 2001). Thus, our research is emphasizing the critical nature of cave microbial communities and suggests that their conservation is important.

2. Threats to Microbial Populations

Cave microorganisms are susceptible to a variety of threats, including human visitation, soil compaction, pollutant spills, cave restoration, and organic carbon enrichment. Whether microorganisms reside in arid-land caves, or those in areas with more rainfall, affects the degree to which these threats are an issue for microbial populations. Rivers and streams running through caves can carry away pollutants and dampen the effects of various threats; however, rivers and streams can also be the vehicle for introducing pollutants. Arid-land and caves with little inflow of moisture, in particular, are much more subject to the effects of organic carbon enrichment and other impacts that result from human visitation of caves. When we visit caves, we shed tens of thousands of skin cells, many of which are life rafts for our own microbial inhabitants, as well as hair and fibers and mud from our clothing (Fig. 2). If we are sick and vomit in the cave, we greatly enrich organic carbon in the habitat. Longer cave trips may bring the issues of urine and feces deposition (Fig. 3). While cricket and beetle feces are a natural part of the ecosystem, human feces are not. There is the matter of scale and the fact that human feces are almost 50% microorganisms. Urine can lead to the buildup of harmful compounds that change the microbial ecosystem (LAVOIE, 1995). As we walk through areas of the cave with soil or detrital material, we cause compaction of the soil, which decreases the available oxygen. Some visitors draw their names and dates in microbial mats (Fig. 4). Our studies (Lavoie and Northup, unpub. data.) suggest that human associated bacteria (e.g. *Staphylococcus aureus*) and fungi are preferentially found in areas with more human impact. If the cave is given time to "rest" (i.e. no human visitation) and we limit the amount of organic carbon buildup, these exotic populations generally die off. However, some exotic populations can persist and damage cultural artworks, such as those found in the caves of France and Spain (JURADO et al., 2008).

Figure 2: Humans leave detritus when they visit caves, such as hair, skin cells, food, and mud from other caves. Illustration by N. Robin Wilson, copyright 1993.

Figure 3: Long caving trips raise the issue of human feces disposal and possible impact as feces volatilize as they leave the body. Illustration by N. Robin Wilson, copyright 1993.

Also, our well intentioned efforts to restore and clean caves can lead to many problems for microbial communities as detailed in BOSTON et al. (2005). By trying to protect pristine pools in Lechuguilla Cave, we unintentionally enriched the amount of organic carbon in the pools from plasticizers in the tubing used to obtain drinking water. This led to a population explosion of a native bacterial population that appears to have then supported introduced *E. coli* in the pool (HUNTER et al., 2004). Through a variety of ways, we provide challenges to subterranean microbial populations. Where native microbial populations reside in oligotrophic habitats within caves, we will see the

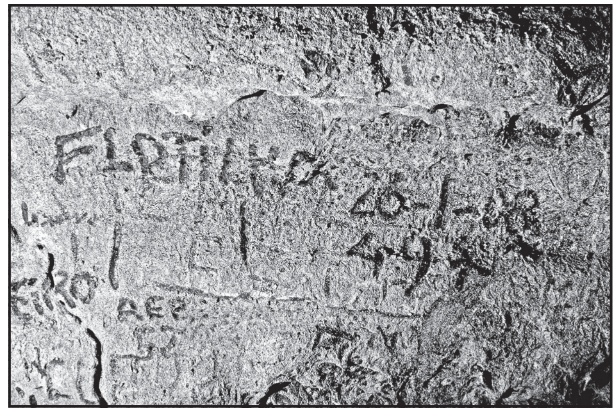

Figure 4: Cave visitors often draw their names in microbial mats that line the walls of lava tubes. Photo copyright 2008 Kenneth Ingham.

most profound effects. Oligotrophic microorganisms don't simply get "fatter" when you feed them more; they often die off, allowing more surface-adapted microorganisms to take their place (KOCH, 1997). Thus, human visitation can introduce new organic matter and exotic microorganisms into caves, which may harm native microbial populations.

3. Is Protection Possible During Active Exploration and Scientific Investigation?

There are some relatively simple things that can be done to protect microbial communities in caves, but the success of these recommendations rests in the acceptance of the value of these microbial populations by cavers, scientists, and other visitors to caves. It is hard to think about and protect things that you cannot even see. If you believe that cave microorganisms are a key component of the cave ecosystem or that an effective treatment for cancer or a new antibiotic that could save your life someday could come from a cave microorganism, then you are likely to be willing to go the extra mile for the microbes. Getting people to this point will take more research into what harms and what protects microbial communities in caves and using that information to educate cave visitors about the problems and solutions. One of the payoffs is that educational programs about cave microorganisms often excite and engage cave visitors—the microbes have wonderful stories to tell.

4. Recommendations

To know how to protect something, you need to understand it. Our knowledge of cave microbial communities is rudimentary, limiting our ability to know precisely what efforts will protect microbial communities in caves. Several laboratories around the world are conducting outstanding culture-independent molecular studies of cave microbial communities to identify novel biodiversity, while others are culturing cave microorganisms to shed light on their physiology and biochemistry, but we need more scientists involved. The first molecular study of microbial diversity was published in 1997, and while many others have followed it, much remains to be learned. Microbial inventories across gradients of depth, nutrient richness, distance from entrances, human impact, etc. are needed to compile a more complete picture of cave microbial communities. Many interesting ecological and evolutionary questions about cave microorganisms await researchers (e.g., SNIDER et al., 2009). Thus, research and inventory are key steps in the journey to protect cave microorganisms. The following recommendations are based on our preliminary investigations and insights, but their effectiveness remains to be tested.

As cavers and visitors to caves, we can take several actions to conserve microbial habitat and microorganisms:

- Establish trails for movement through the cave. When you establish trails, use inert markers that do not enrich organic carbon in the cave and do not degrade. If there are no marked trails, always walk where the "elephant tracks" are.
- For caves in which camping is necessary for exploration, establish camps to concentrate human impact.
- Eat over bags to catch all crumbs. What's a crumb to you is a supermarket to a microorganism.
- Clean your clothes and boots between cave trips to prevent cross contamination between caves.
- Brush your hair to remove loose hairs before going caving.
- Find ways around pristine pools and avoid dipping anything, including yourself, in the pool. Establish a clean pitcher for obtaining water.
- Educate new cavers in the ways to preserve and protect microbial communities.

Scientists, cavers, and cave managers who find unusual deposits that may be microbial should consider establishing a microbial preserve to allow investigation before visitation occurs to any extent. If you see something really intriguing, send a photo to one of the microbiologists around the world who studies these communities in caves. Scientists often study a few areas very intensively and may miss key discoveries. Cavers and scientists should collaborate on microbial discoveries for mutual benefit. Scientists can excite cavers and visitors by providing engaging information about their findings through public talks, articles, and other media that bring the science to the public.

5. Conclusions

Our knowledge of microbial diversity in caves is growing rapidly and revealing a wonderland of microorganisms (Fig. 5) that participate in precipitation and dissolution of cave mineral deposits that have roles in nutrient cycling within the cave ecosystem, that may produce chemical substances of great use to humans, and that serve as an analog for possible life on other planets. These important communities are, however, threatened by some of our actions when we visit or live and work above caves. By being conscious of the ways in which we may enrich organic carbon in caves, we can do much to protect microbial habitats and microorganisms in caves. Are cave microorganisms threatened? In 1997, Jim Staley wrote the following concerning microorganisms in general:

> "Our knowledge of microbial diversity, particularly bacterial diversity, is so meager that we do not yet know if and when most species are threatened."

This is particularly true of cave microorganisms and enhanced efforts to study and understand cave microbial communities are essential to our being able to truly answer the question of whether these populations are threatened.

Acknowledgements

Many cavers over the years have provided immeasurable help in carrying out the various research projects that led to observations that formed the basis for the ideas contained in this manuscript, and in providing leads to new microbial habitats. They include, but are not limited to: Kenneth Ingham for all his great microbe photography;

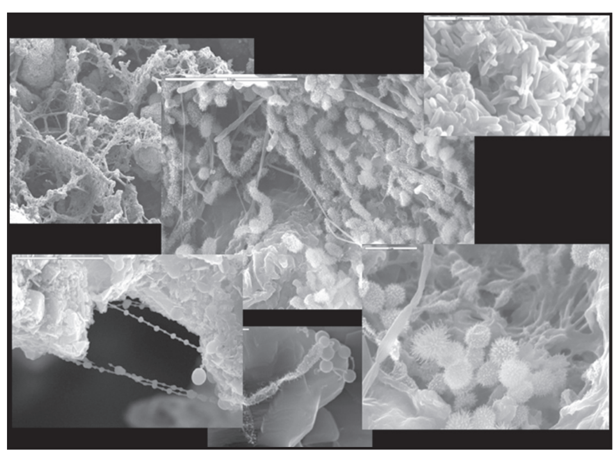

Figure 5: Cave microorganisms represent a wonderland of organisms as seen in these scanning electron micrographs from caves in New Mexico, Arizona, Mexico, and the Cape Verde Islands. Photomicrographs courtesy of Michael N. Spilde.

Val Hildreth-Werker and Jim Werker for photography, engineering, and lots of great research; Andi Hunter, for her invaluable research into the contamination of pools in Lechuguilla Cave; and Penny Boston and Mike Spilde, with whom I have had many stimulating conversations about microbes. The Charles A. and Anne Morrow Lindbergh Foundation, Mammoth Cave National Park, and T & E, Inc. provided financial support for the human impact studies that were carried out in collaboration with Kathy Lavoie who contributed substantially to the ideas on human impact. Thanks go to Leslie Melim and Kenneth Ingham for insightful comments on the manuscript.

References

BOSTON, P.J., NORTHUP, D.E., and K.H. LAVOIE (2005) Protecting microbial habitats: Preserving the unseen. In *Cave Conservation and Restoration*, Hildreth-Werker V., J.C. Werker (Eds), National Speleological Society, Huntsville (AL), p. 61–82.

BOSTON, P.J., SPILDE, M.N., NORTHUP, D.E., MELIM, L.A., SOROKA, D.A., KLEINA, L.G., LAVOIE, K.H., HOSE, L.D., MALLORY, L.M., DAHM, C.N., CROSSEY, L.J., and R.T. SCHEBLE (2001) Cave biosignature suites: Microbes, minerals and Mars. *Astrobiology Journal* **1**, 25–55.

ENGEL, A.S., STERN, L.A., and P.C. BENNETT (2004) Microbial contributions to cave formation: new insights into sulfuric acid speleogenesis. *Geology*, **32**, 369–372.

GONZALEZ, J.M., PORTILO, M.C., and C. SAIZ-JIMENEZ (2006) Metabolically active Crenarchaeota in Altamira Cave. *Naturwissenschaften* **93**, 42–45.

HOSE, L.D., PALMER, A.N., PALMER, M.V., NORTHUP, D.E., BOSTON, P.J. and H.R. DUCHENE (2000) Microbiology and geochemistry in a hydrogen sulphide-rich karst environment. *Chemical Geology* **169**, 399–423.

HUNTER, A.J., NORTHUP, D.E., DAHM, C.N., and P.J. BOSTON (2004) Persistent coliform contamination in Lechuguilla Cave pools. *Journal of Cave and Karst Studies* **66**, 102–110.

JURADO V., SANCHEZ-MORAL, S., and C. SAIZ-JIMENEZ (2008) Entomogenous fungi and the conservation of the cultural heritage: A review. *International Biodeterioration & Biodegradation*. **62**, 325–330.

KOCH, A.L. (1997) Microbial physiology and ecology of slow growth. *Microbiology and Molecular Biology Reviews* **61**, 305–318.

LAVOIE, K.H. (1995) The effects of urine deposition on microbes in cave soils. In *Proceedings of the 1993 National Cave Management Symposium: held in Carlsbad, New Mexico, October 27-30, 1993,* Pate, D.L. (Ed.), National Cave Management Symposium Steering Committee, Huntsville (AL), p. 302–11.

NORTHUP, D.E., and K.H. LAVOIE (2001) Geomicrobiology of caves: A review. *Geomicrobiology Journal* **18**, 199–222.

NORTHUP D.E., and W.C. WELBOURN (1995) Conservation of invertebrates and microorganisms in the cave environment. In *Proceedings of the 1993 National Cave Management Symposium: held in Carlsbad, New Mexico, October 27-30, 1993,* Pate, D.L. (Ed.), National Cave Management Symposium Steering Committee, Huntsville (AL), p. 292–301.

SIMON, K.S., PIPAN, T., and D.C. CULVER (2007) Conceptual model of the flow and distribution of organic carbon in caves. *Journal of Cave and Karst Studies* **69**, 279–284.

SNIDER, J.R., GOIN, C., MILLER, R.V., BOSTON, P.J., and D.E. NORTHUP (2009) Ultraviolet radiation sensitivity in cave bacteria: Evidence of adaptation to the subsurface? *International Journal of Speleology* **38**, 1–12.

SPILDE, M.N., NORTHUP, D.E., BOSTON, P.J., SCHELBLE, R.T., DANO, K.E., CROSSEY, L.J., and C.N. DAHM (2005) Geomicrobiology of cave ferromanganese deposits: A field and laboratory investigation. *Geomicrobiology Journal* B, 99–116.

STALEY, J.T. (1997) Biodiversity: Are microbial species threatened? *Current Opinion in Biotechnology* **8**, 340–345.