Microbially-Enhanced Weathering in Subsurface Habitats: Sulfur-Oxidizing Bacteria and The Cave Environment

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While most caves and karst features form from carbonic acid dissolution of carbonate rocks, caves can also form by sulfuric acid dissolution (Egemeier, 1981). Sulfuric acid is derived from hydrogen sulfide-rich waters that rise up into carbonate strata and become oxidized: 1) through abiotic reactions from mixing of waters having different concentrations of hydrogen sulfide or mixing reduced and oxygen-rich waters (Palmer, 1995), as well as 2) from biological oxidation reactions (Altas & Bartha, 1998).

One of the major problems interpreting caves formed by sulfuric acid speleogenesis is recognizing the mechanisms of sulfuric acid formation. However, in modern caves containing sulfidic waters, there is a wide range of microorganisms in these systems. A vast majority of them are sulfur-oxidizing bacteria that live within cave springs and streams, as well as growing on cave-wall surfaces (Table 1). Some of these bacteria are reportedly acidophilic due to the production of sulfuric acid (Hose & Piscarowicz, 1999; Vlăsceanu et al., *in press*). Therefore, sulfur-oxidizing bacterial populations can be examined as a means of understanding sulfuric acid speleogenesis. Additionally, studying the microbial communities in sulfidic caves can provide a better appreciation of other sulfidic habitats, relative microbial abundance and viability in the subsurface, and potential biogeochemical interactions pertinent to global sulfur cycling. Unfortunately, although sulfur-oxidizing microorganisms have been identified, few studies have addressed the possible geomicrobiological impacts that the microbial communities may have on the cave environment. For these reasons, this work examined sulfur-oxidizing bacterial populations from four sulfidic cave systems and tested whether their metabolic activity can enhance cave formation and modification.

The caves used in this study include: **Movile** Cave, Romania; Grotta Grande del Vento - Grotta del Fiume (Frasassi caves), Italy; Cesspool Cave, Virginia; and Lower Kane Cave, Wyoming. Sub-aqueous and sub-aerial microbial habitats are ubiquitous in these caves. Movile Cave contains the only floating mats, but also has wall biofilms and anaerobic, submerged mats. The Frasassi caves contain thick, filamentous submerged mats in very turbulent water, as well as extensive wall biofilms associated with elemental sulfur and gypsum mineralization. Several delicate filamentous and web-like mats form in Cesspool Cave, predominately limited to the sediment interface in shallow, slow-moving pools. Some mats can also be found in rifle zones and forming thin wall mats near the water. Biofilms and mats in Lower Kane Cave are diverse, including thick filamentous mats in fast-moving water coming from spring discharge areas, red and black mats in pools, and as biofilms on gypsum and elemental sulfur cave-wall surfaces.

TABLE 1 Composite list of known active sulfidic caves and documented sulfur-oxidizing microorganisms.

Cave and location	Microbes reported *	References
Movile Cave, Romania	Thiobacillus thioparus LV43 (G)	Vlăsceanu et al 1997
	Thiobacillus spp. (M)	Engel 1999
Cesspool Cave, Virginia	Beggiatoa, Achromatium, Thiothrix (M)	Hubbard et al. 1990
	Thiobacillus spp., Thiothrix spp., Thiovulum spp. (G)	Engel 1999
Parker Cave, Sulphur River, Kentucky	Thiobacillus spp., Thiothrix spp., Thiomicrospira spp. (G)	Angert et al. 1998
Frasassi Caves, Italy	Thiobacillus spp. (M and G)	Vlăsceanu et al., in press
Misc. springs and caves, Florida	Thiothrix spp. (M and G)	Brigmon et al. 1994
Submarine caves, Cape Palinuro, Italy	Thiothrix spp., Beggiatoa (M)	Mattison et al. 1998
Cupp-Coutunn Cave, Turkmenistan	Thiobacillus ferrooxidans, Thiobiacillus thiooxidans (G)	Maltsev et al. 1997
Lower Kane Cave, Wyoming	Thiobacillus spp. (M)	Engel 1999
Stinkpot Cave, Wyoming	Unknown	Hill et al. 1976
Cueva de Villa Luz, Mexico	Unknown	Hose & Picarowisz 1999
Crystal Beach Spring Cave, Florida	Unknown	Garman 1999

*(M) - microscopic identification and Culturing; (G) - genetic identification

Chemoautotrophic, elemental sulfur- and thiosulfate-based media were used to grow sulfur-oxidizing bacteria from the caves at pH values of 4 and 6.

Thiosulfate-based media were the most successful for achieving enrichment diversity, although growth was observed on all media and pH combinations used. The greatest numbers of bacteria were obtained from filamentous mats from Lower Kane Cave and the cave-wall biofilms from the Frasassi caves. The least number of cultured bacteria were from the Movile Cave wall mats and mats from Lower Kane Cave pools.

A summary of successful laboratory enrichment data shows that several morphotypes were observed, although rod-shaped microorganisms dominated the cave samples and pH enrichments (Figure 1). Short rods were, on average, less than 1 Fm in length, and long rods were greater than 1 μm. All the rods observed were Gram negative. At pH 6, filamentous bacteria were observed from Movile Cave floating mat, Movile Cave submerged mats, Cesspool Cave wall mat, Lower Kane Cave black mats, and Lower Kane Cave stream mats. Minor occurrences of Gram positive cocci and fungi were also observed in enrichment cultures. At pH 4, no filamentous bacteria were enriched for, although there were minor amounts of vibrio/spiril morphotypes, as well as cocci and fungi. Isolation of bacterial strains resulted in only short or long rods. The majority of the isolated colonies were small and transparent, however some were pigmented (brown, black, white, yellowish-orange). Most of the pigmented colonies were strains isolated from Lower Kane Cave black and red mats, and from Frasassi cave-wall biofilms.

Molecular phylogenetic analysis of some of the laboratory strains suggested that most are closely related to several species of the genus Thiobacillus (from the Frasassi caves and Cesspool Cave). Clones obtained from direct PCR amplification of 16S rDNA gene sequences were most similar to sequences of sulfur-oxidizing, filamentous bacteria, including *Thiothrix and Thiovulum* spp. (Engel, 1999).

Individual strains were evaluated for sulfuric acid generation using two methods to detect for acid and to quantify acidification rates. These methods involved incorporating either a pH detector (bromocresol green) or calcium carbonate into solid media. Of 70 strains isolated in the laboratory from the four caves, 47 generated acid on the bromocresol green plates. However, only 30% of all the clones were capable of dissolving supplemental calcium carbonate in the growth media. The Frasassi cave-wall biofilms had the most strains that generated acid and dissolved calcium carbonate (Vlăsceanu et al., *in press*). Based on comparisons with *in situ*, abiotic dissolution rates reported by Galdenzi et al. (1997) and Sârbu (1996) for the Frasassi caves and Movile Cave respectively, preliminary rates of microbially-mediated, sulfuric acid rock dissolution were significantly faster than previously measured abiotic dissolution rates for the cave systems.

Results of this study indicate that there is considerable diversity in populations of sulfur-oxidizing bacteda living in karst systems and that their metabolic activity can have a significant impact on carbonate rock weathering. If active sulfidic karst is characterized by sulfuric acid reactions that are derived from both abiotic and biotic processes, it can be proposed that ancient systems, such as Carlsbad Cavern and Lechuguilla Cave, had the same processes occurring during times in their formation histories. Therefore, any future interpretation of ancient karst development should include a geomicrobiologiCal component.

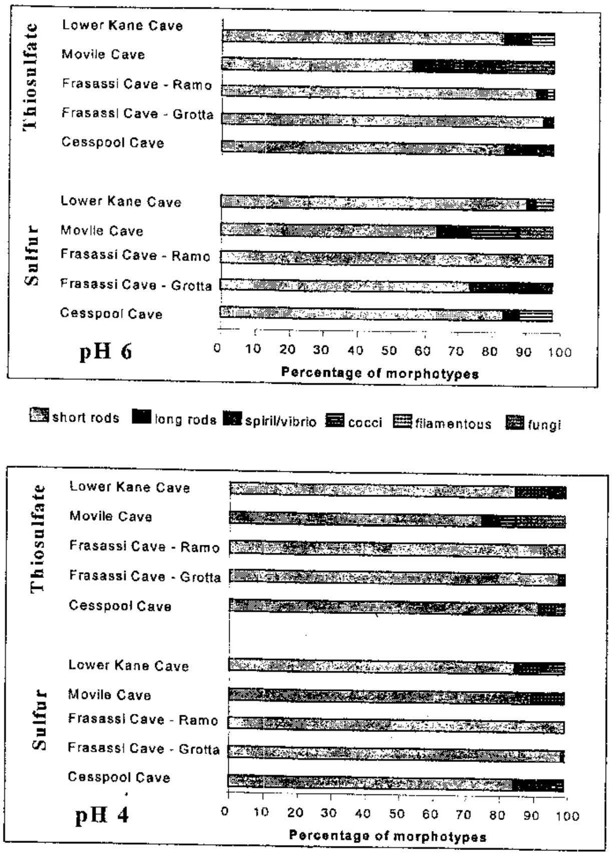

Figure 1. Summary of averaged morphological data from the four cave systems, organized by enrichment media (thiosulfate or elemental sulfur) and pH 6 (upper graph) or pH 4 (lower graph). Ramo: Ramo Sulfureo site in the Frasassi caves; Grotta: Grotta Sulfurea site. in the Frasassi caves.

References

ANGERT, E..R., NORTHUP, D.E., REYSENBACH, A.L., PEEK, A.S., GOEBEL, B.M., PACE, N.R., 1998. Molecular phylogenetic analysis of a bacterial community in Sulphur River, Parker Cave, Kentucky. American Mineralogist, 83: 1583-1592.

ATLAS, R., BARTHA, R., 1998. Microbial Ecology, 4th ed., Menlo Park, Benjamin Cummings, 425-437.

BRIGMON, R.L., MARTIN, H.W., MDRRIS, T.L., BITTON, G., ZAM, S.G., 1994. Biogeochemical ecology of Thiothrix spp. in underwater limestone caves. Geomicrobiology Journal, 12: 141-159.

EGEMEIER, S.J., 1981, Cavern development by thermal waters. NSS Bulletin, 43: 31-51.

ENGEL, A.S., 1999, Geomicrobiology of Sulfidic Karst Systems. Masters thesis, University of Cincinnati, 88 p.

GALDENZI, S., MENICHETTI, M., FORTI, P., 1997. La corrosione di placchette calcaree ad apera di acque solfuree: Dati spetimentiali in ambiente ipogeo. Proceedings of the 12th International Congress of Speleology, La-Chaux-de-Fonds, Switzerland, 1: 187-190.

GARMAN, K.M., 1999. Estimates of seepage rates of reduced, saline groundwater into the Dragon's Lai Tunnel of the Crystal Beach Spring Cave System. NSS Guidebook, Filer, Idaho, p. 58.

HILL, C., SUTHERLAND, W., TIERNEY, L., 1976. Caves of Wyoming. Geological Survey of Wyoming Bull,, 59:229 p.

HOSE, L., PISAROWICZ, J., 1999. Cueva de Villa Luz, Tobasco, Mexico: Reconnaissance study of an active sulfur spring cave and ecosystem. Journal of Cave and Karst Studies, 6: 13-21.

HUBBARD, D., HERMAN, J., BELL, P., 1990. Speleogenesis in a travertine scarp: Observations of sulfide oxidization in the subsurface, in Herman, J., and Hubbard, D., (ads.): Travertine-marl: Stream Deposits in Virginia. Virginia Division of Mineral Resources, Pub. 101. Charlottesville, VA: Dept. of Mines, Minerals, and Energy, Division of Mineral Resources: 177-184.

MALTSEV, V., KORSHYNOV, V., SEMIKOLENNYKH, A, 1997. Cave chemolithotrophic soils; Proceedings of the 12th International Congress of Speleology, La-Chaux-de-Fonds, Switzerland, 1: 29-32.

MATTISON, R., ABBIATI, M., DANDO, P., FITZSIMONS, M.,PRATT, S., SOUTHWARD, A., SOUTHWARD, E., 1998. Chemoautotrophic microbial mats in submarine caves with hydrothermal sulphidic springs at Cape Palinuro, Italy. Microbial Ecology, 35: 58-71.

PALMER, A., 1995. Geochemical models for the origin of macroscopic solution porosity in carbonate rocks, in Budd, A., Sailer, A., and. Harris, P., eds., Unconformities and Porosity in Carbonate Strata. American Association of Petroleum Geologists Memoir 63, Tulsa, Oklahoma: 77-101.

SÂRBU, S., 1996. A Chemoautotrophically Based Groundwater Ecosystem, Ph.D. thesis, University of Cincinnati, 86 p.

VLASCEANU, L., POPA, R., KINKLE, B., 1997. Characterization of Thiobacillus thioparus LV43 and its distribution in a chemoautotrophically based groundwater ecosystem. Applied and Environmental Microbiology 63:3123-3127.

VLĂSCEANU, L., SÂRBU, S., ENGEL, A.S., KINKLE, B., Acidic, cave-wall biofilms located in the Frasassi Gorge, Italy. Geomicrobiology Journal, in press.