

MICROBIAL DIVERSITY IN OZARK REGION CAVES. M. Moreno¹, M. Powers¹, V. M. McQueen¹, M. M. Kuehl¹, H. C. Ong^{1, 2} and D. J. Thomas^{1, 3}. ¹Lyon College, Science Division, 2300 Highland Road, Batesville, Arkansas, USA; ²han.ong@lyon.edu; ³david.thomas@lyon.edu.

Caves, caverns and other karst formations often have unique microbial ecosystems [1, 2]. The lack of sunlight precludes photosynthesis, except at cave openings. Most cave ecosystems are heterotrophic, and depend upon organic materials that fall in through cave openings, carried by water, or deposited by cave animals that travel to the surface. However, some cave microbes are autotrophic, and use chemosynthesis to drive biosynthetic reactions [3, 4]. Microorganisms also have been implicated in the formation of many speleothems [1, 5].

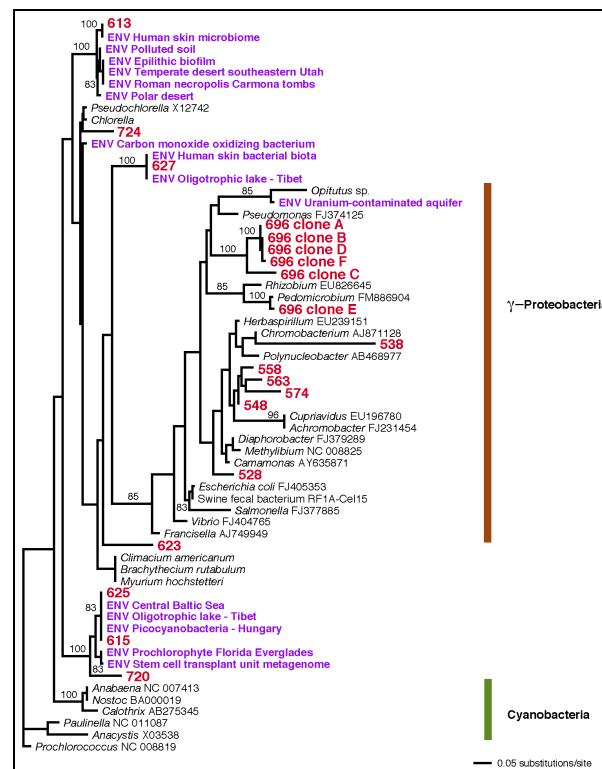
Human exploration of caves disturbs the often-fragile ecosystems. Even when extreme precautions are taken, evidence of human microbial contamination can be found [6]. In this respect, caves provide models for the forward-contamination of extraterrestrial sites by future human explorers.

Caves on other worlds – such as Mars – may provide protected sites for extraterrestrial life forms [7]. While the surface of Mars is inhospitable to life, as we know it, the subsurface could support a microbial ecosystem. Collapsed lava tube caves on Mars have been identified from orbit [8]. Intact cave systems might still support life. Additionally, fossilized speleothems could be biomarkers of extinct life [9]. Extraterrestrial caves (e.g., on Mars or the Moon) might also provide shelter for human explorers [8]. Intact caves could be sealed with airlocks and pressurized with a breathable atmosphere. Such shelters could provide relatively large living spaces that provide shielding against solar and cosmic radiation.

Cave openings provide a narrow environment in which photosynthesis occurs, but at low levels. Reduced light intensity and illumination period select for organisms that can survive in reduced-light habitats – mainly non-vascular plants and epilithic cyanobacteria and algae. This situation is analogous to a hypothetical Europa ecosystem [10] where photosynthesis might occur in transient liquid water environments within newly-formed cracks in Europa's icy surface. Although most caves have milder climates than Europa, the light regimes are similar, and the organisms must cope with light limitation in similar ways.

We report the progress of an ongoing, two-year-old project to isolate and identify photosynthetic, chemosynthetic and other microorganisms in cave environments using metagenetic and classical microbiological methods. Microbes were – and continue to be – isolated from water and soil samples in a number of Ozark caves: Bate Cave (Cord, AR), Blanchard Spring Caverns (Fifty-six, AR), Blevins Cave (Salado, AR), Blowing Cave (Cushman, AR), Bone Cave (Batesville, AR), Cave Point Cave (Pleasant Grove, AR), Indian Rocks Caves (Fairfield Bay, AR), Logan Point Cave (Pleasant Grove, AR), Meacham Cave (Batesville, AR), and Tumbling Creek Cave (Protom, MO). Samples were either plated for culture-dependent DNA isolation and sequencing, or subjected to culture-independent procedures, which involve isolating total DNA from raw samples, PCR-amplifying 16S and/or 23S rRNA sequences,

cloning the amplified products, and sequencing all unique clones. Identifications by DNA sequences were confirmed with microscopic observations and phylogenetic analyses. Cyanobacterial, eubacterial and algal sequences were successfully isolated by culture-dependent and -independent methods, and will continue to be sequenced and monitored for spatial-temporal distributions.



16S rRNA phylogenetic analysis of eubacteria and cyanobacteria. The maximum likelihood phylogenetic analysis was performed using PAUP v. 4.0b10. The tree was estimated using the HKY85 substitution model, set transition to transversion ratio and approximated gamma distribution. All trees were swapped by random stepwise addition with tree-bisection-reconnection (TBR) branch swapping. Bootstrap values were obtained using the same HKY85 substitution model, conducted for 100 replicates. Only bootstrap values greater than 70% are shown. Numbered strains (red) are cave isolates. Known cultured and environmental isolates (purple) are provided for reference.

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