VARIATIONS IN BACTERIAL COMMUNITY STRUCTURE ON TEN DIFFERENT SPELEOTHEMS IN KARTCHNER CAVERNS, ARIZONA

A. Legatzki1*, M. Ortiz1, J. W. Neilson1, R. R. Casavant2, B. M. Pryor3, L. S. Pierson III1, and R. M. Maier1

1Department of Soil, Water, and Environmental Science, University of Arizona, Tucson, AZ, USA (* correspondence: legatzki@email.arizona.edu)
2Arizona State Parks, Phoenix, AZ, USA
3Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson, AZ, USA

Abstract

Kartchner Caverns is a 3.9 km long wet living carbonate cave in southwestern USA near Benson, Arizona. The cave represents an oligotrophic environment with high humidity (average 99.4%) and elevated CO2 [1]. Because of its unique geology, Kartchner Caverns contains minerals from six different chemical classes: carbonates, sulfates, oxides, nitrates, silicates and phosphates, and is considered as one of the top ten caves in the world in terms of mineral diversity [2]. Furthermore Kartchner is also characterized by its variety of speleothems (secondary mineral deposits). In 2006, the cave was added to the National Science Foundation’s Microbial Observatory Program. One goal of our studies in Kartchner is to characterize the heterogeneity of bacterial communities on speleothems. The objective of this study was to explore both, intra- and inter-speleothem variability in the bacterial community structure. Ten different formations located in a single cave room within an area of approx. 10 m (length) x 2 m (width) were examined. A chemical elemental profile of a surface sample scraped from each formation was performed using ICP-MS analysis. The analysis revealed differences in the elemental content of the ten formations. Bacterial DNA community fingerprints were generated from each speleothem using DGGE analysis of PCR amplified 16S rRNA gene fragments. The intra-speleothem analysis revealed that the community profiles from each speleothem using DGGE analysis of PCR amplified 16S rRNA gene fragments were more similar to each other than to profiles from different speleothems. For the inter-speleothem analysis, bacterial community clusters were observed which appear to be influenced by the spatial location of the formation in the room.

Methodology

Characterization of bacterial community structure

Three (study 1) or six (study 2) cotton swabs (30 cm² area per swab) per sample were taken from each of the formations analyzed. Total genomic DNA was isolated by phenol-chloroform extraction. A 336 bp bacterial 16S rDNA gene fragment (incl. V3/V7) was amplified with primers 1076IF and 1406RGC [3] and DGGE analysis (7% acrylamide, 45-65% urea-formamide gradient) was performed. DGGE community profiles were analyzed with Quantity One 4.6.2 software.

Physical and chemical characterization

The color of the formations was determined by Minolta CR-200 Chroma Meter in Munsell colors, and varied from 0.016% in formation F1 to 0.033% in formation A. Calcium was a major element of the formations comprising 39-52% of the total weight. The other elements were measured in trace concentrations.

Conclusions

Study 1: The bacterial community structure from samples taken along the vertical axis of the same speleothem were more similar to each other than to those from different speleothems.

Study 2: From the tested environmental variables (organic carbon, Ca, Fe, P, Zn, Co, Mg, and relative location) only the relative location influenced the bacterial community structure of the ten formations significantly. Drip lines might have an influence on the bacterial community structure of cave formations.

References


Acknowledgements

This research is funded by Microbial Observatory Grant 06CRB064300 from the National Science Foundation. We thank Dr. C. Raunusse and Dr. M. Melding from the University of Arizona, SWES department for help with the color measurements and Dr. M. Palmer from the Oklahoma State University, Department of Botany for discussions about the statistical analyses.

Fig. 1: a) picture of all ten formations (9 stalactites + 1 busea) including their colors (Munsell color system) and b) a map with the relative location of the formations and potential drip lines (DL).

Fig. 2: a) the sampling locations along stalactite F (F1-F5) for study 1, and b) DCA of bacterial DGGE band profiles from four samples from stalactitic W and five samples from stalactite F and H, respectively. The samples were taken along the length (from top to tip) of the stalactites.

Fig. 3: a) Principle component analysis (PCA) of surface material from all ten formations analyzed by organic carbon and elemental content. The colors of the symbols are based on their determined Munsell colors, and b) bacterial DGGE band profiles of the ten formations. Lane L is a DGGE ladder prepared from cultured cave bacteria.

Fig. 4: CCA (canonical correspondence analysis) of bacterial DGGE band profiles of the ten formations with selected environmental variables.

Fig. 5: CCA (canonical correspondence analysis) of bacterial DGGE band profiles of the ten formations with selected environmental variables.

Fig. 6: DCA (detrending correspondence analysis) of bacterial DGGE band profiles of the ten formations with selected environmental variables.