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

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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 CO_2 [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 element 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 the same formation are 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.

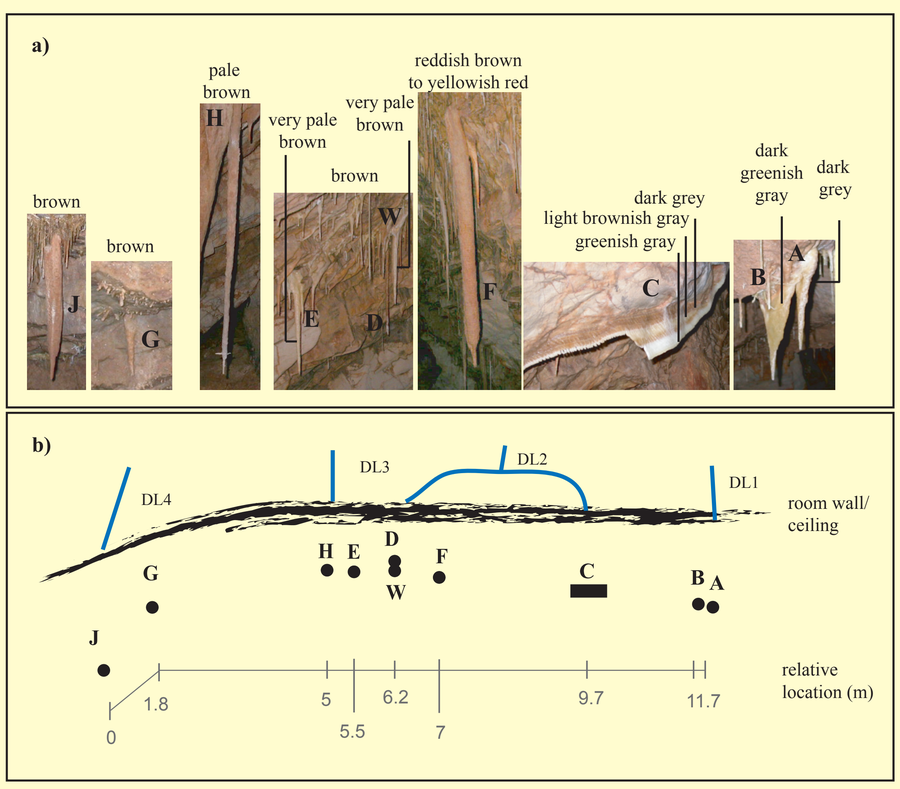

Fig. 1: Shows **a)** pictures of all ten formations (9 stalactites + 1 bacon) including their colors (Munsell color system) and **b)** a map with the relative location of the formations and potential drip lines (DL).

Methodology

Characterization of bacterial community structure

Three (study 1) or six (study 2) cotton swabs (30 cm^2 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 rRNA gene fragment (incl. V7/V8) was amplified with primers 1070F and 1406R-GC [3] and DGGE analysis (7% acrylamide, 45-65% urea-formamide gradient) was performed. DGGE community profiles were analyzed with Quantity One ® 4.5.2 software.

Physical and chemical characterization

The color of the formations was determined by Minolta CR-200 Chroma Meter in Munsell color system. Organic carbon concentration was detected manometrically after dissolving of 350 – 500 mg surface material in 3 N HCl. Elemental analysis was performed by ICP-MS of approx. 200 mg surface material after digestion with concentrated HNO_3 [4].

Statistical analysis

Statistical analyses were performed using Canoco for Windows 4.5 (http://www.canoco.com).

References

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[2] Hill & Forti (1997) *2nd Edition of cave minerals of the world.* 350-354.
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Study 1: Is the superficial bacterial community structure speleothem-specific?

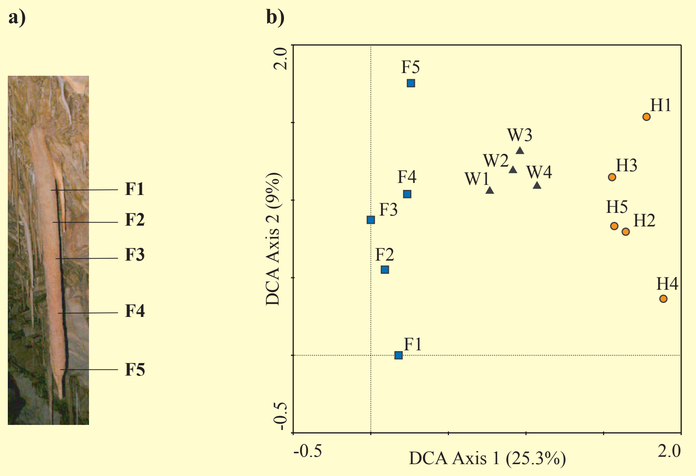

Fig. 2: Shows **a)** the sampling locations along stalactite F (F1-F5) for study 1, and **b)** DCA of bacterial DGGE band profiles from four samples from stalactite W and five samples from stalactite F and H, respectively. The samples were taken along the length (from top to the tip) of the stalactites.

DCA (detrending correspondence analysis)**:** For three speleothems studied samples from the same speleothem clustered together along the first axis and indicating a smaller difference in community structure between samples from the same speleothem compared to samples from one of the other two speleothems.

The distribution of the sample points along the second axis (explains only 9% of the variation) indicates variation among communities sampled from the same speleothem. There is more variation in speleothem F and H than in W communities.

Could this correspond to the fact that speleothem F (119 cm) and H (117 cm) are more than twice as long than speleothem W (41 cm)?

CCA (canonical correspondence analysis)**:** confirmed the hypothesis that sample origin (meaning stalactite F, W, or H) had a significant effect on the bacterial community structure ($p = 0.001$).

Study 2: Do selected chemical or physical properties of the ten formations effect their superficial bacterial community structure?

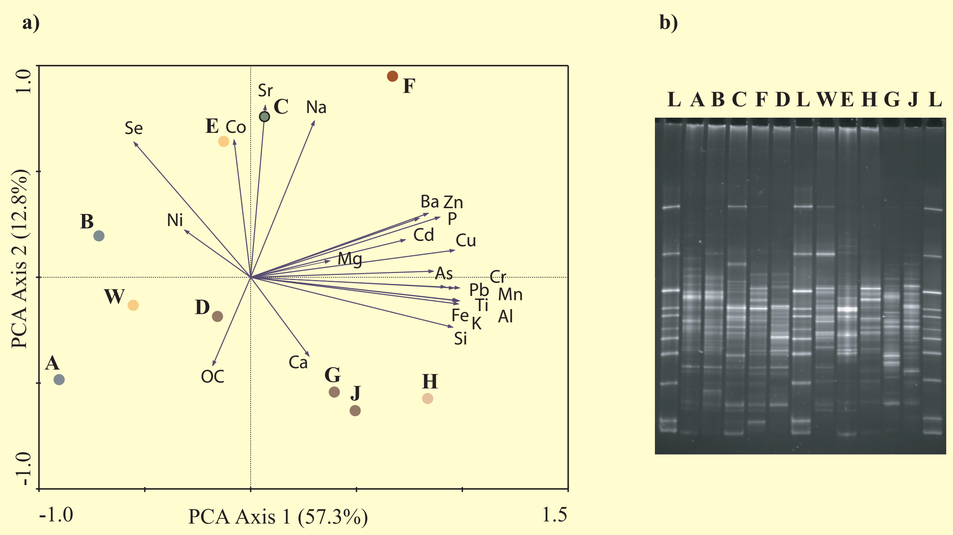

Fig. 3: Shows **a)** Principle component analysis (PCA) of surface material from all ten formations analyzed by organic carbon and elemental content. The colors of the symbols are based on their determined Munsell colors, and **b)** bacterial DGGE band profiles of the ten formations. Lane L is a DGGE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

Chemical profiles (organic carbon and elements) as well as bacterial community structure profiles were generated for each of the ten formations. The organic carbon concentration for all formations was low and varied from 0.016% in formation F 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 concentations.

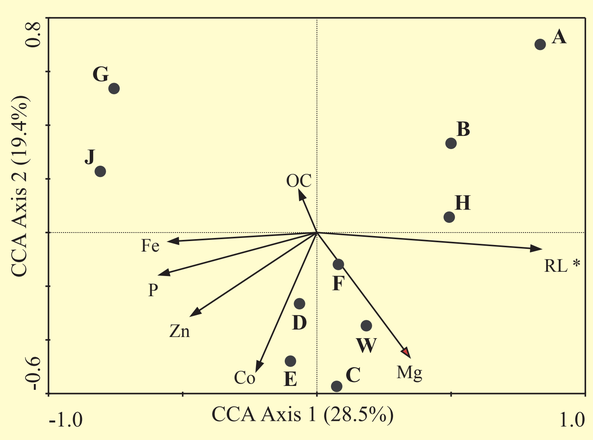

Fig. 5: CCA of bacterial DGGE band profiles for all ten formations with selected environmental variables.

Environmental variables selected as most important were tested for their influence on the bacterial community structures of the ten formations. These included: organic carbon, Fe, P, Zn, Co, Mg, and the relative location (RL). All chemical variables selected are known to play a role in general metabolic processes of bacteria. CCA revealed that relative location was the only variable that affected the bacterial community structure of the formations significantly (*), ($p = 0.005$).

These results led to an evaluation of the physical structure of the cave room. An unique observation were the patterns and lines of soda straws (typically indicating drip lines of water entering the cave) along the cave ceiling in close approximaty to the ten formations (Fig. 1b).

CCA was used to test the hypothesis that the suggested drip lines have an influence on the bacterial community structure of the ten formations. The drip lines influenced the bacterial community structure significantly ($p = 0.001$).

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.

Please visit also our poster "Pyrosequencing reveals the diversity and variability of bacterial communities on speleothem surfaces in Kartchner Caverns" - presented by Marian Ortiz