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Ecophysiological groups of bacteria from cave sediments as potential indicators of paleoclimate

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ABSTRACT

Six bacterial ecophysiological groups from Pleistocene sediment samples collected from two Romanian caves were identified and evaluated for their potential contribution to the interpretation of paleoclimate. Denitrifiers and nitrite oxidizers were present in the culture plates of all samples, the ammonia oxidizers were absent, and the iron-reducers were confirmed solely in the deepest sample of one of the caves. The aerobic mesophilic heterotrophs were much less abundant in sediments with high clay content, while the ammonifiers were positively correlated to the organic matter content measured in the sediments. In both caves optically stimulated luminescence (OSL) dating, geochemical, sedimentological and grain size analyses were performed and results were correlated to the different groups of bacteria. Results point to the importance of surface conditions during the time of sediment inflow inside the caves, although the bacterial communities have continuously been shaped since deposition. The present study aims to connect features of the paleoclimate with preliminary data on bacteria from old cave sediments.

1. Introduction

Sediments which exhibit limited vertical mixing, such as clay-rich anoxic deposits, and with slow microbial evolution provide favorable conditions for paleoenvironmental reconstruction (Dong et al., 2010). Such sediments can be found in caves, typically populated by microorganisms that have adapted to extremely resource-limited environments (Fredrickson et al., 1995), through different mechanisms, such as slow metabolism and slow growth. Moreover, in many regions sediment accumulations in rock shelters and caves are some of the thickest available continental records, therefore holding the potential for characterizing past landscapes and their evolution (Bertran et al., 2008). When successfully dated, cave sediments may provide valuable paleoclimatic and paleo-environmental information proxies, such as their geochemistry, mineralogy, sedimentology, and environmental magnetism (Sasowsky and Mylroie, 2004; White, 2007; Moldovan et al., 2011; Constantin et al., 2014).

Microbe-derived organic matter can become a major portion of the total organic matter that remains in sediments, and can be used as proxy for paleoclimatic records (Meyers, 1997). Viable heterotrophs were discovered in the Neogene permafrost sediment in Central Yakutia (Zhang et al., 2013), while Christner et al. (2003) demonstrated that ice sheets up to 750,000 years old have the potential to contain viable bacteria. Microorganisms of ocean sediments catalyze ongoing processes that have demonstrated to affect Earth's surface and even global climate, a large fraction of the sub-seafloor prokaryotes being represented by living bacteria found in very old (16 Ma) and deep (>400 m) sediments (Schippers et al., 2005). Inagaki et al. (2001) discovered microbial relicts in sediments older than 2 Ma in the West Philippine Basin, and used the results to reconstruct past environmental conditions and past volcanic activity in the area. Inagaki et al. (2005) observed that the microbial community of a core sample of black shale was consistent with the inferred environmental conditions, and it could be used for reconstructing global environments during the Cretaceous. Weijers et al. (2009) and Nieman et al. (2012) used bacterial specific membrane lipids (GDGT) from microorganisms of lake sediments as a paleoenvironmental proxy in lakes. The distribution of the bacterial membrane lipids in lake sediments can be linked to the paleotemperature, climate

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humidity, and soil pH variations for applications in paleoclimatic studies.

Subsurface microorganisms are often anaerobic chemolithotrophs, very distinct from the surface ones (Dong and Yu, 2007) primarily because of the oligotrophic and dark space they live in. Microbial activity may influence various environmental factors including energy source, donors and acceptors of electrons, nutrients and temperature, thereby affecting their capacity of adaptation to the environment (Vieira et al., 2007). Environmental fluctuations cause modifications in species abundance and community structure (Judd et al., 2006), some microorganisms remaining metabolically active, others surviving critical changes through dormancy stages like endospores do (Nicholson et al., 2000). Organic matter fluctuations will affect community composition, but strong changes in the microbial community structure depend on the dormant species able to use the new organic matter input (Judd et al., 2006). Subsurface environments can severely limit the supply that a microorganism receives. Therefore, even minor perturbations in physicochemical properties of subsurface sediments can lead to major changes in the activity and composition of the microbial community (Brockman et al., 1998). Consequently, microorganisms of cave environments can reflect nutrient fluctuations and thereby environmental changes.

The present study explores the possibility of using microbial communities of cave sediments in paleoenvironmental researches. Previous investigations on microorganisms in the studied caves used the molecular methods and revealed the presence of microbial DNA solely in the more recent layers, while for the deeper and older layers no DNA was amplified (Epure et al., 2014). This study proposes a simple, relatively rapid and cost-effective method of sediment screening in order to obtain preliminary data on the bacterial activity that may be used in paleoenvironmental assessment.

2. Materials and methods

The sediment samples were collected from two caves located in the Western Carpathians of Transylvania (Romania) (Fig. 1a): *Peştera cu Apă din Valea Leşului* (Lesu; N 46° 49′ 528″/E 22° 33′ 419″; 650 m altitude; 800 m length; Fig. 1b), which is crossed by a stream with silty-sandy sediments, deposited along the main gallery, in an overbank environment, and *Peştera Urşilor de la Chişcău* (Ursi; N 46° 33′ 14.23″/E 22° 34′ 10.07″; 428 m altitude; 1500 m length; Fig. 1c) that has two levels, with the lower one preserved as a scientific reserve, and drained by a subterranean stream that cuts its overbank silty-clay sediments. The mean annual air temperature inside both caves ranges between 8.5 and 10 °C.

2.1. Sampling and protocol for microbiological analysis

A total of 6 samples were aseptically collected from different vertical depths (Fig. 2) and 50 cm in depth from the side face of the sediment block, by sidewall coring. The analyzed sediment samples consisted of the last 3–4 cm of the 10 cm-long cores. The following samples were obtained: LC60: 55 – 60 cm depth, LC75: 70 – 75 cm depth, LC105: 100 – 105 cm depth (Lesu), and UC70: 65 – 70 cm depth, UC170: 165 – 170 cm depth and UC210: 205 – 210 cm depth (Ursi). Samples were preserved on ice during transportation to the laboratory, and then maintained at 4 °C until processed.

Samples were subjected to bacteriological analyses. Six ecophysiological groups of bacteria were determined. The aerobic mesophilic heterotrophs (AMH) were cultured in nutrient agar plates (Atlas, 2010). For seeding the culture environment, dilutions of 10^{-3}, 10^{-4} and 10^{-5} were prepared for each sample. Three plates were prepared for each dilution and were inoculated under the flow hood with 1 ml of each dilution. The protocol was repeated for all six samples. Incubations were carried out at 27 °C for 2 days and the colonies were counted after 24 and 48 h, respectively. 10 ml of liquid culture medium was prepared in tubes for determining the number of ammonifiers (AM) (in alkaline peptone water; Atlas, 2010) and denitrifiers (DN) (Pochon, 1954), respectively; 9 ml of liquid culture medium were prepared in each tube for determining the number of iron-reducers (IR) (Lu et al., 2008), whereas the ammonia oxidizers (AO) and nitrite oxidizers (NiO) were cultured in tubes containing 2 ml of liquid culture medium (Atlas, 2010). The tubes containing the growth media were then autoclaved. For seeding the culture environment, decimal dilutions of 10^{-3}, 10^{-4} and 10^{-5} were also prepared. Blank controls were included for each sample. 1 ml of each dilution was then inoculated into five tubes for each medium. The protocol was repeated for all six samples. Tubes were then incubated at 22 °C for 10 days. Except for the AMH (where the method of successive dilutions was used), the most probable number of bacteria was calculated according to the statistical table of Alexander (1965).

2.2. Sampling and analytical protocol for geochemical analyses

The geochemical analyses were performed on samples collected at 5 (Ursi) and 10 (Lesu) cm intervals from freshly exposed surfaces, after digging a ~20 cm deep sampling trench. 500 mg of each sample was grinded using an agate mortar and analyzed by X-Ray Fluorescence (XRF), with a Horiba XGT-7000 X-ray Analytical Microscope (technical specifications: X-ray tube-Rh target, tube voltage: 50 kV, tube current: 1 mA; fluorescent X-ray detector: Peltier-cooled Silicon Drift Detector (SDD); transmitted X-ray detector: NaI(Tl) scintillator; X-ray guide tube: monocapillary 10 μm/100 μm without filter). Elemental peaks were automatically identified and quantitative analyses were sensitive at ppm level.

Loss on Ignition (LOI) analyses were performed by subjecting the samples to the following methodology: drying in oven for 4 h at 60 °C; grinding; drying in oven for 3 h at 105 °C; thermal treatment in furnace for 6 h at 550 °C (assessment of organic matter-gas and interstitial water content (OM)) and, subsequently, for 3 h at 950 °C (assessment of inorganic CO_2 content and other gases). The material lost at 550 °C and 950 °C represents the LOI value.

2.3. Sedimentological analysis

Grain size measurements on fine sediment were performed by treating ~5 g of the bulk sample, for 14 days, in a plastic box, with ~0.4 ml of a 1% solution of $Na(PO_3)n$, $n \approx 25$ - Graham's salt (Merck). A quantity of ~2.5 g sample was later extracted from the box and treated again with ~0.2 ml of 2% solution of Graham's salt. Each sample was analyzed on a HORIBA Partica LA-950V2 laser scattering particle size distribution analyzer to reveal grain size fractions. The coarser samples were analyzed by vibrating dry sieving of ~100 g of the bulk sample and weighing the sediment quantity retained on each sieve, on an OHAUS Scout digital balance, down to the 500 μm fraction, which was subjected to the same procedure as the fine samples. Calculations and plots were done using the GRADISTAT 8 software (Blott, 2010); we applied the Folk and Ward (1957) method, and logarithmic statistics.

Sediments from both caves have been analyzed in terms of thickness, grain size and internal structures of depositional units. Grain size analysis was done macroscopically for the fraction>2 mm and with a HORIBA laser machine for the fraction<2 mm. The granulometric scale uses the typical ranges as follows: gravel > 2 mm, sand (2–0.063 mm), silt (0.063–0.002 mm) and clay < 0.002 mm.

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