COMPARISON OF BACTERIAL AND ARCHAEAL COMMUNITIES FROM DIFFERENT HABITATS OF THE HYPOGENIC MOLNÁR JÁNOS CAVE OF THE BUDA THERMAL KARST SYSTEM (HUNGARY)

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Abstract

The Molnár János Cave is part of the northern discharge area of the Buda Thermal Karst System, and is the largest active thermal water cave in the capital of Hungary. To compare the prokaryotic communities, reddish-brown cave wall biofilm, black biogeochemical layers, and thermal water samples from the phreatic mixing zone of the cave were subjected to three investigative approaches, scanning electron microscopy, cultivation, and molecular cloning. According to the SEM images, multilayer network structures were observed in the biofilm formed by iron-accumulating filamentous bacteria and mineral crystals. Cultivated strains belonging to Aeromonadaceae and Enterobacteriaceae were characteristic from both water and subaqueous biofilm samples. The most abundant molecular clones were representatives of the phylum Chloroflexi in the reddish-brown biofilm, the class Gammaproteobacteria in the black biogeochemical layer, and *Thiobacillus* (Betaproteobacteria) in the thermal water samples. The reddish-brown biofilm and black biogeochemical layer's bacterial communities proved to be somewhat more diverse than that of the thermal water. The archaeal 16S rRNA gene clone libraries were dominated by thermophilic ammonia-oxidizer *Nitrosopumilus* and *Nitrososphaera* phylotypes in all three habitats. Considering the metabolic characteristics of known species related to the detected clones, it can be assumed that these communities may participate in the local sulfur and nitrogen cycles and may contribute to microbial mediated sulfuric acid speleogenesis.

Introduction

Under the residential area of Budapest, the capital of Hungary, extensive hypogenic cave systems can be found (Leél-Őssy, 1995). They are part of the Buda Thermal Karst System at the northeastern margin of the Transdanubian Central Range (Fig. 1). In addition, they are located on the border of confined and unconfined carbonate aquifers that serve as the discharge zone of thermal water with different origins and temperatures (Mádl-Szőnyi and Tóth, 2015). In the system, a southern (Gellért Hill) and a northern subsystem (Rózsadomb) have been separated in terms of the flow systems and types of discharged water. Furthermore, major differences can also be observed in the cave-formation processes (Erőss et al., 2012).

Molnár János Cave belongs to the northern (Rózsadomb) discharge area (Fig. 1), and it is the largest active thermal water cave in Europe (Goldscheider et al., 2010). Discharges of lukewarm (20–35°C) and warm to hot (40–65°C) springs are characteristic for this area, and corrosion proved to be the dominant cave forming process (Erőss et al., 2012). The lukewarm springs tend to have lower than 1000 mg L^{-1} total dissolved solids, while the hot waters contain more than 1200 mg L^{-1} (Papp, 1942). Bodor et al. (2015) proved that in the Rózsadomb area infiltration from precipitation does not affect the physical and chemical parameters of the discharged waters. First Erőss et al. (2008) published the characteristic chemical data of the cave's spring water (pH: 6.94; T: 21.7°C; EC: 932 μS cm^{-1}), and later Bodor et al. (2014) found that the temporal variability of the parameters of the spring cave water was negligible.

The chemical characteristics of such hypogenic cave waters are influenced by the type of host rock and the composition of the released gases (Kumaresan et al., 2014). In hypogenic cave systems, microbes can be found in different mineral deposits, streams, snottites, or on cave walls and corrosion residues. Based on the 16S rRNA gene-sequence analysis, members of the phylum Proteobacteria are frequently the most abundant in these habitats. Sulfur-oxidizing bacteria belonging to the classes of Beta-, Gamma- and Epsilonproteobacteria can play an important role in sulfuric acid speleogenesis (Engel, 2011).

In the speleogenesis of Molnár János Cave, microbiologically enforced corrosion was also assumed (Erőss, 2010; Borsodi et al., 2012). In a preliminary study, Firmicutes-related aerobic and anaerobic bacteria, probably participating in local iron and sulfur transformations, were found to be predominant besides Proteobacteria in the iron-rich reddish-brown biofilm formed on the cave wall of the cave (Borsodi et al., 2012).

In the Molnár János Cave, prokaryotes inhabit different habitats, such as the cave walls, where reddish-brown subaqueous biofilm developed, and the black biogeochemical layer can be observed at distinct places on the cave walls under phreatic conditions and in the cavities of the cave, which is almost completely filled with thermal water. As the

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Figure 1. The location of Buda Thermal Karst and Rózsadomb in the Transdanubian Range, Hungary.
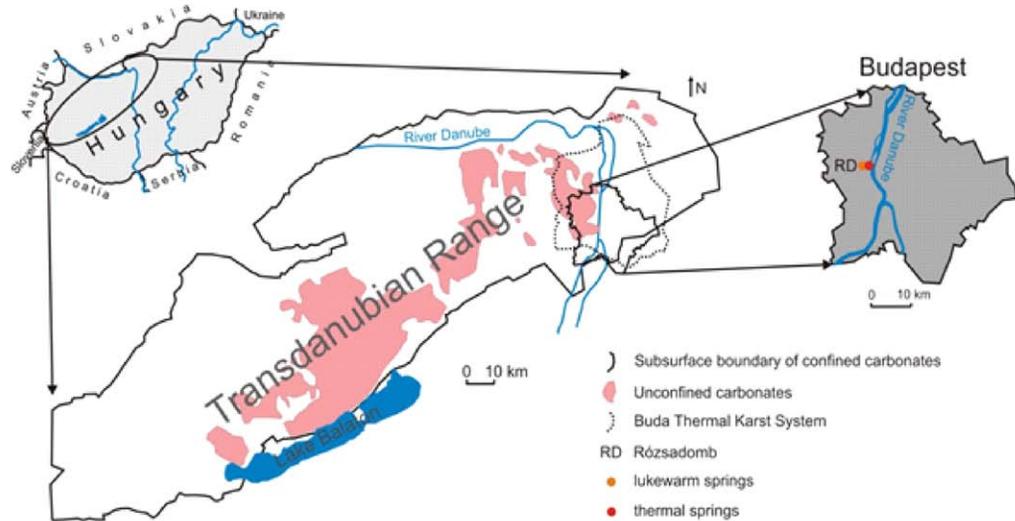
cave is located under a heavily populated area of the capital of Hungary, anthropogenic effects may also have an influence on the composition of the cave microbiota.

The aim of the present research was to gain information about the structure of the prokaryotic communities inhabiting the thermal water, the subaqueous biofilm, and black biogeochemical layers found in the Molnár János Cave mixing zone. To get a more detailed picture about the morphological and genetic diversity of bacterial and archaeal communities, SEM, cultivation, and molecular cloning methods were applied.

Materials and Methods

Cave Description and Sampling

The caves of the Rózsadomb are located in the northern part of the Buda Thermal Karst System (Fig. 1). Chambers can be found mostly at the boundary of the Szépvölgy Limestone and the Buda Marl, and the most frequent minerals are calcite ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) (Leél-Őssy, 1995). Currently, the Molnár János Cave is nearly 6 km in length, and the explored maximum depth of the cave is 100 meters under the Rózsadomb (Erőss, 2010). The host rock of the cave is marl, sometimes with considerable (up to 80%) carbonate content. Considering the increasing number of cave divers that regularly visit the Molnár János Cave, the direct human impact on the cave ecology cannot be negligible. The temperature of the cave's water is 23–26°C near the surface, and ~19°C at 25–35 m depth in the cave due to the mixing of thermal and lukewarm components (Erőss et al., 2008).

Samples for the microbiological examinations were collected from the Molnár János Cave in December 2012. The sampling process was conducted in collaboration with divers. Submerged reddish-brown biofilm (MJB) and black biogeochemical layer (MJD) samples were collected with the help of sterile scalpels, while thermal water (MJW) filling the cave was collected using sterile bottles. According to the divers, the black biogeochemical layers are sporadically observed throughout the whole submerged cave system. At the time of sampling, the measured water temperature, pH, and conductivity values were 20.1°C, 6.9 and 1006 µS cm^{-1}, respectively. Relatively high sulfate (121 mg L^{-1}) and sulfide (6.9 mg L^{-1}) concentrations were measured in the cave's water; similar to the values reported by Anda et al. (2015). The total organic carbon and the total nitrogen concentrations of the samples were 1.4 and 1.8 mg L^{-1}. Concentrations of NH_4^+-N, NO_3^--N, and NO_2^--N were less than 0.01, 1.7, and 0.007 mg L^{-1}, respectively. Other detected physical and chemical parameters are reported by Dobosy et al. (2015).

Scanning Electron Microscopy

For scanning electron microscopy, mucilaginous reddish-brown biofilm samples were filtered onto 0.2 µm polycarbonate filters (Millipore). All subsequent steps were the same as previously described (Anda et al., 2014, 2015).

Cultivation

For cultivation, we used tenfold serial dilutions from the biofilm and thermal-water samples using sterile water and then plated onto R2A (DSMZ Medium 830) and *Sphaerotilus-Leptothrix* ferrous sulfate (DSMZ Medium 803) media (www.dsmz.de/?id=441). The R2A medium was chosen because, like cave environments, it also contains relatively small amounts of organic compounds as compared to other media. It provides the possibility for the cultivation of slow-growing oligotrophic bacteria. The ferrous sulfate medium was chosen for cultivation because results of the previous

studies indicated that bacterial iron oxidation may play an important role in the speleogenesis of the Buda Thermal Karst System (Borsodi et al., 2012; Anda et al., 2014, 2015). Following a 14-day incubation at 20°C, discrete bacterial colonies with different morphology were isolated.

Bacterial DNA Extraction and PCR Amplification

The community DNA was isolated using Ultra Clean Soil Kit (MO Bio Inc., CA, USA) according to the manufacturer's instructions, checked in agarose gel (1%) stained with ECO Safe Nucleic Acid Staining Solution (Avegene, Taiwan), and visualized by UV excitation. DNA of pure cultures was isolated using G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, South Korea) according to the manufacturer's instructions. The primers and temperature protocol used for bacterial PCR and the construction of 16S rRNA clone libraries was the same as previously applied by Anda et al. (2015).

Identification of Bacterial Strains and Molecular Clones

PCR products were grouped according to their Amplified Ribosomal DNA Restriction Analysis pattern produced with enzymes *Msp*I and *Bsu*RI (Fermentas, Lithuania) as described by Anda et al. (2015). The identification of ARDRA group and sequencing reaction were performed as previously described by Anda et al. (2015). Taxonomic relationships of the sequences were determined using the EzTaxon database (Kim et al., 2012) and Basic Local Alignment and Search Tool (BLAST) program (Altschul et al., 1997).

The 16S rRNA gene sequences (on average 800 to 900 bp long) were submitted to the GenBank under accession numbers LN998831-LN998895 for the MJBB (Molnár János reddish-brown biofilm Bacteria) clones, LN998896-LN998926 for the MJDB (Molnár János black biogeochemical layer Bacteria) clones, LN998927-LN998931 for the MJWB (Molnár János thermal water Bacteria) clones, LN998932-LN998939 for the MJBA (Molnár János reddish-brown Archaea) clones, LN998940-LN998947 for the MJDA (Molnár János black biogeochemical layer Archaea) clones, LN998948-LN998960 for the MJWA (Molnár János thermal water Archaea) clones, LN998961-LN998974 for the MJB (Molnár János reddish-brown biofilm) strains, and LN998975-LN998982 for the MJW (Molnár János thermal water) strains.

Results

Scanning Electron Microscopic Observations

The SEM analysis (Fig. 2A-F) revealed a multilayer network architecture of microbial biofilm formed by filamentous bacteria and other cells, together with mineral crystals and organic matter matrix. The structurally complex biofilm was ordered in layers (yellow arrows) and interconnected (red arrows) with filamentous bacteria together with EPS (Fig. 2C). Intertwined filamentous bacteria similar to groundwater-fed, iron-rich microbial mats (Schieber and Glamoclija, 2007) and wastewater biofilms (Felföldi et al., 2015) were also observed. On some micrographs, taken with the vCD detector, very small spheroid shaped precipitates (Fig. 2C-D) and filamentous bacteria (Fig. 2F) can be seen much brighter (white) compared to their environment. The brightness means that the precipitates carried particles containing elements of higher atomic number, such as heavy metals. The elements of greater atomic number may indicate iron compounds accumulated by microbes as previously shown by EDX analysis in an electron microscopic study on the iron-rich biofilm of the Rudas-Török spring cave in the Buda Thermal Karst (Borsodi et al., 2012). It is also apparent that some parts of the biofilm were covered by a transparent substance consisting of elements of small atomic number (C, H, O, N) that could be the residue of the biofilm extracellular polymeric substance. The EPS covered surface of filamentous forms is clearly visible in the greater magnification of Figure 2E.

Cultivation-Based Bacterial Diversity

Altogether 66 bacterial strains were isolated from the biofilm (MJB) and water (MJW) samples using two different media (Table 1). The bacterial strains were identified as representatives of phyla Proteobacteria, Firmicutes, and Actinobacteria based on 16S rRNA analysis. From the ferrous sulfate and R2A media 9 and 8 genera were identified, respectively (Table 1). Among them representatives of the genus *Aeromonas* were found on medium R2A from the MJB sample. In the case of the MJW sample, representatives of the genera *Raoultella*, *Enterobacter*, and *Citrobacter* (Enterobacteriaceae) were cultivated from both media. Among the bacterial strains, representatives of the genus *Brevibacillus* (Firmicutes) and genera *Janibacter* and *Micrococcus* (Actinobacteria) were also cultivated from the MJB sample.

Molecular Clones of Bacteria

The bacterial 16S rRNA gene based clone libraries containing 200 members were created from each of the three samples. Different numbers of phyla were detected per sample: 3 from the water (MJWB), 6 from the black biogeochemical layer (MJDB), and 9 from the reddish-brown biofilm (MJBB). The relatives of the following phyla and classes were found Betaproteobacteria, Gammaproteobacteria, Nitrospirae, Chloroflexi, Firmicutes, Deltaproteobacteria, Ac-

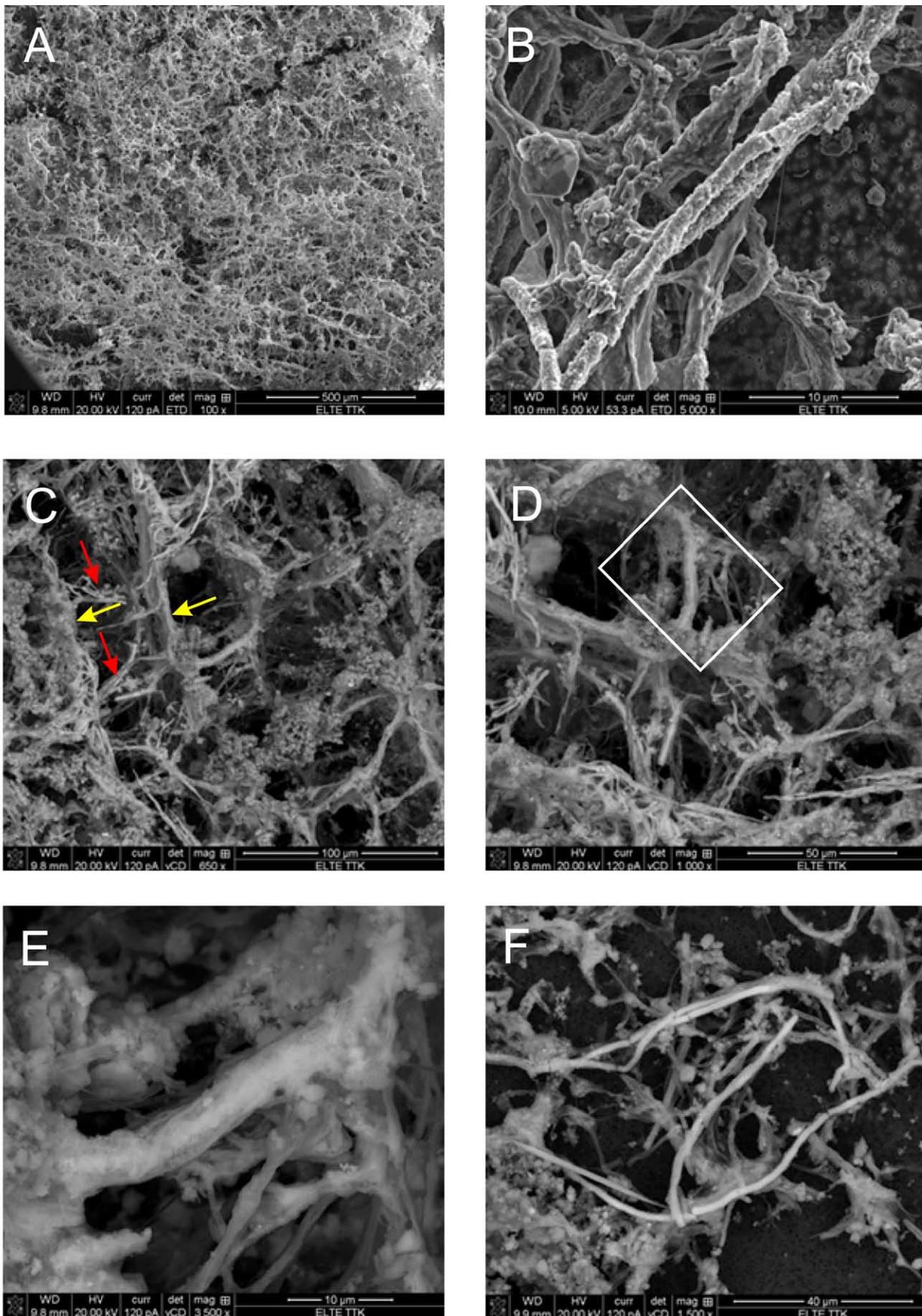D. Anda, G. Krett, J. Makk, K. Márialigeti, J. Mádl-Szőnyi, and A. K. Borsodi.

Figure 2. Scanning electron micrographs of naturally grown biofilm samples from Molnár János Cave. Scale bar (A) 500 μm, (C) 100 μm, (D) 50 μm, (F) 40 μm, (B, E) 10 μm.

idobacteria, Alphaproteobacteria, Actinobacteria, Epsilonproteobacteria, Gemmatimonadetes, Spirochaetes, and Verrucomicrobia (Fig. 3). Among them representatives of the class Betaproteobacteria and the phylum Nitrospirae could be identified in all the three samples.

The MJWB clone library was dominated (96.7%) by phylotypes belonging to the class Betaproteobacteria. These molecular clones showed the highest sequence similarity (95.42%) to the genus *Thiobacillus*. In the MJBB clone library, representatives of the phyla Chloroflexi (33.6%) were the most abundant. Within this phylum, phylotypes (12.5%) related to the order Anaerolineales were identified. In the MJDB clone library, the dominance (29.1%) of the genus *Methylohalomonas*-related (Gammaproteobacteria) clones was found. The second most abundant group of the molecular clones showed the highest sequence matching (95.88% and 95.33%) with the widespread species of *Nitrospira moscoviensis* (Ehrich et al., 1995) and *Nitrospira calida* (Lebedeva et al., 2011).

Figure 3. Taxonomic classification of 16S rRNA gene sequences of the bacterial clone libraries from the Molnár János Cave.
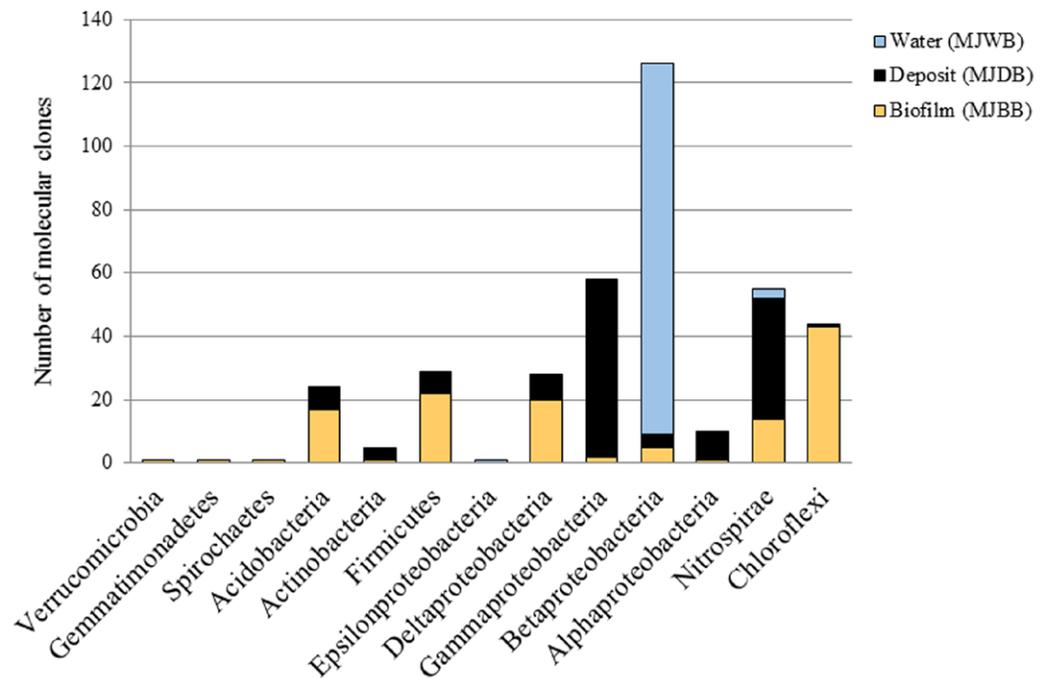
Molecular Clones of Archaea

Three archaeal 16S rRNA clone libraries were also constructed from the reddish-brown biofilm (MJBA), the black biogeochemical layer (MJDA), and the water (MJWA) of Molnár János Cave. Each clone library contained 100 members. The 16S rRNA gene sequences of the representative clones belonged to the phyla Euryarchaeota and Thaumarchaeota (Fig. 4). In all three libraries, the phylum Thaumarchaeota (MJWA: 68.2%, MJBA: 97.85%, MJDA: 100%) was the most abundant. Members of the phylum Euryarchaeota were only found in the case of the MJBA (2.15%) and MJWA (31.8%) samples. In the MJWA clone library, phylotypes were closely related to the "*Candidatus* Nitrososphaera gargensis" and *Nitrososphaera viennensis* (with 97.36% and 97.79% sequence similarities).

In both clone libraries (MJBA, MJDA) from the surface associated samples, representatives of "*Candidatus* Nitrososphaera gargensis" (MJBA: 35.48%, MJDA: 16.7%), *Nitrososphaera viennensis* (Stieglmeier et al., 2014) (MJBA: 32.3%, MJDA: 46.6%), and "*Candidatus* Nitrosoarchaeum limnia" (MJBA: 29.03%, MJDA: 23.3%) were the most abundant.

Discussion

The Molnár János Cave, belonging to the Rózsadomb discharge area of the Buda Thermal Karst System, is the largest active thermal water system in Europe where microorganisms can be studied. This study looked at three ecological niches within the cave system: thermal water, subaqueous black biogeochemical layer, and reddish-brown biofilm on the subaqueous cave wall. The Proteobacteria was one of the most abundant bacterial phylum in the studied habitats of the cave, as revealed both by cultivation and molecular cloning. However, the dominant members of the classes Gamma- and Betaproteobacteria showed different distributions according to the sample types and methods used for examinations. The presence of the genus *Aeromonas* was identified among the strains isolated from the reddish-brown biofilm (Table 1). This biofilm growth pattern differs from previous studies that found *Aeromonas* species to be common members of freshwater planktonic communities (Langó et al., 2002). The occurrence of these indicator organisms in the

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Figure 4. Taxonomic classification of 16S rRNA gene sequences of the archaeal clone libraries from the Molnár János Cave.
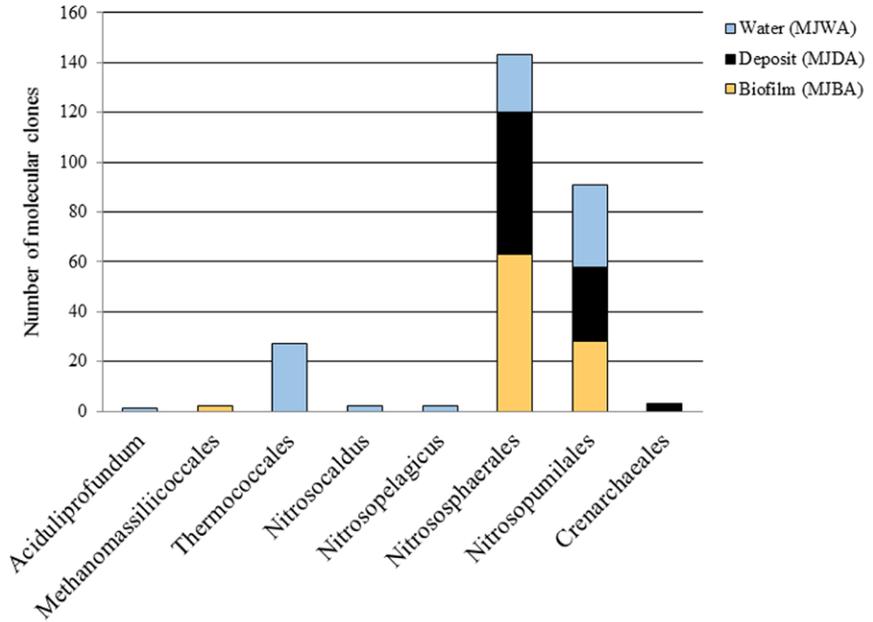

Table 1. Cultivated strains from the thermal water and reddish-biofilm samples inhabiting the Molnár János Cave. Notes: R: R2A medium, I: ferrous-sulfate medium, B: reddish-brown biofilm sample, W: thermal water sample.

Taxonomic Identification	Representative Strain (Accession Number)	Similarity % (bp)	Origin of the Sample	Media	
				R	I
Alphaproteobacteria					
Brevundimonas bullata (D12785)	MJW-S108 (LN998978)	100% (0/1090)	W	+	
Caulobacter henricii (AJ227758)	MJB-S139 (LN998974)	98.50% (10/667)	B		+
Paracoccus yeei (AY014173)	MJW-S104 (LN998975)	99.78% (2/914)	W	+	
Betaproteobacteria					
Xylophilus ampelinus (AF078758)	MJB-S116 (LN998968)	97.78% (21/944)	B		+
Variovorax boronicumulans (AB300597)	MJB-S128 (LN998972)	98.83% (10/853)	B		+
Vogesella alkaliphila (HE819389)	MJB-S123 (LN998970)	98.64% (13/955)	B		+
Gammaproteobacteria					
Aeromonas veronii (X60414)	MJB-S102 (LN998961)	99.71% (3/1044)	B	+	
	MJB-S104 (LN998962)	100% (0/1010)	B	+	
	MJB-S108 (LN998965)	100% (0/966)	B	+	
Citrobacter freundii (ANAV01000046)	MJB-S137 (LN998973)	99.80% (2/983)	B		+
Citrobacter youngae (AJ564736)	MJW-S106 (LN998976)	99.78% (2/915)	W	+	
	MJW-S123 (LN998981)	99.79% (2/931)	W		+
Enterobacter ludwigii (AJ853891)	MJW-S107 (LN998977)	98.76% (10/806)	W	+	
	MJW-S122 (LN998980)	99.24% (8/1047)	W		+
Pantoea dispersa (DQ504305)	MJB-S107 (LN998964)	98.67% (14/1054)	B	+	
Raoultella terrigena (Y17658)	MJW-S109 (LN998979)	99.39% (5/822)	W	+	
	MJW-S126 (LN998982)	99.43% (6/1050)	W		+
Firmicutes					
Brevibacillus fluminis (EU375457)	MJB-S119 (LN998969)	97.96% (20/981)	B		+
Brevibacillus ginsengisoli (AB245376)	MJB-S125 (LN998971)	97.41% (27/1042)	B		+
Actinobacteria					
Janibacter terrae (AF176948)	MJB-S105 (LN998963)	99.90% (1/992)	B	+	
Micrococcus yunnanensis (FJ214355)	MJB-S113 (LN998966)	99.81% (2/1046)	B		+
	MJB-S114 (LN998967)	99.90% (1/991)	B		+

cave water possibly suggests human influence, because their growth is facilitated by organic matter input (Araujo et al., 1989). The water of Molnár János Cave is currently thought to be supplied by karst and basinal fluids according to the numerical flow simulation (Mádl-Szőnyi and Tóth, 2015). Nevertheless, in the caves of Buda, the role of sewer leakage cannot be excluded under the residential area (Virág et al., 2009; Bergmann et al., 2011). In this study, representatives of Enterobacteriaceae (Gammaproteobacteria) were identified in the water from the cave, but presence of fecal coliforms was not verified by molecular or culturing methods, indicating that the infiltration from the surface was negligible. Members of the family Enterobacteriaceae with facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped cells have been found in the caves of the Slovak Karst, too. In that case correlation was found between biological pollution and agricultural activity (Seman et al., 2015).

The similarity values of the partial 16S rRNA gene sequences of molecular clones to previously described species were below the (>97%) identification level (Stackebrandt and Goebel, 1994) in almost all cases. The sequence analysis of the representative molecular clones (selected by ARDRA grouping) resulted in the closest matches (with 87% to 99% sequence similarities) mainly to uncultured clone sequences from different environmental sources such as caves, thermal springs, soils, and sediments (Hansel et al., 2008; Lin et al., 2012; Ivanova et al., 2013).

In the water clone library (MJWB), a high proportion (94.2%) of molecular clones was found to be related to *Thiobacillus* species (Betaproteobacteria). The genus *Thiobacillus* consists of chemolithotrophic sulfur-oxidizing bacteria with diverse metabolisms that are a common constituent of sulfuric freshwater, marine, and subsurface habitats (Kelly and Wood, 2000). The bacterial sulfur metabolism is significant in different caves, so sulfur-oxidizing bacteria have a crucial role in sulfuric acid speleogenesis. During their metabolism, as a by-product of sulfide oxidation, the bacteria produce sulfuric acid that dissolves carbonates (Engel, 2011; Kumaresan et al., 2014). In the water of Molnár János Cave, relatively high amounts of sulfide were measured, similar to that of the thermal water of Városliget-II well, another member of the Buda Thermal Karst System. The phylotypes related to the species of *Thiobacillus aquesulis* were the most abundant according to the 16S rRNA gene sequences (Anda et al., 2015). By comparing these results against the *Thiobacillus*-related representatives from these habitats (MJWB-C7 and VLWB-C7), a 99% sequence matching was found, demonstrating the dominance of the same phylotype in both Buda Thermal Karst System communities.

In the black biogeochemical layer clone library (MJDB), the majority of the clones (29.1%) was associated with the genus *Methylohalomonas* (Gammaproteobacteria), which is distantly related (90.18% sequence similarity) to the moderately halophilic and obligate methylotrophic *M. lacus*. Previously Chen et al. (2009) described the dominance of methylotrophic organisms from the Movile Cave, Romania. The facultative methylotrophic bacterium *Methylobacterium radiotolerans* has also detected from the thermal water of the T6 karst well of Harkány Spring, located in southwestern Hungary. It indicates that biological metabolism of methane may also be important in these karst cave environments (Miseta et al., 2012).

The reddish-brown biofilm clone library (MJBB) was dominated by bacteria belonging to the phylum Chloroflexi. The filamentous bacteria were observed in the SEM images. Among them, the order Anaerolineales was identified in the highest proportion (37.2% of the phylum Chloroflexi). Members of the phylum Chloroflexi, such as *Bellilinea caldifistulae* (Yamada et al., 2007), are also prevalent in the biofilm developed on the inner surface of an outflow pipeline of the Városliget-II thermal well (Anda et al., 2015). *Chloroflexus* spp. were also identified from hypogenic caves, such as the Lower Kane Cave in Wyoming, Frasassi Cave System in Italy, and Cueva de Villa Luz in Mexico (Engel, 2007). In addition, several bacterial clones from the reddish-brown biofilm library showed high sequence matching (81.03-94.07%) with different thermophilic sulfate reducing species of the genera *Desulfuromonas*, *Desulfomonile* (Deltaproteobacteria), *Thermanaeromonas* (Firmicutes) and *Thermodesulfovibrio* (Nitrospirae). Sulfur and sulfate reducing Deltaproteobacteria (including the genus *Desulfuromonas*) have also detected in the biofilm of the Rudas-Török spring cave and Diana-Hygieia thermal spring, in the southern area of the Buda Thermal Karst System (Borsodi et al., 2012; Anda et al., 2014). During a previous study of the reddish-brown biofilm of Molnár János Cave, the majority of the molecular clones were related to thermophilic aerobic and anaerobic bacteria belonging to phylum Firmicutes (Borsodi et al., 2012). The members of this phylum (representatives of orders Clostridiales and Thermoanaerobacterales) were confirmed to be significant in this clone library, as well.

Studying the role of the ammonia and nitrite-oxidizing bacteria and ammonia-oxidizing archaea in cave environments and thermal springs has become a research hotspot recently (Chen et al., 2009; Tetu et al., 2013; Ortiz et al., 2014). Thermophilic nitrite-oxidizing bacteria (genus *Nitrospira*) often appear in different spring caves (Marks et al., 2012; Anda et al., 2014). In the black biogeochemical layer of Molnár János Cave, the phylum Nitrospirae was the second most abundant group. The molecular clones showed the highest sequence matching (95.88%) with the species *Nitrospira moscoviensis* described from a corroded iron pipe (Ehrich et al., 1995). Previously, the phylum Nitrospirae was also found to be dominant in the water and biofilm of another thermal spring of the Buda Thermal Karst System (Anda et al., 2014). Besides the nitrite-oxidizing bacteria, ammonia-oxidizing archaea also play an important role in the nitrogen cycle of different karstic environments (Ortiz et al., 2014; Stahl and de la Torre, 2012; Bartossek et al.,

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2012). Among the ammonia oxidizing prokaryotes, chemolithoautotrophic ammonia-oxidizing archaea are dominant, and these microorganisms can be found in a wider range of habitats, such as hot springs and acidic soils, than ammonia-oxidizing bacteria (Stahl and de la Torre, 2012). The functional and taxonomic composition of the speleothem metagenome of Kartchner Caverns (Arizona, USA) and the Nullarbor Plain aquatic Weebubbie Cave, Australia, shows a dominance of ammonia-oxidizing archaea belonging to the phylum Thaumarchaeota. The Thaumarchaeota are recognized to be dominant players in global nitrification (Stahl and de la Torre, 2012). In the case of the Kartchner Caverns, Thaumarchaeota reads were classified as *Nitrosopumilus maritimus* (13%) and *Nitrososphaera gargensis* (4.7%). The former is an ammonia-oxidizing marine archaeon (member of group I. 1a), while the latter can often be found in terrestrial environments (Ortiz et al., 2014; Tetu et al., 2013). Representatives of *Nitrososphaera gargensis* were also found in high proportion in the Molnár János Cave (brown biofilm 33 clones, black biogeochemical layer, and water 15 clones) samples.

Conclusions

Prokaryotic communities participating mainly in the sulfur cycle of Molnár János Cave located in the Buda Thermal Karst System showed habitat dependent distribution. The reddish-brown biofilm formed on the subaqueous cave wall was dominated by anaerobic bacteria belonging to the filamentous Chloroflexi and sulfate reducing Deltaproteobacteria, while aerobic sulfur-oxidizing *Thiobacillus* (Betaproteobacteria) were the most abundant in the water of the cave. In addition, *Aeromonas* and the family Enterobacteriaceae were also detected here, indicating human impact. In the black biogeochemical layer, the majority of the phylotypes were associated with methylotrophic species. On the contrary, phylotypes related to ammonia-oxidizing archaea (*Nitrosopumilus* and *Nitrososphaera*) and nitrite-oxidizing bacteria (Nitrospirae) were found to be abundant in all three habitats. Therefore, it can be assumed that nitrogen biotransformation may also be an important microbial process beside the sulfuric acid speleogenesis in the studied hypogenic cave.

Acknowledgements

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