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Microbial diversity on the rock paintings in Magoura Cave, Bulgaria

ABSTRACT

The Magoura Cave contains an impressive display of prehistoric art. The different ecological niches in cave caverns and galleries may be exploited by a large variety of microorganisms and their growth can lead to both aesthetic and structural damages of the paintings mentioned. For this reason the microbial diversity in the cave and the role of microbial communities in rock paintings destruction is of great interest. The main objective of this research is connected with investigation of the microbial diversity of the rock paintings in the cave. The investigations are focused on characterization of the microbial communities and determination of the dominant microbial groups. Eleven different physiological groups of microorganisms have been tested by using of the elective nutrient media and specific conditions of cultivation. The quantitative analysis of the each group was done by the classical methods. The microbial studies reveals that on the rock paintings present continuously stable microbial population with stable qualitative and quantitative composition. In some samples are found high levels of sulphate-reducing bacteria, denitrifying bacteria, ammonifying bacteria and silicate bacteria. These taxonomic groups are involve in the biodeterioration processes.These results must be taken in mind during the conservation activities connected with paintings in the cave.

Key words: biodiversity, biodeteriogens, prehistoric paintings, microbial monitoring

Introduction

The Magoura Cave is located in north-western Bulgaria close to the village of Rabisha, at 18 km from the town of Belogradchik in the Vidin Province. The cave contained an impressive display of prehistoric art located in lateral caverns connected to several galleries. More than 700 paintings, represent different cultural and hunting scenes. The drawings were made by guano feces from cave dwelling bats, which chemical composition includes mainly ammonium oxalate, urate, and phosphates as well some earth salts and impurities. Guano has also a high concentration of nitrates (Emerson et al., 2007). Considering this composition, guano is not immune material to microbial attack. Given the wide range of organic and inorganic molecules that are present in bat guano paintings, many different types of microorganisms may grow on such substrates provided that favorable environmental conditions (humidity, temperature, and pH) (Barton et al., 2004), which growth will lead to both aesthetic and structural damages. As aesthetic damage one must consider discoloration, stains and formation of biofilm on the painted surface, whereas as structural damage one must consider cracking and disintegration painted layer, resulting in detachment of the layer itself from the rock surface (Ciferri, 1999).

Show cave managements including artifcial lighting and the maximum number of daily visitors produce striking differences as well. Caves can be difficult to navigate and often require physical modification to allow easy access for visitors. Visitors in tourist caves have direct physical effects such as the introduction of concrete and steel structures; transport of mud, dust, and nutrients; installation of lights and the exhalation of water vapour and carbon dioxide into the air. Indirect physical effects include alteration of the microclimate, both through physical modifications that change the ventilation regime and through the presence of visitors leading to changes in temperature, humidity and CO_2 within the cave environment (Allemand & Bahn, 2005; Schabereiter-Gurtner et al., 2002). Anthropomorphic changes to cave physical environments to aid access or to reduce backtracking can have adverse effects on the internal microclimate of cave systems with subsequent changes to the cave environment affecting the quality of decorations and cave art and the diversity of cave microflora.

Considering these facts, a necessity of long-term analysis of the composition of, and the variations in, microbial population of the painted areas as well as of unpainted gallery walls and flour surrounding environment emerged.

The microbial diversity in caves and the role of microbial communities in rock paintings destruction is a topic of present interest in Bulgaria, Europe and the rest parts of the world. The caves are colonized by a variety of cold adapted microorganisms (Barton & Northup, 2007; Engel et al. 2004). The availability of valuable information about composition of the microbial communities in rock art caves emphasizes the interest in understanding of their complexity.

There is great media interest in the conservation of paleolithic paintings and the possible application of new technologies in their preservation (Bastian & Alabouvette, 2009; Chelius & Moore, 2004; Holmes et al., 2001). However, the design of novel, scientifically based conservation strategies requires, from the microbiologist point of view, knowledge on the microbial communities developing in an environment (i.e., Magoura Cave). At present, the microorganisms composing these microbial communities and their natural functions are poorly studied.

Hypogean environments, such as caves containing prehistoric paintings, are not exempted containing diverse communities of microorganisms (Gonzalez et al., 2006; Schabereiter-Gurtner et al., 2002; Zimmermann et al., 2005). Recent publications have mentioned an accelerated expansion of microorganisms in these systems (Allemand & Bahn, 2005; Holden, 2002).

This gap in our knowledge needs to be filled before deciding which technologies are to be applied and which microorganisms should be the targets of conservation initiatives.

The objective of this study were to characterize the permanent microflora in Magoura cave by classical cultivation scheme. The availability of valuable information about composition of the microbial communities in rock art caves will emphasize the interest in understanding of their complexity.

Materials and Methods

Taking of the samples and their preservation till further manipulation is done according standard regulations of microbiological analysis (Bulgarian State Standard). Prior analysis no preservatives were added, because the main goal of the investigation is to quantify only viable cells. Priority for sampling had locations with signs of possible biodeterioration activity (paintings itself, wet wall surfaces, spots with different color).

Samples from surface areas were taken by sterile cotton swabs (Constix® Swabs, Contec, Canada) (Figure 1).

Figure 1. *Locations of sampling in Magoura cave*

Aiming to estimate dynamics of microbial population inhabiting Magoura Cave, four subsequent sampling were done in the course of twelve months.

Nutrient media and Sample cultivation

According presumption of most probable groups with contribution to deterioration processes several target groups were subjected to analysis with relevant medias as follows:

- Nutrient Agar (BBL™ Nutrient Agar). Used for cultivation of heterotrophic aerobic bacteria.
- Nutrient Agar+YGC (Sigma-Aldrich). Used for estimation of the total number of spore forming bacteria.

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- Nutrient agar (BBL™ Nutrient Agar) with indicator-phenol rot. Used for cultivation and determination of amonificatosrs.
- Saratchandra medium (Saratchandra, 1979). Used for cultivation of nitrificators.
- Giltai medium used for cultivation of denitrificators.
- Potato Dextrose agar (Acumedia). Used for cultivation of silicate bacteria.
- Saburo Agar (Sigma-Aldrich). Used for cultivation of fungi.
- Gause I agar (Sigma-Aldrich). Used for cultivation of actinomyces.
- Pfenning's nutrient medium (Sigma-Aldrich). Used for cultivation of sulfate-reducing bacteria.
- Waksman medium (Waksman, 1979). Used for cultivation of neutrophilic thio-bactera.
- 9K medium (Silverman & Lundgren, 1959). Used for cultivation of acidophilic thio-bacteria.

Methods for typing by classical microbiological scheme

Isolation and further taxonomical determination of isolates was based on classical microbiological scheme according Bergey's manual of determinative bacteriology (1989).

Morphological analysis includes gram staining, cell shape, motility and spore-forming.

Cultural examination was based on ability to growth on the relevant media.

Biochemical analyses includes utilization of different substrates (indole, H_2S, glucose, lysine and ornithine).

Results and Discussion

In each sampling campaign 15 samples were taken from different locations in cavern area. The comparative quantitive analysis clearly demonstrates that each sample, resp. location possess unique microbial population structure and specific ratios between different target groups.

The analysis of first sample revealed presence of all tested physiological groups with dominant place of psychrophiles and oligicarbophiles. The other stable component of the detected microflora in this location are amonificators, denitrificators and sulfate-reducing bacteria (1.0 x 10^{4} -1.0 x 10^{5}CFU/ml) (Figure 2).

In second sample again all groups are presented but the structure is little bit different – again the predominant group is psychrophiles, but next to it are actinomyces and fungi followed by silicate bacteria. This could be explained by the location and the gathering place for tourist group near the staircase (Figure 3).

Comparative analysis of rest twelve samples revealed the same composition with slightly variations in number of actinomyces and fungi group.

The analysis of the results consigns several conclusions about the characteristic of inhabiting microflora in Magoura Cave in terms of long-time observation. There is relatively stable microflora, presented by different taxonomic groups. The constant taxons include dominantly heterotrophic bacteria, amonificators and silicate bacteria.

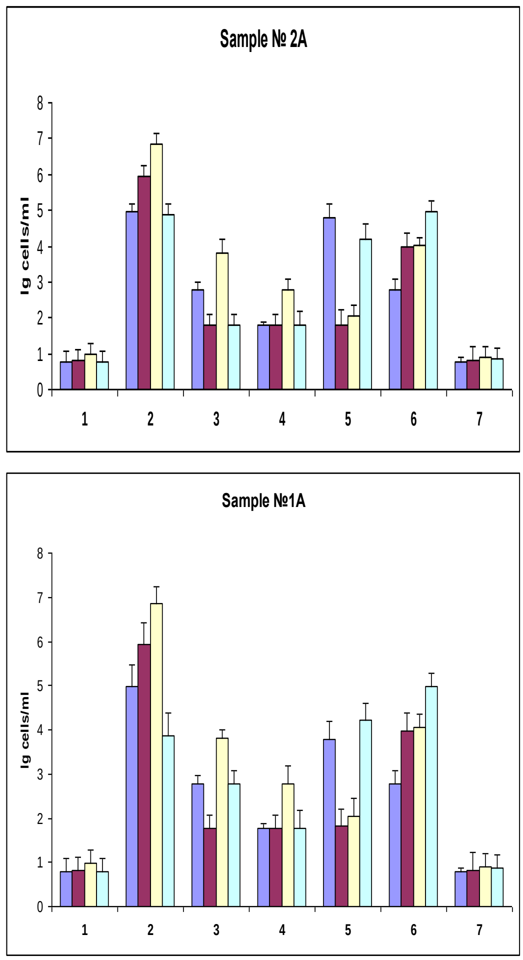

Figure 2. *Sample №1 (A/B), Groups Microorganisms: 1 – Obligate psychrophiles, 2 – Facultative psychrophiles, 3 – Sporeforming bacteria, 4 – Nitrificators, 5 – Denitrificators, 6- Amonificators, 7 – Urobacteria*

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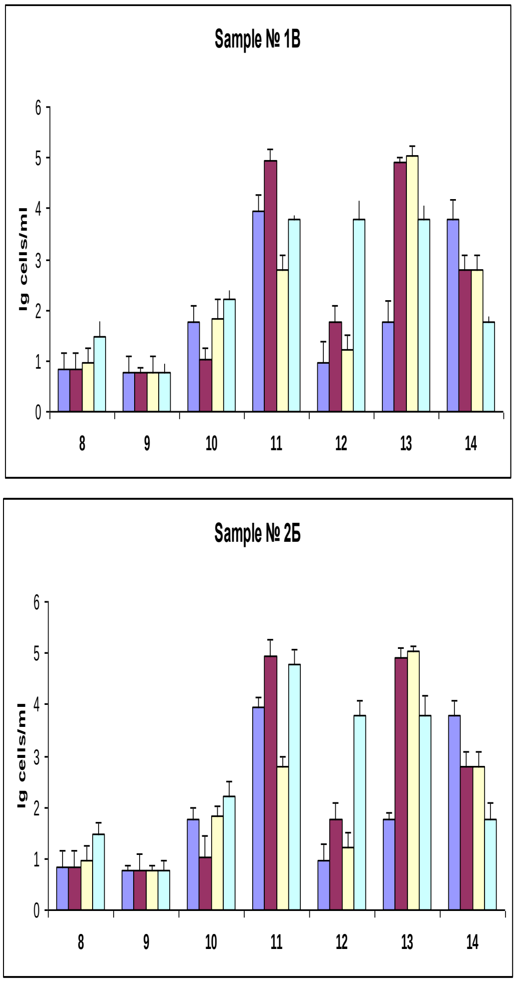

Figure 3. *Sample №2 (A/B), Groups Microorganisms: 8 – Neutrophilic ironbacteria, 9 – Acidophilic iron bacteria, 10 – Oligocarbofiles, 11 – Silocate bacteria, 12 – Sulphate-reducing bacteria, 13- Actinomices, 14 – Fungi*

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