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DETERIOGENIC EFFECT OF MICROBIAL COLONIZATION OF PREHISTORIC PAINTINGS IN MAGOURA CAVE, BULGARIA

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Abstract: The Magoura Cave is located in North-Western Bulgaria. The cave contained an impressive display of prehistoric paintings, made by the guano faeces of cave dwelling bats. Many different types of microorganisms may grow on such substrates under favourable environmental conditions and have a biodeterioration effect on the paintings.

The aim of the present study is focused on complete characterization of the microbial communities inhabiting the Magoura cave and determination of the most active biodeteriogens.

The comparative analysis of the microbial colonization clearly demonstrates that each sample possesses unique microbial population structure and specific ratios between different target groups.

The monitoring studies reveal that in the cave present continuously stable microbial population with steady qualitative and quantitative composition. In some samples high levels of sulphate-reducing bacteria, denitrifying and ammonifying bacteria as well as silicate bacteria were found. Some of these bacteria are typical biodeteriogens and it is possible they take part in the destruction of the paintings.

Based on the results obtained, special treatments must be developed for the restriction of the microbial colonization and the conservation of the unique rock paintings.

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. The drawings were made by guano faeces from cave dwelling bats, which chemical composition containing mainly ammonium

oxalate, urates, and phosphates as well as high concentration of nitrates (Emerson et al., 2007).

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 favourable environmental conditions (humidity, temperature, and pH) (Barton et al., 2004). The different ecological niches in cave caverns and galleries may be exploited by a large variety of microbial species, 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).

Microbial processes act in conjunction with physical and chemical deterioration, and in most instances are inseparable from it. The material composition of the paintings as well as the surrounding environment can, in part, determine the community of microorganisms and the mechanism of deterioration. However, microorganisms can also alter the physical and chemical properties of a material surface, thereby affecting other deterioration mechanisms.

With a few exceptions, most of the investigations worldwide, concerning characterization of the microbial flora present in cave galleries have been limited to selected groups of microorganisms rather than to all types of species that might be present on a given environment (Groth et al., 1999). Despite the fact that the majority of the cave isolates belongs to well known species, more than 30% of them present poorly investigated taxons, which metabolic characteristics still have to be defined. Besides, focusing on the metabolite active microbial groups will comprehend further to our understanding of deterioration processes in cave drawings (Schabereiter-Gurtner et al., 2002).

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 (Engel et al. 2004; Barton and Northup, 2007). The availability of valuable information about the composition of the microbial communities in rock art caves emphasizes the interest in understanding their complexity.

The aim of the present study is focused on the complete characterization of the microbial communities inhabiting the Magoura cave and determination of the most active biodeteriogens.

MATERIALS AND METHODS

Sampling, Transportation, Preliminary manipulation

Taking of the samples and their preservation until further manipulation is done according to standard regulations for microbiological analysis (Bulgarian

State Standards). Priority for sampling had locations with signs of possible biodeterioration activity (paintings itself, wet wall surfaces, spots with different colour) (figure 1).

Ten samples in total were taken from different locations in the cavern area by sterile cotton swabs (Constix® Swabs, Contec, Canada).

Nutrient media and Sample cultivation

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

A) Nutrient Agar (BBL™ Nutrient Agar). Used for the cultivation of heterotrophic aerobic bacteria.

B) Nutrient Agar+YGC (Sigma-Aldrich). Used for estimation of the total number of spore forming bacteria.

C) Nutrient agar (BBL™ Nutrient Agar) with indicator-phenol rot. Used for the cultivation and determination of ammonifyers.

D) Saratchandra medium (Saratchandra, 1979). Used for the cultivation of nitrifiers.

E) Giltay medium used for cultivation of denitrifiers.

F) Potato Dextrose agar (Acumedia). Used for the cultivation of silicate bacteria.

G) Saburo Agar (Sigma-Aldrich). Used for the cultivation of fungi.

H) Gause I agar (Sigma-Aldrich). Used for the cultivation of Actinomyces.

I) Pfenning's nutrient medium (Sigma-Aldrich). Used for the cultivation of sulphate-reducing bacteria.

J) Waksman medium (Waksman, 1979). Used for the cultivation of neutrophilic thiobacteria.

K) 9K medium (Silverman and Lundgren, 1959). Used for the cultivation of acidophilic thiobacteria.

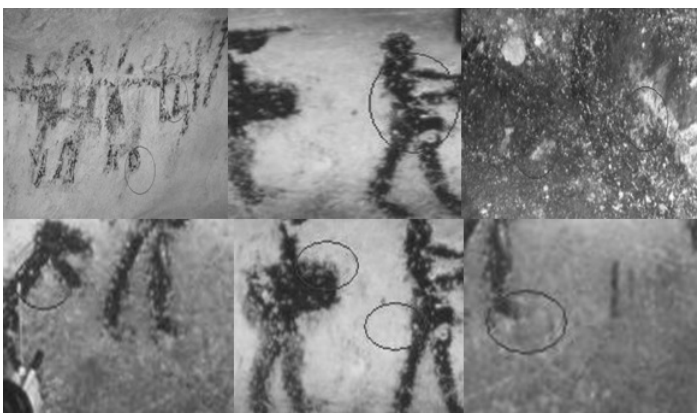


Figure 1. Sampling locations in Magoura cave.

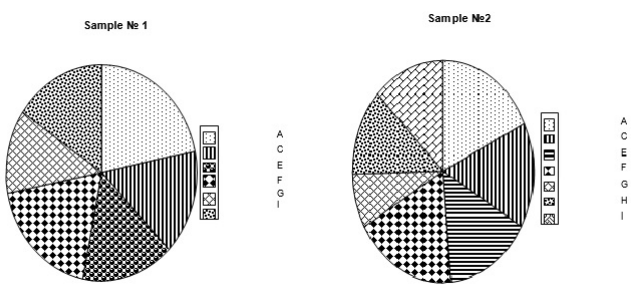
The quantity of the microorganisms of the target groups was estimated by the most probable number method under standard conditions for the cultivation of each microbial group.

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

RESULTS AND DISCUSSION

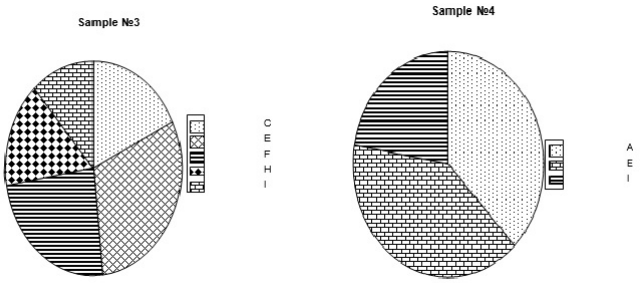
The comparative quantitative analysis of the microbial colonization of the samples taken from different locations in the cavern area clearly demonstrates that each sample location possesses the unique microbial population structure and specific ratios between different target groups of microorganisms.

Comparative analysis of **Sample №1** (from the entrance area, rock surface) reveals the presence of 6 from 11 target groups. This abundance might be explained by location, where a lot of visitor groups packed. The predominant group is heterotrophic aerobic bacteria (±1.0 x 10^7 CFU/ml), followed by silicate bacteria (±1.0 x 10^6 CFU/ml). The other components of the detected microflora in this location are ammonifiers, denitrifiers and sulphate-reducing bacteria (1.0 x 10^4 -1.0 x 10^5CFU/ml). The absence of Actinomyces is clearly demonstrated.

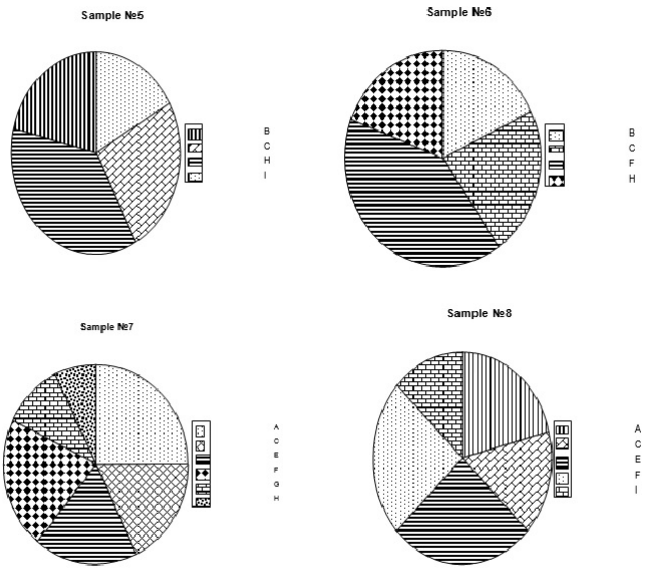


Sample №2 originates from similar location, which is confirmed by the same ratio composition of target groups. The only significant difference is the presence of Actinomyces (±1.0 x 10^4 CFU/ml).

Sample №3 is taken from the paintings, closest to the entrance. The dominant microbiota is formed of ammonifiers, denitrifiers and silicate bacteria. The next **sample №4** is taken from the painting in the same area. Relatively low rates of microbial colonization in these samples suggest positive tendency of low-risk status of this area from bio deterioration of tested drawings. This tendency is confirmed from the analysis of next two samples, taken from the adjacent pictures, where only 5, respectively 3 groups are presented. The only common group for these two samples is the group of sulphate-reducing bacteria (1.0 x 10^2 -1.0 x 10^3 CFU/ml).

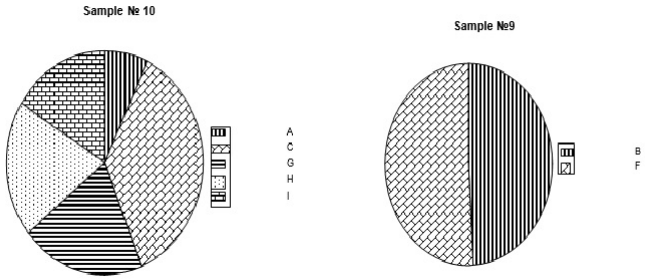


The different origin of samples №6 and №7 (guano deposits) explains the different microbial microflora content with dominant presence of heterotrophic bacteria and ammonifiers.



Sample №8 is taken from the small water deposit, beneath the rock cliff which explains the presence of silicate bacteria.

Significantly low microbial colonization is established in samples №9 and №10 where only a few representative groups are found, mainly ammonifiers and heterotrophic bacteria.



The almost unified character of microbial colonization was established and in the next two samplings of the microbiological monitoring performed.

CONCLUSIONS

The analysis of the results consigns several conclusions about the characteristic of inhabiting microflora in Magoura Cave. There is relatively stable microflora, presented by different taxonomic groups of microorganisms. The constant taxons include predominantly heterotrophic bacteria, ammonifiers and silicate bacteria. Some of these bacteria are typical biodeteriogens and it is possible to take part in the destruction of the paintings.

Based on the results obtained, special treatments must be developed for the restriction of the microbial colonization and the conservation of the unique rocks paintings.

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REFERENCES

1. Emerson, J. and Roark, A. 2007. Composition of guano produced by frugivorous, sanguivorous, and insectivorous bats. *Acta Chiropterologica*, 9: 261-267.
2. Engel, A.S., Porter, M.L., Stern, L.A., Quinlan, S., Bennett, P.C. 2004. Bacterial diversity, and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic "Epsilonproteobacteria". *FEMS Microbiology Ecology*, 51: 31-53.
3. Barton, H.A., Taylor, M.R., and Pace, N.R. 2004. Molecular phylogenetic analysis of a bacterial community in an oligotrophic cave environment. *Geomicrobiology Journal*, 21: 11-20.
4. Barton, H. and Northhup, D. 2007. Geomicrobiology in cave environments: past, current and future perspectives. *Journal of Caves and Karst Studies*, 69: 163-178.
5. Ciferri, O. 1999. Microbial degradation of paintings. *Applied Environmental Microbiology*, 65: 879-885.

6. Groth, I., Vettermann, R., Schuetze, B., Schumann, P., Saiz-Jimenez, C. 1999. Actinomycetes in Karstic caves of Northern Spain (Altamira and Tito Bustillo). *Journal of Microbiological Methods*, 36: 115–122.
7. Schabereiter-Gurtner, C., Saiz-Jimenez, C., Pinar, G., Lubitz, W., Rolleke, S. 2002. Cave Palaeolithic paintings harbour complex and partly unknown microbial communities. *FEMS Microbiology Letters*, 211: 7–11.