Hyphomycetous fungi in the Cave of Doña Trinidad (Ardales, Malaga, Spain)

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Cave environments often contain rock art paintings. However, microbial deterioration is threatening the preservation of these sites. Most of the well known caves with paintings (Altamira, Lascaux, etc.) are affected by progressive biodeterioration. At present, the conservation and preservation of the paintings and engravings of these environments are being actively encouraged by scientists, conservators and general public, because of their high artistic value.

The Cave of Doña Trinidad (Ardales, Málaga, Spain) contains numerous paintings and engravings, some of them dated back about 20,000 years old. A microbiological study revealed that bacteria are widespread in caves. However, scarce information is available on the presence of fungi in caves. Besides bacteria, fungi can also play a crucial role in the colonization of caves with paleolithic paintings, such as the case of the Cave of Lascaux, France. Fungi are ubiquitous protagonists in subterranean environments and fulfil a range of important ecological functions although current understanding of fungal biodiversity in caves is limited.

In the present study, the fungal diversity of some soils, animal excrements and air samples from the Cave of Doña Trinidad, were investigated by 18S rDNA-based denaturing gradient gel electrophoresis (DGGE) analysis and by culturing methods. The fungal isolates were characterized by morphology and identified by sequencing parts of the small ribosomal subunit (18S) and internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). Among the isolates members of the genus Fusarium, Arthoderma, Aphanoascus, Aspergillus and Penicillium were found to be dominant. In addition to this anamorphs of ascomycetes, the genus Trichosporon was the only member detected during this study belonging to basidiomycota.

From previous DNA based molecular studies we showed that the genus Fusarium was a major component of the total microbial community in a cave wall. Further research will be focused on colonization patterns carried out in the laboratory by this fungal species.

Keywords: Cultural Heritage; Hypogean Environment; Fungi

near alkylbenzene sulfonate reactor

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eering of São Carlos, University of São Paulo, São

onic surfactant more widespread in detergents strial wastewater. Currently, LAS represents more 000, its world-wide consumption was around 2.5 dable and it is removed from wastewater through ues). LAS in wastewater plants can cause some e microorganisms responsible for the biological e LAS degradation in anaerobic conditions, with s immobilization. It had been used four horizontal e first one was inoculated with sludge from UASB ulum was from UASB reactor used in pig farming th polyurethane foam as support material. R3 and ge. However, R3 was filled with charcoal and R4 ort material. All reactors were feed with syntbetic ation time (HDT) was 12 hours and COD influent ded, biomass samples were collected, analyzed by and partial sequencing of 16S rRNA. The organic igh pressure liquid chromatography (HPLC) and rs were able to remove LAS. After 313 operation nd R3 and R4, after 343 operation days degraded gene fragment sequence showed that all reactors clones from R1 and R2 reactors was related with ble for LAS degradation. Biomass samples for R3 ivating bacteria of Delta Proteobacteria Class. The Class. Beyond *Clostridium*, other sulfate reducing on because they were identified in all evaluated the bacteria pertaining to the Delta-Proteobacteria

r alkylbenzene sulfonate; DGGE; 16S rRNA.

Microbial communities and biogeochemistry in different volcanic environments from Canary Islands (Spain)

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The microbial communities of different sites located within the Canary Islands were studied in relationship to the geochemical characterisites of this volcanic environment. In this study, we focused on the microorganisms thriving in terrestrial environments, either from boreholes or water mining activities at these islands. Molecular methods were used to detect the presence of microorganisms in water samples. Two boreholes were studied and were located in Tenerife Island near the summit of Teide Volcano (MM at 2264 m and EP 2133 m above sea level) and water was collected from 450 m (MM) or 370 m (EP) depth at the aquifer water surface. At the water surface, CO_2 content was over 25-fold atmospheric CO_2 content and temperature around 13ºC (MM) or 11ºC (EP). A highly diverse microbial community was detected and was composed by a large number of bacterial divisions, such as, Firmicutes, Gammaproteobacteria, Actinobacteria, Betaproteobacteria, Bacteroidetes, Epsilonproteobacteria, Verrucomicrobia, Nitrospirae, Chloroflexi, Alphaproteobacteria, and several uncharacterized candidate divisions (OP11, OD1, and OP10). Water is a scarce resource in Canary Islands and search for water sources is an important activity in this archipelago. One of these mines located in La Palma Island was visited and water temperature was 30ºC. Some members of the microbial communities were detected and consisted mainly on Alpha and Gammaproteobacteria as the most important groups, and Nitrospirae, Deltaproteobacteria, Verrucomicrobia, Firmicutes, Betaproteobacteria, and Acidobacteria. Results shows the presence of very diverse microbial communities which might have significant consequences for the geochemistry of these underground water sites.

Keywords biogeochemistry; microbial communities; volcanic environments; boreholes; water mining

Microbial communities from caves with paleolithic paintings

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Recent studies on the microorganisms involved in biodeterioration are revealing the existence of highly diverse and complex microbial communities. Pioneering analysis of microbial communities were carried out through the culture of microorganisms. At present, the use of molecular methods based on the detection of specific sequences of nucleic acids have allowed us to detect a large number of microorganisms which had not previously been discovered. The results obtained during our latest studies showed the presence of numerous microorganisms never or rarely reported before in caves with rock art paintings. Among these microbial groups, the presence of sulfate-reducing bacteria (mainly belonging to the Deltaproteobacteria), Bacteroidetes, Chloroflexi, Crenarchaeota, Gemmatimonadetes, Nitrospirae, Planctomycetes, Verrucomicrobia, and several uncultured bacterial candidate divisions have been frequently detected. The metabolic capabilities of the microorganisms that have not been previously cultured are generally unknown, and most of the microbial groups mentioned above have never, or rarely, been cultured. Consequently, there is no information on their metabolic potential, and their potential role on the deterioration of the studied paintings is unknown. In the last years, several attempts are been reported to approach the role of specific microorganisms in the environment using a variety of novel approaches. In this study, molecular methods based on the use both of DNA and RNA have been applied to detect the presence of microorganisms (DNA based) in the studied sites and those showing significant metabolic activity (RNA based) in the studied samples. Examples of complex microbial communities in the caves will be shown. Due to the high microbial diversity and the scarcity of current knowledge on most environmental microorganisms, specially those never or rarely cultured, paleolithic painting conservation is a serious challenge from the microbiological perspective. Filling the gaps in our knowledge of microbial diversity and metabolism is a requirement to understand the potential role of microbial communities on cultural heritage.

Keywords microbial communities, caves, paleolithic paintings; biodeterioration; microbial diversity

Microbial Cu-leaching and siderophore production under anaer conditions

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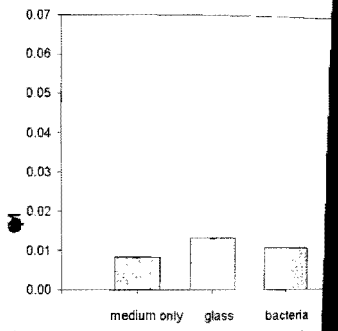
In Sweden spent nuclear fuel will be encapsulated in Cu-canisters and stored in geological envi with access to anaerobic groundwater. It is therefore important to understand and quantify m effects on material durability, such as Cu-corrosion, and mechanisms behind radi transportation to the biosphere. A mineral glass with trace amounts of Cu was incubated anaer in chemically defined medium for 3 weeks with the facultative anaerobe *Pseudomonas flu* isolated from groundwater. *Pseudomonas fluorescens* and most other bacteria produce iron(III) chelators, so called siderophores, under aerobic conditions. Siderophores are known to chel metals as well, but with less affinity. Chemical analyses done with ICP-MS show about 4 tim Cu concentrations in cultures supplemented with glass than in sterile glass controls. What mec that lie behind this is still to be revealed.

In order to increase the reactive surface a second set with crushed glass and bacteria was conducted. Although growth was very poor – anaerobic production of the microbial pyoverdine type siderophore was indicated by both spectrofluorometry and absorbance spectrometry in presence of glass. This is noteworthy as siderophores are not supposed to be excreted by bacteria at anaerobic conditions. However these findings suggest that a water soluble Cu-pyoverdine complex is formed as pH was the same in both sterile controls and in inoculated samples.



As the common groundwater bacterium used in the study survives and grows in environments that are considered to be used as nuclear fuel waste repositories it would consider its effect on the Cu canisters used for the burnt out waste.

The anaerobic production of pyoverdine siderophores is an important new finding that sho taken into account in this perspective as even poor growth result in excretion of detectable ligands that could be used for metal mobilization from the surroundings.

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