MULTIDISCIPLINARY RESEARCH FOR THE CONSERVATION OF CULTURAL HERITAGE

Advanced Research Training on the Conservation of Cultural Heritage (MEST-CT2004-51395) is a Host Fellowship for Early Stage Research Training. This Marie Curie Action, funded by the European Commission, started in May, 2005. The main objective is the training of researchers in the early stage of their professional career. The training is focused on the acquisition of integrated humanistic, scientific and technological competencies in the field of Cultural Heritage. This research training on specific and inter-connected areas, which likely corresponds with some of the most innovative, in terms of research and application of novel instrumentation, is developped in a multidisciplinary environment which includes archaeology, chemistry and material science, physics, geology and biology.

The CSIC Thematic Network on Cultural Heritage, as host institution of this Marie Curie Action, dedicates this issue of COALITION to the dissemination of some of the preliminary results obtained by the fellows.

Thus, Ekaterina V. Akatova writes on the microbial communities of a restored tomb in the Roman Necropolis of Carmona (Seville), Solenne Gaspard presents a paper on the application of LIBS to cultural assets, Liz K. Herrera reports on a study of baroque artworks by non-destructive techniques, Zuzana Jurasekova shows the application of vibrational spectroscopy for identification and characterization of natural dyes employed in cultural assets, Joeri Kaal describes the fire history of Galicia, Francesca Stomeo analyses the bacterial communities from Doña Trinidad Cave (Ardales, Spain), and Malgorzata Walczak reports on the characterization of modifications in polymer films by means of LIF.

COALITION editors

ANALYSIS OF THE MICROBIAL COMMUNITIES FROM A RESTORED TOMB IN THE NECROPOLIS OF CARMONA (SEVILLA, SPAIN)

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Introduction

The protection and conservation of the European Cultural Heritage is a matter of global relevance. Increasing deterioration of materials (stone, brick, leather, paper, wood, paintings, metals, etc.) is causing great concern. Atmospheric pollution, urbanization, tourism, groundwater fluctuations or inappropriate conservation treatments all play a role which need to be investigated.

Sometimes tourism causes a negative impact on the monuments and constitutes a real danger for the preservation of cultural sites. Some sites, like subterranean monuments (caves, catacombs, tombs, etc.) are exposed to intense modifications of its microclimate due to visits (i.e., lamps, ventilation, openings to the exterior, doors, etc.). That modifications result in change of microclimate and the appearance of conspicuous biofilms that cover walls and ceilings, in contrast with areas restricted to visitors in which biofilms were not observed (Sanchez-Moral et al. 2005).

The colonization of stones by microorganisms has been reviewed extensively (Saiz-Jimenez 1994, 2001). Visible microbial growth and associated pigments leads to undesirable aesthetic changes in historical monuments, as well as its deterioration and, occasionally, destruction. One of the first colonizators are phototrophic microorganisms, such as cyanobacteria and algae (Ortega-Calvo et al. 1993). Bacteria and fungi are heterotrophic microorganisms that benefit from organic matter synthesized by phototrophs.

The biodeteriorative mechanisms include the excretion of aggressive metabolism products such as organic acids and inorganic acids, as well as the mechanical destruction by

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penetration of the organisms into the substrate.

There is a need of safeguard and protection of the European Cultural Heritage and this only can be done by promoting the application of science and technology.

Ideally, an inventory of the existing microorganisms associated with the damage of selected objects of art should be included in any restoration project. The effectiveness of restoration treatments depends on the methods and the products chosen. Generally the treatment should kill all microorganisms but does not give any kind of protection for future recolonisation, and so the restoration efforts do not always obtain the expected result, and sometimes they even accelerate the deterioration process.

This study aims to analyze the microbial communities developing on mural paintings and walls of the Tomb of Servilia (Roman Necropolis of Carmona, Seville, Spain), after a restoration process; and to compare with those microbial communities that were present before the treatment.

Study site

The Roman Necropolis of Carmona (Seville, Spain) represents one of the most significant burying sites in Southern Spain used during the 1st and 2nd centuries A.D. At the time of its use, cremation predominated over the ritual of burial and therefore the site consists mainly of underground family chambers that contain a number of niches holding the funerary urns.

The form of the Tomb of Servilia differs greatly from those of the other tombs. It is a monumental structure reproducing a luxurious underground mansion in Hellenistic style. It consist of a covered gallery and a funeral chamber that has a trapezium-shaped ground plan and is covered by a pointed vault. Because of its monumental form the tomb is thought to have belonged to a powerful Roman family. The wall of the funeral chambers, were decorated with rich mural paintings, although, at present, only a few fragments remain.

The Roman Necropolis was carved into a largely soluble calcarenite of the Messinian-Lower Pliocene that is highly porous and is easily affected by weathering and processes of microkarstification. One of the most characteristic effects of these processes is the crystallization of abundant salts at specific sites as a consequence of evaporation.

Methods

In order to determine the changes of the microbial communities after restoration treatment we applied culture-independent techniques along with bioinformatics and statistics methods, which allow the detection of microorganisms *in situ* without the requirement for growth on specific culture media (Gonzalez and Saiz-Jimenez 2004). Moreover, it is demonstrated, that cultivation methods recover less than 1% of the total species of the microorganisms present in environmental samples (Giovannoni et al. 1990, Ward et al. 1990).

PCR-amplification of the 16S rRNA-encoding gene (16S rDNA) fragments is followed by separation of these fragments by Denaturing Gradient Gel Electrophoresis (DGGE). To identify the microorganisms, cloning of 16S rDNA PCR products was performed and selected clones analyzed following the protocols described by Gonzalez et al. (2003).

Results and discussion

DGGE analysis of 16S rDNA fragments was used to examine the effects of the restoration treatment on microbial communities of the Tomb of Servilia. In Figure 1 are shown the DGGE patterns of the 16S rDNA fragments (primers 341F-GC and 518R) amplified from three samples: before treatment (BS), and at 8 (U21) and 20 (YYY) months after restoration treatment. Each of the three samples produced a distinct DGGE fingerprint.

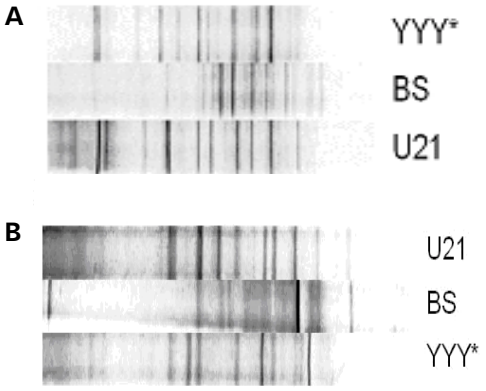

Figure 1. Microbial communities from the Tomb of Servilia. A: DGGE patterns of bacterial communities, B: DGGE patterns of phototrophic communities. BS is a sample taken before restoration, U21 was taken 8 months after restoration, and YYY was obtained 20 months after restoration.

By comparing DGGE profiles of the microbial communities representing each sample, one can observe the increasing in the number of microorganisms after biocide treatment (Figure 1 A). Most of these microorganisms persist after 20 months of treatment. The use of biocide did not eliminate the microbial community from the Tomb of Servilia, rather introduced an extra element of risk for the conservation of the tomb. Such element is the selection of new microorganisms which has resulted in a different community structure at the treated site.

This fact denotes that the recolonization of the tomb walls, over 20 months, lead to a more complex microbial community than the one existing before restoration treatment. It indicates that the restoration treatment (cleaning, use of quaternary ammonium compounds and consolidation) had not any definite action on the prevention of bacterial growth. Moreover, the data suggest that the microbial community present before restoration treatment was reestablish shortly after the treatment.

The monitoring for phototrophic members of the microbial communities denoted important changes (Figure 1 B) between the samples before and 8 months after treatment, in a way similar to that the described for bacteria.

Sequencing of libraries of samples before restoration treatment, resulted in detecting Actinobacteria (54,5 %), Proteobacteria (22,8 %), Bacteroidetes (14 %), and Cyanobacteria (8,7 %). The bacterial community of the sample, taken 8 months after restoration treatment contained Actinobacteria (37 %), Proteobacteria (31,5 %), Cyanobacteria (14 %), Bacteroidetes (8,5 %), Firmicutes (6 %) and Nitrospirae (3 %). The bacterial community of the sample taken 20 months after restoration treatment contained bacteria phylogenetically related to Actinobacteria (44 %), Bacteroidetes (34,5 %), Proteobacteria (15,5 %) and Cyanobacteria (6 %). These results suggest a decreasing of Gram-positive bacteria and an increasing of Gram-negative bacteria in the microbial community of the sample taken 8 months after restoration when compared with the untreated one. On the other hand, the reverse is true for the microbial community of the samples taken 20 months after restoration. Since quaternary ammonium compounds are degraded by Gram-negative bacteria (McBain et al. 2004), the biocide can be used as carbon source. The exhaustion of the biocide-carbon source, promoting the growth of Gram-negative bacteria, will lead to the predominance of Gram-positive bacteria in the microbial community develoved after 20 months treatment, similar to that observed in the microbial community before treatment.

Actinobacteria was the most dominant group in all samples. The abundance of Actinobacteria in subterranean environments has been previously reported in numerous studies and this group constitute common members of the microbial community (Monte and Ferrari 1993, Groth et al. 1999, Laiz et al. 2002). Since the Actinobacteria are generally heterotrophic microorganisms, their growth must be dependent on organic matter being transported from the surface into the tomb.

Hence, although Actinobacteria take part in biodeterioration processes, they, and particularly *Streptomyces*, may act as antagonists to many different fungi that makes them promising candidates for biocontrol agents (Getha and Vikineswary 2002, Sharma et al. 2005).

In spite of DGGE banding patterns are subjected to PCR bias due to DNA extraction methods, potential preferential amplification, and the formation of chimeras (Wintzingerode et al. 1997), generally, the banding patterns obtained in this study can be considered to reflect the most abundant rRNA types in the community.

Our data have shown i) that the application of quaternary ammonium compounds had non mid- or long-term biocide effects on the microbial communities thrieving on mural paintings, and ii) that the effectiveness of restoration treatments can be evaluated by using a genetic fingerprint.

In conclusion, the monitoring of restored monuments over time and the knowledge on microbial communities structure and biodiversity can lead to better understanding, interpretation and prediction of the effects that biocide treatments may produce on Cultural Heritage assets.

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APPLICATION OF LASER INDUCED BREAKDOWN SPECTROSCOPY (LIBS) TO CULTURAL HERITAGE

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Introduction

Laser Induced Breakdown Spectroscopy (LIBS) is an analytical technique in which a powerful laser beam is focused on a sample. As result of irradiation, some amount of material is ablated and a plasma is formed. Light emitted by the plasma is composed of spectral lines characteristic of the elements present in the sample. By analyzing this light, it is possible to deduce the elemental composition of the material (Cremers 2006).

LIBS features several advantages that are interesting for the analysis of Cultural Heritage objects. The analysis can be performed in situ and only requires an optical contact with the object. The technique doesn't need sampling, nor sample preparation. Furthermore, LIBS is a very rapid technique, as the information is recorded with a single laser pulse measurement. The technique is micro destructive, as the material ablated from the surface is minimal. In addition, LIBS allows