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A concerted action from the European Commission (EVK4-CT-1999-2001) on molecular microbiology as an innovative conservation strategy for indoor and outdoor cultural assets

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Short Communications

ALTERATION OF STONE MONUMENTS: PHOTOTROPHIC MICROBIODETERIOGENS

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Monuments located outdoors are affected not only by physical and chemical weathering but also by biological activities of stone-dwelling microorganisms, among which the phototrophs often prevail (Gómez-Alarcón et al. 1995; Urzì et al., 1994; Ortega-Calvo et al. 1993). One of the consequences of microbial development is the formation of thick patinas with intense pigmentation varying from green to dark-green or dark red, which considerably alter the aesthetic appearance of the monuments, like those covering the statues shown in Figures 1-2.

Fig 1. Sandstone statue, Boboli Gardens, Florence (Italy).

Microbial biofilms do not only produce aesthetic damage, but can cause stone surface weathering, enhancing the loss of stone particles from the crystalline structure (Krumbein, 1988).

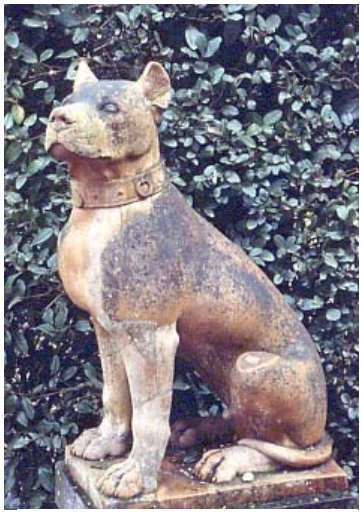


Fig 2. Brick statue, Boboli Gardens, Florence (Italy).

In fact, the microorganisms can develop into the porosity and the first layers of stone material (Fig 3), and produce scaling and detachment

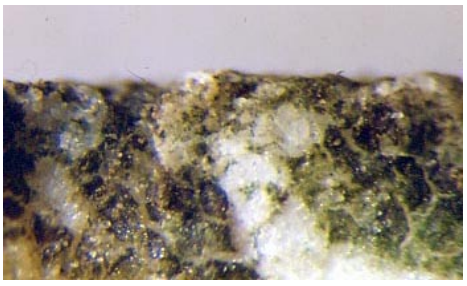


Fig. 3. Green phototrophic micro-organisms developed into the surficial layers of a Marble statue (x 40).

Phototrophic microorganisms are usually prevalent in microbial biofilms (Figs 3, 4). Many phototrophic microorganisms are able to produce dark coloured sunscreen pigments, as shown in Figure 4 (right).

These substances which protect the cells against UV radiation and high light intensities contribute to darken the biofilms of the stone surface.

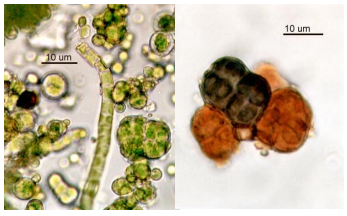


Fig 4. Phototrophic biocoenoses (bar 10 µm).

The phototrophic microorganisms present in the biofilms collected from Italian stone monuments are prevailingly constituted by chlorophyta and cyanobacteria. These are usually identified following traditional techniques, but more recently molecular techniques have been introduced for a proper identification and with the objective to recognise these microorganisms directly on stone monuments. As example we report the procedure used for the identification of cyanobacteria by detecting the intergenic spacer (PC-IGS) between genes encoding for phycocyanin (a cyanobacterial specific protein) and using as references the cyanobacterial strains isolated from Italian monuments and PCC strains (Tomaselli *et al.* 2000).

The cyanobacterial strains were isolated from colonies developed on agarised cultural media (BG-11$_0$ and BG-11, Rippka *et al.*, 1979) in Petri dishes inoculated with samples collected from several Italian stone monuments. The isolated strains were identified according to the diagnostic keys reported in the 3rd volume of Bergey's Manual of Systematic Bacteriology (Castenholz & Waterbury, 1989). The cyanobacterial strains were purified by repeatedly cells washing followed by streaking in Petri dishes on the agarised cultural media. These strains purified and characterised constitute now a specific collection of phototrophic stone microbiodeteriogens.

The molecular analysis was performed both on some axenic strains of the collection (Table 1) and on the biofilms collected from stone monuments (Table 2).

Table 1. Cyanobacterial strains used and generic assignment.

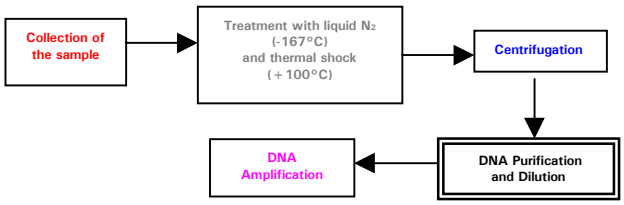
Strain	Origin	Assignment
C6	Leaning Tower (PI)	*Synechococcus* sp.
C8	Leaning Tower (PI)	*Leptolyngbya* sp.
C9	Leaning Tower (PI)	*Pleurocapsales*
C11	Leaning Tower (PI)	*Pleurocapsales*
Li-f	Medici Fortress (LI)	*Plectonema* sp.
Li-m	Medici Fortress (LI)	*Myxosarcina* sp.
Vol	Roman statue Volterra (SI)	*Phormidium* sp.
Peg	Boboli Garden statues (FI)	*Phormidium* sp.
Mu-2	Boboli Garden statues (FI)	*Pleurocapsales*
Mu-c	Boboli Garden statues (FI)	*Pleurocapsales*
Mu-sc	Boboli Garden statues (FI)	*Scytonema* sp.
Mu-pl	Boboli Garden statues (FI)	*Plectonema* sp.
Bg-c	Boboli Garden statues (FI)	*Pleurocapsales*
PCC 6307	Pasteur Culture Collection, Paris	*Synechococcus*
PCC 6308	Pasteur Culture Collection, Paris	*Synechocystis*
PCC 73106	Pasteur Culture Collection, Paris	*Gloeocapsa*
PCC 6306	Pasteur Culture Collection, Paris	*Leptolyngbya*

Table 2. Biofilms and their origin.

Biofilm	Origin
Dark patina	Marble substrate, Cascine Park (FI)
Dark patina	Sandstone substrate, Cascine Park Pyramid (FI)
Green patina	Sandstone substrate, Cascine Park Pyramid (FI)

The axenic cyanobacterial strains were aseptically grown in liquid media under controlled conditions. Cells of each strain were collected by centrifugation and the genomic DNAs were extracted to perform ARDRA (Amplified Ribosomal DNA Restriction Analysis) (Lamenti *et al.* 1999). The biofilms collected by scraping the stone surface were treated, in order to extract the DNA, with different methods: thermal shock; ultrasonic waves (Sonier); homogenisation in mortar with a buffer used for cell lysis or with liquid nitrogen. Among the tested methods we found that the most efficient procedures to extract the DNA was to pestle the patina in mortar with liquid nitrogen followed by the application of thermal shock to the suspension (Graph 1).

Graph 1. Scheme of treatment of biofilm samples for DNA extraction.



The extracted DNAs of axenic cyanobacterial strains were amplified for the 16S gene with the universal primers and for the intergenic region between the *cpc*A and *cpc*B genes encoding for the phycocyanin (PC-IGS), then the amplified products were digested with restriction endonucleases.

The restriction profile analysis of the amplified 16S genes for the strains reported in Table 1, permitted the construction of the dendrogram showing the similarities among the strains (Fig. 5).

This preliminary characterisation at genomic level (ARDRA) of phototrophic micro-organisms isolated from Italian monuments permitted us to have an indication of the biodiversity of culturable cyanobacterial strains present in the biofilms. A more extensive isolation and

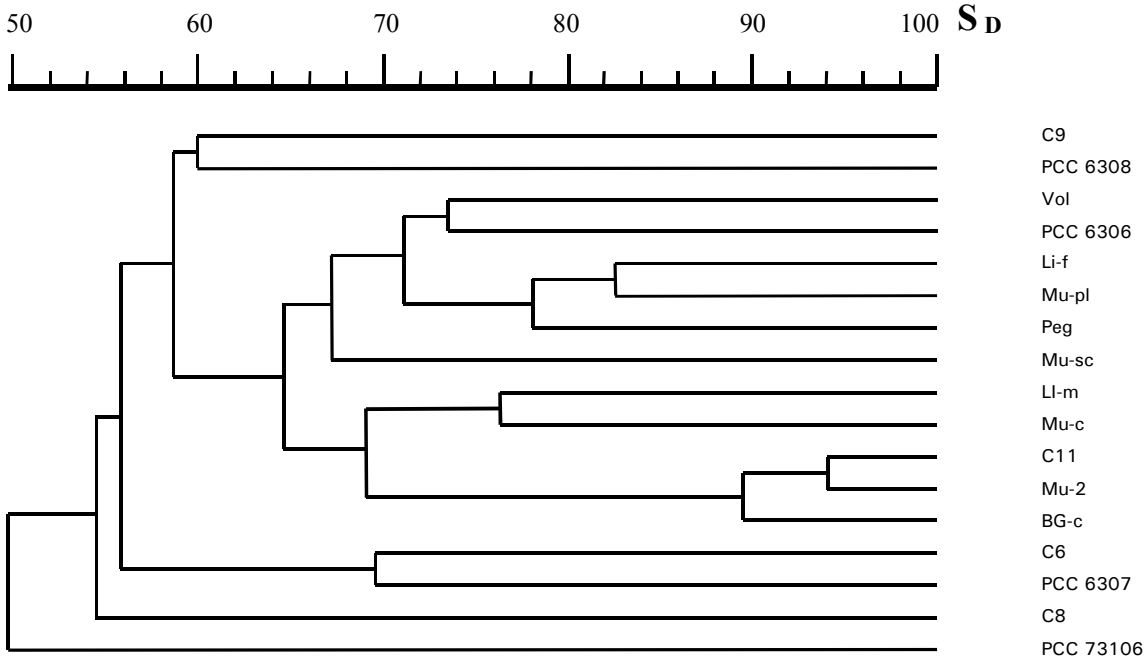


Fig. 5. UPGMA dendrogram of cyanobacterial 16S ARDRA on combined profiles from five endonucleases. S_D , Dice similarity coefficient (%).

purification work is ongoing in order to clarify the genetic structure of the sampled cyanobacterial biofilms.

The DNAs extracted from patinas with different methods were tested for the quality and amplified for PC-IGS. Although DNAs extracted from patinas treated with liquid nitrogen resulted to be of good quality and gave positive results for the amplification with universal primers, it failed to be amplified for PC-IGS. This fact could be explained by the presence of dead cyanobacterial cells in the biofilm or of cyanobacterial strains different from those used as reference, which could have mutations in the sequences where primers link.

On the other hand, the positive amplification for PC-IGS of the isolated strains suggests that such PCR target could be useful to detect the presence and to identify cyanobacterial strains directly in biofilms, provided that a specific data bank of cyanobacterial biodeteriogens is available.

Acknowledgements

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MICROBIAL CONTAMINATION AND INSECT INFESTATION IN SPANISH MUSEUMS, ARCHIVES AND LIBRARIES

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Over the last 10 years a survey has been carried out in Spanish museums, archives and libraries to detect the most common microorganisms and insects involved in the deterioration of historical objects. The results showed about 40 different strains of microorganisms identified from bioaerosols, in museums, and 75 from in archives and libraries. It was also found that biodeterioration of cellulose and proteinaceous materials was produced by 30 species of insects. However in general, insect infestation presented higher incidence than microbial contamination in the deterioration of objects located in cultural institutions.

Very often, collections are exhibited in historical buildings that maintain micro-environments appropriated for the development of fungi, bacteria and insects, on their objects including cultural properties made of cellulose; books, textiles, furniture, paintings, wood sculptures, altar pieces, and proteinaceous materials such as parchment, vellum, leather, mummy skin, and synthetic materials.

Many fungal and bacterial species start their development depending on the available moisture on the surface of an object. In this context, scantly research has been done on the effect of moisture content in a material and the appropriate water activity which determines the water available for the germination of microbial spores and indicates the risk of microbial contamination in a support. In addition, air ventilation should be taken into account. It contributes to inhibit microbial growth in both environment and objects.

In Spanish museums and archives the most common species of microorganisms isolated in recent searches belong to: *Alternaria solani, Alternaria tenuis, Aspergillus niger, Aspergillus luteus, Aspergillus flavus, Aspergillus fumigatus, Aureobasidium pullulans, Cladosporium herbarum, Cladosporium cladosporoides, Chaetomium globosum, Chaetomium sp., Fusarium roseum, Fusarium solani, Geothrichum sp. Gliocadium sp., Mucor racemosus, Penicillium glaucum, Rhizopus oryzae, Penicillium frequentans, Penicillium notatum, Penicillium griseofulvum, Penicillium chrysogenum, Rhizopus nigricans, Stachybotrys sp., Trichoderma viride, Trichothecium sp., Ulocladium, sp.*

These microorganisms produce deterioration of paper, adhesives and plastic materials. In this context, fungi with potential patological effects to people have been described including: *Alternaria solani, Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor, Aspergillus luteus, Chaetomium globosum, Cladosporium cladosporoides, Fusarium solani, Mucor racemmosus, Penicillium glaucum, Rhizopus oryzae, Trichoderma viride.*

In proteinaceous materials, it has been found that anaerobic bacteria are the most deleterious to parchment. Collagen can be hydrolysed by collagenase produced by bacteria such as *Clostridium*. Strains of *Bacillus*, *Pseudomonas*, *Sarcina* and *Bacteroides* induce collagen degradation in anaerobic conditions. Other proteins and lipids of parchment may also be altered by enzymes that are products of aerobic fungal and bacterial species. *Bacillus subtilis* exhibits very high activity in hydrolyzing native collagen which occurs above 95% RH.

In addition, several species corresponding to *Actinomyces* have been detected on organic and inorganic materials.

In the literature it has been reported microorganisms isolated from both environment and objects (cellulose and proteinaceous) located in museums and archives. They are as follows:

Fungi

Acremonium sp., Alternaria tenuis, Alternaria solani, Alternaria alternata, Aspergillus niger, Aspergillus flavus, Aspergillus tamari, Aspergillus versicolor, Cladosporium elatum, Cladosporium cladosporoides, Cladosporium herbarum, Cephalosporium sp., Curvularia lunata, Chaetomium globosum, Chaetomium succineum, Fusarium roseum, Fusarium solani, Fusarium oxysporum., Geothrichum sp., Gliocadium sp., Mixotrichum sp., Monilia macrospora, Mucor racemosus, Mycoderma sp., Myrothecium verrucaria, Ophistoma sp., Paecylomyces variabilis, Penicillium bevicompactum, Penicillium frequentans, Penicillium chrysogenum, Pestalotia oxyanthi, Phoma glomerata, Rhizopus nigricans, Trichothecium roseum, Trichothecium sp., Trichoderma viride, Trichoderma longibrachiatum, Trichoderma lignorum, Ulocladium botrytis, Verticillium chlamydosporium, Verticillium albo-atrum, Scopularioupsis brevicaule, Scopulariopsis acremonium, Stachybotrys atra, Spicaria sp.

Bacteria

Aeromonas caviae, Aeromonas sp., Bacillus subtilis, Bacillus cereus, Bacillus circulans, Cellulomonas sp., Cellulomonas cellasea, Cellulomonas cellulans, Cellvibrio mixtus, Chromobacterium sp., Cytophaga aurantiaca, Flavobacterium breve, Micrococcus luteus, Micrococus roseus, Micrococcus varians, Pseudomanas fluorescens, Pseudomonas elongata, Streptococcus sp., Streptomyces rimosus, Staphylococcus sp., Clostridium sp., Vibrio sp. Xanthomonas sp.

Different studies related to biodeterioration in European museums showed similar microbial species isolated from organic materials. However, more research is required to understand the biological activity of specific strains on museum objects exposed to particular microclimatic conditions. It is also necessary: determine levels of water activity in relation to the nature of the object, enzime production and metabolites excreted by microorganisms involved in biodeterioration, thresols for the development of specific organisms in both objects and environment and identification of species dangerous for people and for historic materials.

In the archives analysed it was recently detected a significant increase of professionals suffering physiological illnesses relating to indoor air pollution. In fact, it has been reported that spores and hifae fragments included in airborne biological particles play an important role in allergies, skin and systematic mycoses. Consequently, the pathology of the isolated species should be considered to establish regulations and guidelines in preventive conservation in museums, archives and libraries.

Insects

In Spain the most serious bio-deterioration problems produced by insects were found in Galicia and on the Mediterranean coast, basically in the Levant and southern Andalusia. This is due to the climatic factors of high relative humidity (RH) and moderate temperatures, the inappropriate maintenance and micro-environmental conditions of the museum or archive buildings themselves.

It has been found a progressive infestation of art collections by *Anobium punctatum, Lyctus bruneus, Hylotrupes bajulus, Anthrenus flavipes, Attagenus unicolor, Tineola biselliella,* and specially by the termite *Reticulitermes lucifugus*. The latter was detected on the Mediterranean coast and in the southern, north-western and central regions of Spain.

Species of the Anobiidae family are commonly found in works of art made from pine, oak, walnut, cedar, cherry, holm-oak, cork and chestnut. Cerambycidae insects were isolated from pine, cherry, oak, holm-oak, cork and walnut wood. Dermestidae species are mainly isolated from textiles made of silk and wool, and from wooden objects made with the help of organic adhesives.

At present, microbial and insect taxonomic studies in biodeteriorated

objects are being carried out using conventional methods. However, very often these methods are time-consuming to develop and ineffective to determine some specific strains. For this reason, more accurate and rapid analyses including molecular techniques to optimize diagnostic studies on biodeterioration of Cultural Heritage.

Reports

SYMPOSIUM ON TECHNOLOGY AND THE PROTECTION OF CULTURAL HERITAGE MATERIALS

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Conservation of natural resources has been studied intensively in the United States during the past quarter century, with significant positive results for preservation of the natural environment. However, research into conservation of cultural heritage materials, and particularly biodeterioration processes, has not had a high priority. Funding for research is minimal, and there are few laboratories with active programs. A symposium was held at the annual conference of the American Association for Advancement of Science, held in San Francisco in February 2001. The symposium title was "Technology and the Protection of Cultural Heritage Materials". The objective was to make American scientists aware of the current challenges and innovations in the field of cultural heritage materials research.

The symposium was organized by Ralph Mitchell. In his introduction he described some of the recent major advances in microbiological research. These included the use of molecular biology and biotechnology methods of analysis, biochemical processes involved in deterioration, and novel approaches to control of biodeterioration. Participants described innovations in the fields of diagnosis and control of deterioration of works of art, historic documents, natural history and museum collections, and historic buildings and archeological sites.

Barbara Berrie of the National Gallery of Art in Washington described the application of chemical analytic techniques in the reversal of deterioration of works of art. One example showed how an understanding of corrosion processes could be utilized to control deterioration of metal objects. She also described the use of enzymes to clean works of art.

Norbert Baer from New York University discussed innovations being utilized to protect historic documents in the National Archives. He explained how the U.S. Declaration of Independence is being analyzed for deterioration, using modern non-invasive methods. His presentation also included a description of methods being used to preserve historic film and audio tapes being stored in the National Archives.

Carolyn Rose of the Smithsonian Institution Natural History Museum emphasized the need to move from remedial measures to preventative strategies to protect ethnographic and archeological objects. She described the use of computer tomography for early detection and the application of laser cleaning techniques.

The Getty Conservation Institute's extensive efforts to provide long-term preservation of Mayan archeological sites were described by the Institutes director, Tim Whalen. He demonstrated how a multidisciplinary approach could be used as a model for preservation of archeological sites.

The conservation of library, archival and museum collections was discussed by Jim Reilly of the Rochester Institute of Technology. He showed how the application of decay kinetics could be used to develop a predictive model for decay of organic objects in collections. The model is being tested on a wide range of objects in museums and archives.

It was clear from the positive response of the audience that these presentations provided our scientific colleagues with a new insight into conservation research. Further symposia at scientific conferences are planned as a means of increasing conservation research programs in the United States.

Participants

Norbert Baer. Conservation Center. New York University

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Carolyn Rose. Department of Anthropology. National Museum of Natural History. Smithsonian Institution. Washington, DC

Timothy Whalen. Getty Institute of Conservation. Los Angeles, CA

Forthcoming Activities

Steering Commitee meeting S2. Seville, Spain, September 28-30, 2001.

COALITION Workshop 2: Novel molecular methodologies. Luckenwalde, Germany, March/April 2002.

Call for papers

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