The European Commission
Community Research
Energy, Environment and Sustainable Development

COALITION

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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1. Presentation of COALITION

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Microorganisms can be responsible for the destruction of monuments, buildings and cultural heritage assets, including the decay of concrete, cement, stones, mural paintings, etc. These microorganisms are not only related with biodeterioration but water-damaged indoor building materials are frequently colonized by complex bacterial communities and may emit mixed bioaerosols into the indoor air. Therefore, historic buildings with a history of moisture damage must be investigated from two point of views: conservation of the cultural heritage and for the etiology of health symtoms associated with indoor air.

The study of microbial communities is usually accomplished by using traditional culture methods. However, it is believed that less than 10 % of the extant microorganisms have been discovered in individual ecosystems, and that culture methods are partially inadequate for studing microbial natural community compositions. In the study of cultural assets the situation is particularly worrying because traditional culture methods are biased by the limitations imposed by the conservation of the work of art and the very rare availability of often extremely small samples. To the limited information provided by traditional culture techniques the constraints originated by the sampling methods, the reproducibility of the physiological niches, etc. are added. Therefore, it can be assumed that probably 90 % or more of the microorganisms present in cultural assets remained undiscovered. This is of great importance, as most restoration works apply conventional biocides which probably are not suitable for the unknown and complex microbial communities growing on and beneath the surfaces. Current restoration efforts can conceivably have an opposite effect, particularly if these measures use substrates which support the growth of microorganisms and, consequently, accelerate the deterioration process. Often not sufficiently respected, microbial colonization should be taken into consideration when planning the restoration.

Molecular ecology techniques so far have usually been applied to aquatic environments. Aquatic and soil molecular ecology is much more advanced than the attitude in monuments and in the cultural heritage protection, as the cultural heritage poses many more problems in the identification of detrimental biodiversity versus neutral or even protective biodiversity than in any other environment. This is intended to be changed by an European project, whose acronym is COALITION. The project started April 1, 2000 and is funded by the European Commission.

OBJECTIVES

1. To identify, introduce and enhance the use of molecular biology and biotechnology techniques suitable to be of interest in the field of conservation/restoration of the cultural heritage. Common methods include microbial cell counting or isolation and subsequent identification of laboratory pure cultures. These methods are not only time-consuming but also have the disadvantage that relatively large amounts of sample material are needed. It is therefore of great interest to provide fast, straightforward methodologies, as conventional methods are carried out by scientists using complex taxonomic and ecological approaches. A workshop will help to consolidate and improve upon the state-of-the-art of molecular techniques and recommen-dations on its use will be generated. The goal is to identify and enhance the use of a set of methodologies affordable by restoration or maintenance companies. This will be done with inputs from previous and on-going European and national projects. The benefit obtained from the application of these techniques will be: i) the

minimization of sampling, ii) the optimization of information in diagnostic studies on microbial contamination of cultural assets, and iii) to analyse the potential health hazard, such as toxic or otherwise bioreactive metabolites by the organisms present in the objects undergoing restoration. These data will form the basis of guidelines for health protection of restorers and museum workers.

2. To obtain information on the type of microorganisms colonizing different and representative materials, by producing an inventory of the microorganisms associated with the damages to cultural assets. This is a prerequisite to include biodecay as an integral part of the restoration process and will allow i) to design the most effective treatments for eliminating active microbial communities and biodeterioration, and ii) testing the efficiency of biocide and cleaning treatments as well as follow-up of procedures.

3. To disseminate the advantages of using molecular techniques for diagnostic purposes to end users, e.g. architects, restorers, curators, responsible for cultural heritage, etc. This will be achieved by producing guidelines and recommendations for effective evaluation of microbial activities and for safety manipulation of contaminated objects. The data will be discussed in a panel with stakeholders and end-users and the transfer of technology from the research community to the conservation/restoration community will be critically addressed.

It is demonstrated that water-damaged indoor building materials contained substances of microbial origin extremely toxic to eukaryotic cells and large quantities of several microbial species known to be potentially toxic or immunopotentating. These include peptidoglycans, gram-negative endotoxin, β-1,3-D glucans, lipoglycans and teichoic acids and mycotoxins. Therefore, detailed knowledge on the species and hazardous properties of microorganisms, and their emitted metabolites, in historic buildings and cultural objects are of great interest, not only concerning health and safety (including working conditions) but of socio-economic value when decisions are made on the methods of renovation and the suitability of the historic buildings for residential or professional uses and the need for protecting the renovators' health. To this end COALITION will undertake the following tasks:

1. Toxicity and biohazard detection methods which include novel biochemical toxicity tests using specific biochemical activities of spermatozoan and human peripheral blood lymphocytes as toxicity endpoints. The new tests are tools for assessing of immunotoxicity, mitochondrial toxicity, effects on the cellular motility and signal trafficking systems and membrane integrity.

2. Degradative capacities from isolated microbial cultures or directly from material samples will be measured using fluorogenic enzyme substrates and kinetic fluorometry. For instance, infestation of the material by fungi can sensitively be assessed by measuring acidophilic cellulase and chitinase activity.

COALITION will bring together clusters of researchers in different areas, with particular emphasis in molecular microbiology, microbial ecology and biodeterioration and will achieve the exchange of results of ongoing work, the development of new research initiatives, the preparation of ”state of the art” reviews, and the coordination of research on cultural heritage that is already funded by the European Commission. In addition, the project will foster cooperation and dissemination between scientists, conservators and restorers inside and outside the European Union and training of molecular microbiology techniques.

It is expected that the project will greatly increase our knowledge and especially the exchange of views on the appropriate techniques to study the detrimental heterotrophs involved in biodeterioration on a molecular level. The state of the art

in the field of biodeterioration is practically leaping forward through the project inasmuch as it combines the major lab groups in this field existing in Europe.

DISSEMINATION

Electronic newsletter. An electronic newsletter will be used as the communication media of the project which will help in efficient communication between the members, and also will be distributed to those joined the dissemination list.

Web-site. A web-site will be created and linked to the European Commission, General Directorate XII as well as to the on-going projects which already have a web-site. The web-site will contain the objectives, description of the project and the main events to be organized, as well as a call for external involvement.

External involvements. The project is open for those with a interest in being involved in the workshops (independient observers) and conferences or the output of particular themes. A distribution list will be created for those with an interest in obtaining outputs, particularly the electronic newsletter. They will regularly receive workshops announcements, call for papers or reviews and newsletters that are produced by the project.

Advanced course. The project will train and educate young biologists, conservators and restorers in the application of molecular microbiology techniques for conservation diagnosis by organizing an advanced course.

International conference. COALITION will organize an international conference on Cultural Heritage and Molecular Biology in Seville at the end of the year 2003. This will involve contributions and participations from researches, conservators, restorators and cultural heritage authorities and will be the final deliverable of the project. The conference will disseminate the data collected and reviewed by the members along the duration of the project, and the invited lectures and contributions will be published in a special issue of an international journal.

PARTICIPANTS

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2. Two new projects join COALITION

In the last call for research projects (February 15, 2000) a number of proposals were approved and funded by the European Comission. Two of these projects are related with biodeterioration problems and agreed to join COALITION

BIOMEDIATED CALCITE PRECIPITATION FOR MONUMENTAL STONES REINFORCEMENT (BIOREINFORCE-EVK4-2000-22027)

Piero Tiano
C. S. Opere d'Arte, Firenze, Italy

Monumental stone decay is a consequence of the interaction between the material and the environmental parameters such as water, heat, atmospheric pollutants and living organisms. This interaction starts at the stone surface and progress inward leading to progressive increasing of stone porosity and weakening of the cohesion of the mineral structures. Conservation of monuments foresee in most cases the application of consolidating products in order to strengthen the weathered stones and to avoid dwindling of material. Numerous kind of products both inorganic or organic have been used to this aim but almost all have showed, in time, different negative results, such as colour changes, crust formation, glossy appearance and substrate exfoliation. Furthermore, the synthetic organic products are normally formulated and applied in solvent, thus contributing to the increase of the environmental pollution. The efficiency of these products, due to their exposition to the aggressive polluted air, has shown a very short duration.

Actually, after the indiscriminate application of almost all categories of new synthetic resins, the unique treatment applied seems based on ethyl silicate formulates. Even if this kind of product should be used chiefly only on silica based stone material (sandstones), the consolidating action is quite weak due to the nature of the product used. The people involved in monument repair and maintenance are seeking for treatments safer and longer lasting in order to overcame the drawbacks related with the use of not suitable synthetic materials and to increase the time necessary between successive interventions.

The coordinating institution is deeply involved from long time in the conservation of cultural heritage and collaborates with restorers. Our chief interest is to introduce in the field, using scientific and technological approach, safer and improved techniques for the conservation of monumental stones. For these reasons we suggest the use of alternative treatments based on new calcite precipitation bio-induced by water soluble macromolecules. This method, once successful applied on monumental stones, will induce the formation, inside the stone porosity, of new calcite crystals, contributing to increase the stone cohesion. These new crystals behave in the same way of the original constituent of calcareous material overcoming the negative effects recorded on monumental stones after the application of organic resins.

Scientific objectives and approach

The objective of the project is to develop and validate a new methodology for monumental stones conservation based on biomineralisation processes. This could satisfy the request for more durable and safer products in order to reduce the costs, delay the maintenance interventions and pose no risk both for the personnel and the environment, conciliating the end-users and stakeholders with the application of innovative treatments.

Actually this type of intervention can be made using bio-mediated processes with the application of either organic-matrix macromolecules extracted from sea shells, or of viable calcinogenic bacteria. Both methods are unsatisfactory and the

biomediated approach needs to be improved; from one side by increasing the very low yield of organic-matrix macromolecules, as actually produced with sea shells extraction, and from the other side avoiding the secondary negative effects due to the application of viable microorganisms.

The molecular biology and the bacterial genetic engineering are the innovative technologies chosen to improve the bioinducing calcite precipitation method. These tools will be applied for finding the genetic expression of crystal formation in bacteria. This will be cloned and the bio-inducing proteins will be produced, in sufficient amounts, in an appropriate expression vector (host cell). At the end of the project we will have a great availability of inducing organic macromolecules produced by biotechnological method. With these a Bio-Mediated calcite Treatment (BMT) will be developed and validated in laboratory and outdoor conditions, evaluating its efficiency in the stone reinforcement, due to new calcite precipitation inside porosity induced by specific bio-derived low cost renewable macromolecules.

Expected impacts

Public and private institutions involved in historical buildings repair and maintenance need safer methodologies for stone materials and the environment. Once the new method is successfully validated we can apply, for the restoration of monumental stones, a new treatment based on a product of the same nature of stone substrate, with the prospective of a longer lasting efficiency and a lower environmental impact. The safeguard of the cultural heritage is awaiting for scientifically endorsed new materials and procedures for conservation and if this problem will not resolved the monumental stones are exposed to a serious chance of loss or damage.

The new method will demonstrate its maximum efficiency for calcareous stones (like marble and limestones), but also for other lithotypes having a certainly carbonatic component (i.e. secondary calcite cement) or with a partially calcitic matrix. The successful of the project will furnish, in very short time, the end-users with a new tool to improve their skilful to perform safer and more reliable restoration interventions of monuments in line with a sustainable development.

Participants

- **CSCOA** - CNR - Centro Studi Opere Arte (Firenze, Italy) (Co-ordinator)
- **WIS** - The Weizmann Institute of Science, Department of Structural Biology (Rehovot, Israel)
- **LCM** - Loughborough University, Department of Chemistry (Loughborough, United Kingdom)
- **DBG** - Dept. of Animal Biology and Genetic, University (Firenze, Italy)
- **UBM** - University of Barcelona, Department of Crystallography and Mineralogy (Barcelona, Spain)
- **TRI** - Trivella spa, (Milano, Italy)
- **QUE** - Quélin SA, (Rueil-Malmaison, France)
- **KIK** - Institute Royale du Patrimonie Artistique (Brussels, Belgium)
- **CPP** - Circles des Parteinaires du Patrimoine, Laboratoire de Recerche des Monuments Historiques, (Champs sur Marne, France)

CYANOBACTERIA ATTACK ROCKS: CONTROL AND PREVENTIVE STRATEGIES TO AVOID DAMAGE CAUSED BY CYANOBACTERIA AND ASSOCIATED MICROORGANISMS IN ROMAN HYPOGEAN MONUMENTS (CATS – EN4-2000-00659)

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The proposal CATS focuses on the control, prevention and monitoring of cyanobacteria-dominated biofilms that cause damage to rock surfaces in Roman hypogea. It develops and integrates physical and biotechnological methods

intended to limit the growth of microorganisms on valuable archaeological surfaces, and applies analytical methods to monitor the presence and the extent of the microbial damage.

The overall objective of CATS will be to achieve a better understanding of biotransformation and biodecay processes of lithic substrata caused by the growth of biofilm-forming cyanobacteria in hypogean monuments. In addition, CATS intends to evaluate the applicability of a two-phase (physical plus biotechnological) strategy to decrease and inhibit the growth of phototrophic and heterotrophic microorganisms that cause severe damage mostly to calcareous rock surfaces in Roman hypogea. Accordingly, CATS will answer the following two major and essential questions in order subsequently to develop control and preventive strategies:

- How does microbial activity alter the mineralogical, textural and geochemical features of rocks?
- What conditions limiting growth of cyanobacteria can be safety applied in Roman hypogea?

To achieve these central objectives different types of microsensors will be developed. These will be used to quantify biologically induced variation of gases and ions on the colonised lithic substrata. Data on the petrological and geochemical characteristics of rocks and on structure, function and diversity of biofilms will be integrated with those obtained using microsensors in order to describe and model the damage of rock surfaces. This part of the project will end with the construction of a multiparametric portable device based on microsensors that will be produced as a new tool for microbial monitoring.

In the other part of the project, a pilot study will be set up to investigate the possibility of using a new lighting system providing wavelengths poorly used by cyanobacterial photosynthesis. This will drastically decrease the growth of cyanobacteria and therefore the quantity of organic matter available to the associated heterotrophic populations. Subsequently, the new lighting system will be experimentally set up *in situ* in order to confirm the laboratory results. At the end of this part, the public response to the innovative strategies proposed will be tested and the benefit to cost ratio of a new illumination system in Roman hypogea will be evaluated.

In addition to the physical approach, newly identified biomolecules related to iron metabolism and cell-to-cell signalling pathways will be checked for their ability to interfere with bacterial and, especially, cyanobacterial metabolism by removing factors indispensable to microbial development. The application of these environmental biotechnologies under laboratory conditions should provide a new method to control and prevent growth of phototrophic biofilms.

Specific objectives of the project are:

- to characterise the geological, geo- and hydrochemical, and physical environment of rocks unaffected or colonised by cyanobacterial communities inside Roman hypogea, and to evaluate possible preferences of cyanobacteria and associated microorganisms for specific lithologies;

- to describe the architecture and functioning of biofilms built by cyanobacteria and associated microorganisms on different types of lithic surfaces;

- to ascertain the most critical physical, chemical and biological factors that control colonisation of rock surfaces;

- to assess and quantify the damage caused by cyanobacterial biofilms to different types of surface;

- to develop new physical methods to control and prevent biofilm growth using wavelengths in the visible part

of the light spectrum that are, at best, poorly used by photosynthesis;

- to identify siderophores and cell-to-cell signalling biomolecules and experimentally to test their potential to interfere with biofilm development;

- to develop an innovative monitoring method using a multiparametric microsensor device for the measurement of biogeochemical parameters on endangered rock surfaces;

- to test the response and expectation of citizens to the innovative strategies proposed.

Participants

- **URTV.DB.LBV** – University of Rome "Tor Vergata", Department of Biology, Laboratory of Plant Biology, (Rome, Italy).
- **UNITOVRM** – University of Rome "Tor Vergata", Department of Science and Chemical Technology (Rome, Italy).
- **CSIC-IRNAS** – Instituto de Recursos Naturales y Agrobiología (Sevilla, Spain).
- **UB** – University of Barcelona, Department of Natural Products, Plant Biology and Soil Science, Faculty of Pharmacy (Barcelona, Spain).
- **UNIME01** - University of Messina, Istituto Policattedra di Microbiologia (Messina, Italy).
- **HKI** – Hans-Knöll-Institut für Naturstoff Forschung e.V. (Jena, Germany).
- **VTT** – Tecnical Research Centre of Finland, VTT Biotechnology, (Espoo, Finland).
- **U W Swansea** – University of Wales Swansea, Biochemistry Research Group (Swansea, United Kingdom).
- **IDRONAUT, S.R.L.** (Brugherio, Italy).
- **PCAS** – Pontificia Commisione di Archeologia Sacra (Rome, Italy).

3. Forthcoming activities

Steering Commitee Meeting S1: Barcelona (Spain), December 1-2, 2000.

First Coalition Workshop: Development, update and preparation of database: Gent (Belgium), March 8-10, 2001.

4. Call for papers

This newsletter is open to external contributions, including short communications and notes (maximum 2 pages), or critical comments (1 page) on the topics covered by COALITION.

5. Dissemination of this newsletter

This first issue is being sent to a wide list of people related with Cultural Heritage. However, for receiving the second and subsequent issues we request you to send us an e-mail to coalition@irnase.csic.es with the message: Subscribe COALITION.