Biodeterioration: An Overview of the State-of-the-Art and Assessment of Future Directions

C. SAIZ-JIMENEZ

Instituto de Recursos Naturales y Agrobiologia, CSIC, Apartado 1051, 41080 Sevilla, Spain

1. Introduction

Throughout history humans have used the most beautiful and durable stones for monumental buildings. Beginning in the sixth century BC, marbles extracted from the Pentelikon quarry became a common building material. Abundant limestone and marble quarries enabled the ancient Greek architects to build the Acropolis and Roman architects to build the Forum. These monuments, sculptures and stony object of art exposed to open air have deteriorated, through the centuries, due to natural causes.

Colonisation of microorganisms on building materials and biodeterioration are usually linked to environmental conditions. The most significant parameters affecting microbial growth are represented by physical factors, mainly moisture, temperature, and light, as well as by the chemical nature of the substratum. In present days, however, industrial and urban activities have modified the composition of the atmosphere, resulting in a more agressive environment. Obviously, these antropogenic compounds have inffluenced to some extent the colonisation and growth pattern of microorganisms in polluted urban environments. To this respect, it is worth of mention the destructive effect of the lichen *Dirina massiliensis* forma *sorediata*, which was observed on Italian, Spanish and Portuguese monuments during the last two decades. Although lichens are usually regarded as agents of major biodeterioration on a geological, rather than historical, time-scale, Seaward and Edwards (1995) concluded that there is strong evidence to suggest that recent environmental changes have been conducive to increasingly detrimental invasion by this aggressive species. This lichen is extending its ecological range in many areas of Europe, probably due to its reproductive strategy, its ability to exploit man-made substrata (Seaward, 1989), and the absence of other competing species resulting from the increasing air pollution.

Although atmospheric pollution is generally recognized as a significant factor in the deterioration of cultural properties, biodeterioration deserves more attention that has received, as in fact, a great variety of organisms colonizing stones, including bacteria, fungi, algae, lichens, mosses and plants have been reported (Saiz-Jimenez, 1994, 2001; Tiano, 1994, 1998).

2. Major gaps in research

According to Amann et al. (1995) among the disciplines devoted to studying the different life forms on our planet, microbiology was the last to be established. In contrast to animals and plants, the small organisms that can visualized only by using microscopes have a morphology too simple to serve as a basis for a sound classification and to allow for reliable identification.

Until very recently, microbial identification required the isolation of pure cultures followed by testing for multiple physiological and biochemical tests. The use of an approach aiming at the isolation of cultivable microorganisms was the major gap in biodeterioration studies on monuments in the last decades. In fact, taxonomists considered monuments as an environment where an array of conventional microbiological approaches could be displayed and, as a consequence, the publication of lists of isolated microorganisms was common from the 60's to early the 90's.

Most microorganisms isolated from weathered stones are usually ubiquitous and their dispersion forms readily distributed through the atmosphere (e.g. species of *Bacillus, Micrococcus* among bacteria, and of *Aspergillus, Penicillium, Cladosporium* among fungi). The isolated species are common in floristic listing and have a worldwide distribution. This could conduce to the erroneous statement that common microorganisms in nature are also the agent of biodeterioration. The situation is more complex, as the presence of microorganisms on decayed material does not necessarily imply that it has caused the damage observed. In fact, its presence could be merely incidental (airborne deposition of spores o propagules) and, therefore, the value of flora listings is small.

Nature can cultivate all extant microorganisms but no laboratory, which is a considerable bias for the searching of biodeteriogens. Culture media with high content of organic substances are generally used to isolate and growth environmental microorganisms. These culture media contain carbohydrates, proteins, vitamins, etc. in concentrations far exceeding those the organism would ever encounter in nature, and to which was adapted for maximum efficiency of utilization. In the laboratory, rich nutrient media are utilized because promotes microbial growth, reduce incubation times and promotes the production of industrially useful metabolites, but when dealing with the study of microbial communities they can mask the real microorganism distribution and isolate selectively airborne propagules and/or fast-growing species instead of the active microorganisms functioning in the ecosystem (Laiz et al. 2001). On the other hand, the abundant production of secondary metabolites (e.g. organic acids), concomitant with rich nutrient media, seems to be a pathological behaviour resulting directly from a metabolic overflow and could not be extrapolated to nature (Saiz-Jimenez, 1995). Therefore, it is clear that most publications on biodeterioration have considerable bias because the list of species only refers to those easily cultivable and omit slow growing and uncultivable microorganims. In addition, the assumption that microorganisms could be deletereous due to the production of organic acids -as observed in laboratory conditions- in most cases is a speculation.

There is an increasing evidence that not all microorganisms from an ecosystem can be cultivated and many unknown species have never been cultivated before for lack of suitable methods. In a classical study, Ward et al. (1990) considered that 80 % or more of the microorganisms remain undiscovered and this raised the question of how well we know the Earth's biota and its biochemical potential. In addition, several reports stated that only a small portion, typically far less than 1% of organisms in the environment can be cultivated by standard techniques and that culturable diversity underrepresents environmental diversity (Pace, 1996). If we extent this to the microbial communities involved in biodeterioration, we must agree that our knowledge of the whole process and the involved agents is very scanty. This was further proved in the last years by the application of novel molecular biology tools in searching of microbes involved in the deterioration of mural paintings. This research was performed in the framework of the European Commission (EC) project "Novel molecular tools for the analysis of unknown microbial communities of mural paintings and their implementation into the conservation/restoration practice", (ENV4-CT98-0705 - MICROCORE). In this project, new DNA based techniques have been applied to mural paintings which allow the identification of individual microbial species in sample material without the cultivation of the organisms.

Rölleke et al. (1996) used a molecular approach based on the detection and identification of DNA sequences encoding rRNA (rDNA) to identify bacteria present on ancient wall paintings, since it was shown that most of these bacteria cannot be cultivated under laboratory conditions. They identified members or close relatives of the genera *Halomonas, Clostridium,* and *Frankia*. Such groups of bacteria were not previously isolated or mentioned as involved in biodeterioration processes of wall paintings. On the other hand, many members of the genera *Bacillus, Micrococcus, Arthrobacter*, etc. which were cultivated and isolated from the samples were not observed using the molecular approach.

In further studies it was found that members of the domain *Archaea* were common to mural paintings in spite of the very different environmental conditions (Piñar et al. 2001). *Archaea* were restricted to extreme environments (high temperature, high ionic strenght, or extremely anaerobic surroundings). This perception, based on the properties of a few cultivated organisms proved incorrect and natural population studies using rRNA gene cloning and sequencing revealed that *Archaea* are abundantly distributed in a wide variety of environments (Pace, 1996). To these environments should be added mural paintings and, probably in general, most deteriorated monuments where efflorescences are abundantly represented.

Not only *Archaea*, but other rare microbes such as members of the genus *Rubrobacter* were identified from mural paintings in Austria and Germany (Shabereiter-Gurtner et al. 2001). The two known species of this genus are thermophilic, and were isolated from hot springs in Japan and Portugal, and thermally polluted effluents in the United Kingdom. The 16S rDNA sequence analyses obtained from the mural paintings revealed that they may either represent new species within the genus *Rubrobacter* or a new genus very closely related to *Rubrobacter.* These data clearly illustrates how much is unknown on the microbial ecology of deteriorated monuments, as the picture obtained using a molecular approach was completely different to those obtained using conventional culture methods. In other words, there was not a correspondence between isolated bacteria and identified DNA sequences. This stressed the need to reconcile the data obtained using both methodologies in order to progress further.

In the study of microbial communities present in 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. According to the above mentioned statements the introduction and dissemination of molecular biology and biotechnology techniques in the whole conservation/restoration process of cultural heritage assets will represent an advancement for this particular field.

In this sense, an EC “Concerted action on molecular microbiology as an innovative conservation strategy for indoor and outdoor cultural assets” (EVK4-CT-1999-20001 - COALITION) was recently launched. One of the objectives of COALITION is 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 microbiological methods are carried out by scientists using complex taxonomic and ecological approaches. The goal is to identify and enhance the use of a set of methodologies affordable by restoration or maintenance companies. 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. The concerted action intend 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.

In the 2001 call for research projects, the EC funded two projects where molecular tools will be used: BACPOLES (EVK4-2001-00009) and VIDRIO (EVK4-2001-00013). In a report from the EC the first project was considered to present a revolutionary method for the prevention of bacterial decay in wooden foundation poles and archaeological sites. BACPOLES will explore the development of protection strategies based on the possibility to use phages (an innovative medical technique new to wood science). VIDRIO will employ

PCR techniques for amplification of bacteria and fungi involved in the deterioration of stained glass window.

3. Biomineralisation: a solution to biodeterioration?

The weathering of building stones is not a recent phenomenon but it was well-known in antiquity. This was the reason why Roman architects coated stones with a sacrificial layer of stucco. Weathering starts as soon as a stone is extracted from a quarry or a mortar is placed in a building. Weathering of building materials is caused by natural environmental factors. Sun, frost, wind, rain, and so forth contribute gradually to this process. The calcareous matrix, due to the calcite leaching process, shows a progressive increasing in its porosity and a significant decreasing of its mechanical characteristics (Amoroso, 1983). Finally, materials are broken down into smaller particles and ultimately into constituent minerals.

Attempts to slow down the deterioration of monuments have been continuously made by the application of conservative treatments with inorganic or organic products (Lazzarini and Laurenzi Tabasso, 1986). The use of the latter present some drawbacks due to their chemical composition and thermal expansion coefficient which are quite different from that of the stone. Besides, these are usually formulated and applied in solvents at very low concentration. In such a way, a high amount of organic solvents are wasted in the environment. The conservative treatments are usually made on monumental stones that are exposed to heavy polluted atmosphere. For this reason and considering the chemical nature of these products, their efficiency in time can be considered very short, and in some cases they can have a detrimental effect for the conservation of the stone material itself.

Microbial precipitation of $CaCO_3$ is a common process in soil, freshwater and marine sediments. Carbonate formation can occur in different ways both as passive (Krumbein, 1972) or active bioprecipitation (Castanier et al. 1989; Cañaveras et al. 2001). Several studies have pointed out the complexity of this phenomenon that can be influenced by the physico-chemical parameters of the environment and it is correlated both to the metabolic activity and the cell wall structure of microorganisms.

Bacterial activity can control the crystallogenesis of carbonates through environmental conditions, or crystals can start to build up on the bacterial wall. In a few steps a kind of cocoon is forming and subsequently biomineral assemblage appears progressively turning into true crystals, either well shaped or poorly organized, trapping bacteria within the mineral structure. Differences in the type of crystal formed (vaterite, aragonite or calcite) depend both on growing features and bacterial strains, different bacteria precipitate different types of $CaCO_3$, and the most common crystalline forms are either spherical or polyedric (Cañaveras et al. 2001). These results indicate that bacteria could play an active role in $CaCO_3$ precipitation and that mineral formation is not only an indirect consequence of environmental changes, but produced by the metabolic activity of bacteria.

Recently new methodologies have been proposed to improve $CaCO_3$ precipitation on calcareous stones, based on the biomediated calcite precipitation (Orial, 1993; Tiano, 1995, 1999; Tiano et al. 1999).These were based on the fact that the formation of minerals by organisms is a common phenomenon and many kind of biomineralization products and processes are present in most classes of organisms. These have be formed during the last 600 billions of years and have contributed to mould the earth and the biological involvement ranges from biologically-induced to biologically-controlled processes.

The growth of new calcite crystals inside stone porosity, a biomineralisation process induced by organic matrix macromolecules (OMM), extracted from marine shells and skeletons represents a new approach to improve the conservative treatments of monumental stones, based on the use of inorganic compounds.

The feasibility study and the results obtained on small limestone samples using the OMM extracted from the shell of a mollusk (*Mytilus californianus*) have shown a good efficiency of the bio-induced calcite precipitation process with respect to the decrease of the amount of water absorbed and the increase of the superficial strength of the stone material treated (Tiano, 1995). Contemporary, another biomineralisation treatment has been developed applying living cultures of selected calcinogenic bacteria (Orial, 1993). The bacterial activity can control the crystallogenesis of carbonates through environmental conditions, or crystals can start to build up on the bacterial wall. In a few days a kind of cocoon is forming and subsequently biomineral assemblage appears progressively turning into true crystals either well shaped or poorly organized, trapping bacteria within the mineral structure (Castanier et al. 1989). This treatment has demonstrated its efficiency (Castanier et al. 2000) even if the application of heterotrophic viable bacteria, inside a monumental stone, need some improvements. In fact, chemical reactions with stone minerals due to metabolic by-products and the possible growth of undesired micro-organisms (mainly fungi) due to the presence of nutrients for bacterial development, could have negative effects on the monumental stone itself (Tiano et al. 1999).

4. Major gaps in research

Years ago, an old method, used by the ancient Romans, based on the application of lime-water, was proposed and experimented on some deteriorated calcareous stones in order to impart a slight water-repellence and consolidating effect (Price, 1984). This method creates a white thin sacrificial surface applicable only on white stones (like marble). Besides, the new calcite has a very weak adhesion with the underlaying mineral structure. According to Tiano (1999) the effectiveness of this latter action was very doubtful.

Tiano et al. (1999) treated stone samples of Pietra di Lecce (bioclastic limestone, consisting almost exclusively of $CaCO_3$), by incubation at 28°C for 15 days with a solution containing 10^6 cells/cm^2 of selected calcinogenic bacteria (*Micrococcus* sp. and *Bacillus subtilis)*. The bacteria were daily soaked with the B4 nutrient medium. The treated samples have shown a clear reduction of the amount of water absorbed, even if this result was partly derived from the presence of a big bacterial mass, due to the microbial growth, rather than to the formation of newly calcite crystals inside porosity. The bacterial calcite crystals have similar characteristics to those obtained with the organic matrix macromolecules. The widespread diffusion of the calcite biomineralization process and the isolation of a bacterial mutant unable to precipitate calcite crystals, suggested the presence of a genetic control for the mineral forming mechanism. Hence the biotechnological approach to the bio-mediated treatment of decayed stones is to individuate the bacterial genetic sequences that control the macromolecules involved in the biomineralization of calcite. Once characterized the process can be easily bio-engineered with a massive production of standardized macromolecules. These can be safely used for monumental calcareous stones restoration, without the dangerous presence of viable microorganisms.

In fact, the main gaps in the use of calcinogenic bacteria are i) the application of the bacterial culture onto the stone surface, ii) the repeatedly application of a nutrient medium for several days into the stone, which conceivably will permit the development not only of calcinogenic bacteria, but other bacteria inhabiting the stone niche, iii) to prove the integration of the bio-induced calcite and the reinformcement of the mineral matrix are required further petrographical, mineralogical and physico-chemical studies, iv) detailed microbial ecology studies are needed in order to ascertain the effects of the introduction of new bacteria into the natural microbial communities, the development of the communities at short, mid and long-term, and the eventual secondary colonisation of heterotrophic microorganisms using bacterial organic matter and dead cells, such as actinomycetes, fungi, etc.

5. Future perspectives

In the last years, biodeterioration studies are developing in parallel with the introduction of molecular tools for the detection of microbial communities in deteriorated assets, and molecular microbial ecology is expected will reach a great importance in this field. Some problems are still present and acting as limiting factor in the wide application of molecular technologies, in general, and in biodeterioration studies, in particular:

1. Most of the application aim to the phylogenetic position, detection and enumeration of microorganisms.
2. No methods were so far satisfactory to detect the microbial activity. Only a few studies exist, in which the metabolic activity of microorganisms using molecular biological methods has been investigated.
3. Sampling methods and procedures to keep samples during the transport to laboratories can enormously change the relationship among the different microflora components.
4. DNA extraction procedure could be not successful to detect microorganisms with tick cell walls, and thus results in understimation of the colonising population.
5. Some methods that study the microbial communities understimate the presence of low amounts of DNA. The quantification of microbes, detected by PCR-based detection systems is still a problem when used for mixed target sequences. Especially, sequences representing the minority of organisms might be overlooked.

What should be done to meet this goal?: First, it is needed an optimisation of protocols concerning sampling procedures, extraction, eventual removal of inhibitors of DNA digestions and PCR amplifications, and PCR. Second, it is necessary to identify the techniques to be used, as well as its pitfalls, limitations and possible solutions. A preliminary survey indicates that the following techniques should be evaluated: DGGE, TGGE, RISA, DNA fingerprinting (RAPD, DAF, AP-PCR).

The use of a new fingerprinting technique called AFLP (Amplified fragment lenght polymorphism) based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA is proposed. The technique involves three steps: i) restriction of the DNA and ligation of oligonucleotide adapters; ii) selective amplification of sets of amplification fragments, and iii) gel analysis of amplified fragments. This method offers the advantage with respect to other fingerprinting methods that it is independent on the reaction conditions. The large number of amplified products obtained can facilitate the possibility to compare microbial populations in different sites, surfaces and localities, as well it can be applied to obtain genomic maps within differentiate at subgeneric level.

There is a need for increasing the use of *in situ* methods, as well. Once the knowledgement of the colonising microflora is obtained, the use of specific single cell probes for *in situ* hybridization can be applied.

It is clear that one important step in the possible application of molecular methods is the optimisation of steady protocols and to promote that different groups can work on different methods and compare their results in order to verify the validity of the methodologies proposed before to apply them in the field of cultural heritage. This only can achieved in the framework of multidisciplinary and multilaboratory studies, such as those involved in EC projects. Therefore, the continuation of this type of programmes is capital for the advancement of our knowledge on biodeterioration of cultural heritage assets.

Molecular biology and bacterial genetic engineering are innovative technologies suggested to improve bioinduction and biomineralisation method. These tools are being used for finding the genetic expression of this mechanism. Preliminary results in the identification of the genes controlling this process have produced bacterial mutants which do not induce calcite precipitation (Perito et al. 2000). These mutants could be useful to identify the genes involved in calcite precipitation and might lead to a low cost renewable source of macromolecules for Bio-Mediated calcite Treatment (BMT) of monumental stones. This is being studied in the framework of the EC project BIOREINFORCE (EVK4-2000-22027).

The passage from laboratory dimension to a real *in situ* application of the bio-mediated calcite precipitation method needs further improvements, and particularly the carefull control and fate of bacteria. This can be overcome if the organic-matrix bioinduced method is industrialized, for which it is needed a constant and regular supply of the organic-matrix itself. However, the source of these calcite precipitating proteins is actually time-consuming and results in low yield extraction from mollusk shells. Moreover, the application of heterotrophic viable bacteria, inside a monumental stone, does not seem appropriate for this field of intervention. In fact, chemical reactions with stone minerals due to metabolic by-products and the growth of fungi, due to the application of organic nutrients for bacterial development, can have negative effects on monumental stone itself.

The widespread diffusion of the calcite biomineralisation process with the introduction in the European market of French and Spanish companies promoting this type of stone treatments in buildings and churches requires the attention of microbiologists and geologists in order to validate the effectiveness of the method. Further studies on testing of the stone performance at mid- and long-term are required. A screening on the microbial communities before and after industrial application of calcinogenic bacteria and their succession and evolution along the time is clearly needed, and this should be carried out in the framework of EC projects.

6. References

[1] Amoroso, G., Fassina, V. (1983) Stone Decay and Conservation, Elsevier, Amsterdam.

[2] Bartolini, M., Pietrini, A.M., Ricci, S. (1999) Irradiation on artistic stoneworks for control of algae and cyanobacteria. In: Of Microbes and Art. The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage, CNR, Florence, Italy, pp. 221-227.

[3] Caneva, G., Nugari, M.P., Pinna, D., Salvadori, O. (1996). Il Controlo del Degrado Biologico. Nardini Editore, Fiesole.

[4] Cañaveras, J.C., Sanchez-Moral, S., Soler, V., Saiz-Jimenez, C. (2001) Microorganisms and microbially induced fabrics in cave walls. Geomicrobiol. J. 18, 223-240.

[5] Castanier, S., Maurin, A., Perthuisot, J.P. (1989) Production bacterienne expérimentale de corpuscules carbonatés, sphéroïdaux à structure fibro-radiarie. Réflexions sur la définition des ooïdes" Bull. Soc. Géol. France, 5, 589-595.

[6] Castanier, S., Le Metayer-Levrel, G., Orial, G., Loubier J.F., Perthuisot, J.P. (2000) Bacterial carbonatogenesis and applications to preservation and restoration of historic property. In: Of Microbes and Art. The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage. O. Ciferri, P. Tiano, G. Mastromei (eds.), Kluwer Academic-Plenum Publisher, New York, pp. 203-218.

[7] Gomez-Bolea, A., Ariño, X., Balzarotti, R., Saiz-Jimenez, C. (1999) Surface treatment of stones: consequences on lichenic colonization. In: Of Microbes and Art. The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage, CNR, Florence, Italy, pp. 233-237.

[8] Krumbein, W.E. (1972) Rôle des microorganismes dans la génèse, la diagénèse et la dégradation des roches en place. Rev. Ecol. Biol. Sol., 3, 283-319.

[9] Laiz, L., Hermosin, B., Caballero, B., Saiz-Jimenez, C. (2001) Facultatively oligotrophic bacteria in Roman mural paintings. In: Protection and Conservation of the Cultural Heritage of the Mediterranean Cities, E. Galan, ed. (in press).

[10] Lazzarini, L., Laurenzi Tabasso, M. (1986) Il Restauro della Pietra. CEDAM, Padova.

[11] Orial G. (1993) Biomineralization as new protective treatment of calcareous stone. In: Proceedings of 2nd International Conference on Biodeterioration of Cultural

Property, Yokohama, Japan, K. Toishi, H. Arai, T. Kenjo, K. Yamano (eds.), pp. 98-116.

[12] Pace, N.R. (1996) New perspective on the natural microbial world: molecular microbial ecology. ASM News 62, 463-469.

[13] Perito, B., Biagiotti, L., Daly, S., Galizzi, A., Tiano, P., Mastromei, G. (2000) Bacterial genes involved in calcite crystal precipitation. In: Of Microbes and Art. The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage. O. Ciferri, P. Tiano, G. Mastromei (eds.), Kluwer Academic-Plenum Publisher, New York, pp. 219-230.

[14] Piñar, G., Saiz-Jimenez, C., Shabereiter-Gurtner, C., Blanco-Varela, M.T., Lubitz, W., Rölleke, S. (2001) Archaeal communities in two disparate deteriorated ancient wall paintings: detection, identification and temporal monitoring by DGGE. FEMS Microbiol. Ecol. (in press).

[15] Price, C.A. (1984) The consolidation of limestone using a lime poultice and limewater. In: Proceedings of Adhesives and Consolidants IIC Meeting, Paris, IC, London, pp. 160-162.

[16] Saiz-Jimenez, C. (1994) Biodeterioration of stone in historic buildings and monuments. In: Biodeterioration Research 4: Mycotoxins, Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration, G.C. Llewellyn, W.W. Dashek and C.E. O'Rear, eds. Plenum, New York, pp 587-603.

[17] Saiz-Jimenez, C. (1995) Deposition of anthropogenic compounds on monuments and their effect on airborne microorganisms. Aerobiologia 11, 161-175.

[18] Saiz-Jimenez, C. (2001) The biodeterioration of building materials. In: Microbiologically Influenced Corrosion, vol. 2, J. Stoecker, ed. NACE, Houston. (in press).

[19] Seaward, M.R.D. (1989) Lichens as monitors of recent changes in air pollution. Plants Today, March-April 1989, 64-69.

[20] Seaward, M.R.D., Edwards, H.G.M. (1995) Lichen substratum interface studies, with particular reference to Raman microscopic analysis. 1. Deterioration of works of art by *Dirina massiliensis* forma *sorediata.* Crypt. Bot. 5, 282-287.

[21] Shabereiter-Gurtner, C., Piñar, G., Vybiral, D., Lubitz, W., Rölleke, S. (2001) *Rubrobacter*-related bacteria associated with rosy discolouration of masonry and lime wall paintings. Arch. Microbiol. (in press).

[22] Tiano, P. (1994) Biodeterioration of stone monuments: a critical review. In: Recent Advances in Biodeterioration and Biodegradation, vol. 1, K.L. Garg, N. Garg, K.G. Mukerji, eds. Naya Prokash, Calcutta, pp. 301-322.

[23] Tiano, P. (1995) Stone reinforcement by calcite crystal precipitation induced by organic matrix macromolecules". Stud. Conserv. 40, 171-176.

[24] Tiano, P. (1998) Biodeterioration of monumental rocks: decay mechanisms and control methods. Sci. Technol. Cult. Herit. 7, 13-32.

[25] Tiano P. (1999) Conservazione dei monumenti: riprecipitazione biomediata di calcite per il restauro di pietre calcaree alterate" OPD Restauro n° 11, 85-97.

[26] Tiano P., Biagiotti, L., Mastromei, G. (1999) Bacterial bio-mediated calcite precipitation for monumental stones conservation: method of evaluation. J. Microbiol. Meth. 36, 139-145.

[27] Ward, D.M., Weller, R., Bateson, M.M. (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345, 63-65.

ANNEX 1

COMMENTS ON THE IMPACT OF THE EUROPEAN COMMISSION RESEARCH PROGRAMMES (1986-2002) ON BIODETERIORATION STUDIES

It must be admitted that EC research programmes had a strong impact on the European Cultural Heritage science and promoted and supported biodeterioration research. A number of innovative projects using new tools, technologies and methodologies were carried out and represented an important advance in this field.

An European Commission report entitled "A Review of the European Commission Research on Environmental Protection and Conservation of the European Cultural Heritage", version October 2000, presented the activities of the programme which started in 1986. Since this time the major achievement was the consolidation of research teams in most European countries, covering diverse areas and promoting multinational and multidisciplinary studies. In this respect, some teams specialized in biodeterioration problems participated actively in the programme. These teams cover most groups of organisms involved in biodeterioration processes and the apply the most recent methodologies (see list and addresses below). Some of these groups are key reference for other scientists and are very active in their fields with many papers published in journals that become listed within major bibliographic data bases.

"Technological Requirements for Solutions in the Conservation and Protection of Historic Monuments and Archaeological Remains" is a recent study funded by the Scientific and Technological Options Assessment Panel of the European Parliament (see http://www.ucl.ac.uk/sustainableheritage/research/projects.htm) The Study discovered many achievements associated with European support for scientific and technological research for the protection and conservation of cultural heritage, including biodeterioration aspects, namely:

- Creation of an active research community
- A body of research of unparalleled and enviable international quality and character
- Ongoing effectiveness of research beyond initial funding
- Substantial rate of publication
- Imaginative tools of dissemination and publication
- Clear spin-offs and contribution to European competitiveness often going outside the European cultural heritage area
- Contribution to emerging European legislation, for example, air quality management.

The Study also uncovered important research gaps that have yet to begin to be investigated. It has also discovered the need for continuing fine scale advancement in areas where researchers have been active for a number of years. The overall picture is that European research in the field of cultural heritage protection must be put on a secure footing if it is to maintain its commanding lead over other regions of the world.

The Study concludes that:

1. It would be invidious to attempt to separate basic and applied research in this area of research. Like any other scientific endeavour, this field needs to integrate basic and applied research if it is to continue to thrive.

2. Small, flexible, focused interdisciplinary teams responsive to European needs, must be sustained, promoted and celebrated as models of sustainability and that what is proposed under the European Research Area (ERA) for large and complex research projects, could inflict serious damage on this area of research.
3. Resources cannot be delegated to Member States because of the interdisciplinary nature of cultural heritage and the need for a co-ordinated pan-European perspective across this research that helps to define the essential character of European cultural heritage. National programmes only serve local needs, leading to loss of strategic output, lessening of competitiveness and risk of duplication.
4. A mechanism needs to be created to help researchers working in this field to communicate and exchange information with related sectors such as construction, urban regeneration, land reclamation and agriculture.
5. There is overwhelming agreement over the need for sustainable research funding for cultural heritage and for an iterative process of exchange among researchers, decision-makers and end-users in order to maximize benefits from project inception through to dissemination, audit and review.

For all these reasons, the most significant recommendation in this Report is the identification of the need for a European Panel on the Application of Science for Cultural Heritage (EPASCH).

The full report can be obtained at: http://www.europarl.eu.int/stoa/publi/pdf/00-13-04_en.pdf

LIST OF BIODETERIORATION RESEARCH TEAMS PARTICIPATING IN PROJECTS FUNDED BY THE EUROPEAN COMMISSION (UPDATED OCTOBER 2000)

1. EUROPEAN COMMUNITY ENVIRONMENTAL RESEARCH PROGRAMME (1986-1990)

1.1 Biodeterioration studies on stone monuments

C. Saiz-Jimenez

Instituto de Recursos Naturales y Agrobiologia

Apartado 1052

41080 Sevilla

Spain

E-mail: saiz@cica.es

2. STEP PROGRAMME SCIENCE AND TECHNOLOGY FOR ENVIRONMENTAL PROTECTION (1989-1992)

2.1 Protection and conservation of historic buildings, monuments and associated cultural property

C. Saiz-Jimenez

Instituto de Recursos Naturales y Agrobiologia

Apartado 1052

41080 Sevilla

Spain

E-mail: saiz@cica.es

2.2 Conservation of granitic rocks and application to megalithic monuments

B. Silva

Departamento de Edafoloxia

Universidad de Santiago de Compostela

Campus Universitario Sur

15706 Santiago de Compostela

Spain

E-mail: edbsilva@uscmail.usc.es

3. EUROPEAN COMMISSION R&D ENVIRONMENT PROGRAMME (1991-1994)

3.1 Interactive physical weathering and bioreceptivity study on building stones, monitoring by computerized X-ray tomography (CT) as a potential non-destructive research tool

J. Swings
Universiteit Gent
Faculteit Wetenschapen
BE-9000 Gent
Belgium
E-mail: jean.swings@rug.ac.be

P. Nimis
Universita degli Studi di Trieste
Dipartimento di Biologia
IT-34127 Trieste
Italy

C. Saiz-Jimenez
Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

W. E. Krumbein
Institut für Chemie und Biologie des Meeres
Carl von Ossietzky Universität Oldenburg
DE-26015 Oldenburg
Germany
E-mail: wek@africa.geomic.uni-oldenburg.de

3.2 Microstructural decay of lithoid monuments, caused by environmental factors, studied using a newly developed radar-aided methodology

C. Saiz-Jimenez
Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

3.3 Atmospheric eutrophication and secular organic pollution (biological and mineralogical reactions of Mediterranean monuments)

W. E. Krumbein

Institut für Chemie und Biologie des Meeres

Carl von Ossietzky Universität Oldenburg

DE-26015 Oldenburg

Germany

E-mail: wek@africa.geomic.uni-oldenburg.de

C. Urzi

Dipartimento di Scienze Microbiologiche, Genetiche e Moleculari

Università degli Studi di Messina

Salita Sperone 31

Villaggio S. Agata Messina

98166 Messina

Italy

E-mail: clara.urzi@unime.it

4. EUROPEAN COMMISSION R&D ENVIRONMENT AND CLIMATE PROGRAMME (1994-1998)

4.1 Assessment of environmental risk related to unsound use of technologies and mass tourism

H. Busse

Institut für Mikrobiologie und Genetik

Universität Wien

AT-1030 Wien

Austria

4.2 Development of evaluation criteria, prediction and control methods concerning sea-salt effects on monument stones

B. Silva

Departamento de Edafoloxia

Universidad de Santiago de Compostela

Campus Universitario Sur

15706 Santiago de Compostela

Spain

E-mail: edbsilva@uscmail.usc.es

4.3 Deterioration of prehistoric rock art in karstic caves by mass tourism: integrated study (environment, geology, geochemistry and microbiology) for their conservation

C. Saiz-Jimenez

Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

4.4 Novel remediation strategies for preservation of marble structures endangered from environmental damages

C. Urzi
Dipartimento di Scienze Microbiologiche, Genetiche e Moleculari
Università degli Studi di Messina
Salita Sperone 31
Villaggio S. Agata Messina
98166 Messina
Italy
E-mail: clara.urzi@unime.it

4.5 Novel molecular tools for the analysis of unknown microbial communities of mural paintings and their implementation into the conservation/restoration practice

W. E. Krumbein
Institut für Chemie und Biologie des Meeres
Carl von Ossietzky Universität Oldenburg
DE-26015 Oldenburg
Germany
E-mail: wek@africa.geomic.uni-oldenburg.de

C. Saiz-Jimenez
Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

J. Swings
Universiteit Gent
Faculteit Wetenschapen
BE-9000 Gent
Belgium
E-mail: jean.swings@rug.ac.be

W. Lubitz

Institut für Mikrobiologie und Genetik
Universität Wien
AT-1030 Wien
Austria
E-mail: oldfox@gem.univie.ac.at

4.6 Development of an innovative water repellent biocide surface treatment for mortars: assessment of their preformance by using modern analytical tools and surface analysis

C. Saiz-Jimenez
Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

C. Urzi
Dipartimento di Scienze Microbiologiche, Genetiche e Molecolari
Università degli Studi di Messina
Salita Sperone 31
Villaggio S. Agata Messina
98166 Messina
Italy
E-mail: clara.urzi@unime.it

E. Bock
Institut für Allgemeine Botanik
Universität Hamburg
DE-22609 Hamburg
Germany

I. Beech
School of Pharmacy and Biomedical Sciences
University of Portsmouth
P01 2DT Portsmouth
Great Britain

4.7 To save the artistic heritage from insect pest without using toxic chemical compounds

N. Valentin
Centro de Investigaciones Biologicas

Velazquez 144
28006 Madrid
E-mail: nieves.valentin@iphe.mcu.es

C. Federici
Istituto Centrale per la Patologia del Libro
Laboratorio di Biologia
IT-00184 Roma
Italy

5. EUROPEAN COMMISSION ENVIRONMENTAL AND SUSTAINABLE DEVELOPMENT (1999-2002)

5.1 Concerted action on molecular microbiology as an innovative conservation strategy for indoor and outdoor cultural assets

C. Saiz-Jimenez
Instituto de Recursos Naturales y Agrobiologia
Apartado 1052
41080 Sevilla
Spain
E-mail: saiz@cica.es

W. E. Krumbein
Institut für Chemie und Biologie des Meeres
Carl von Ossietzky Universität Oldenburg
DE-26015 Oldenburg
Germany
E-mail: wek@africa.geomic.uni-oldenburg.de

C. Urzi
Dipartimento di Scienze Microbiologiche, Genetiche e Molecolari
Università degli Studi di Messina
Salita Sperone 31
Villaggio S. Agata Messina
98166 Messina
Italy
E-mail: clara.urzi@unime.it

W. Lubitz
Institut für Mikrobiologie und Genetik

Universität Wien
AT-1030 Wien
Austria
E-mail: oldfox@gem.univie.ac.at

J. Swings
Universiteit Gent
Faculteit Wetenschapen
BE-9000 Gent
Belgium
E-mail: jean.swings@rug.ac.be

M. Salkinoja-Salonen
Department of Applied Chemistry and Microbiology
Viikinkaari 9
00014 Helsinki
Finland
E-Mail: Mirja.salkinoja.salonen@helsinki.fi

S. Rölleke
Genalysis GmbH
Im Biotechnologiepark
D-14943 Luckenwalde
Germany
E-mail: sabine.roelleke@genalysis.de

P. Tiano
CNR C.S. Opere d'Arte
Via Gino Caponi 9
50121 Firenze
Italy
E-mail: tiano@cscoa.fi.cnr.it

N. Valentin
Centro de Investigaciones Biologicas
Velazquez 144
28006 Madrid
E-mail: nieves.valentin@iphe.mcu.es