

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



Genalysis®



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Short Communications

MICROBIOLOGICAL ASSESSMENT IN CONSERVATION: A SUMMARY OF QUESTIONNAIRE RESULTS

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Introduction to the questionnaire

In August 1999 a questionnaire was sent out to a variety of international specialists who encountered biodeterioration through their work in the conservation of cultural material. The objectives of this questionnaire were to review the role of microbiological assessment in conservation, and the approaches and techniques currently in use or being developed. Of the respondent population 51% were specialists in microbiology and/or molecular biology while 24 % were conservation specialists.

The development of the questionnaire, collation of results and interpretation of findings formed part of the research for a final year diploma dissertation entitled 'The significance of appropriate sampling and cultivation in the effective assessment of biodeterioration', undertaken at the Conservation of Wall Painting Department, Courtauld Institute of Art, London.

The scope and content of the questionnaire aimed to provide:

- ◆ a profile of the respondents and their roles in the conservation of cultural material;
- ◆ an overview of objectives, priorities and factors influencing the assessment of microbiological impact;
- ◆ an insight into the approaches and techniques employed in the investigation of microflora;

- ◆ opinions on the respective roles of specialists in conservation projects incorporating microbiological investigations; and
- ◆ to determine the influence of investigation findings on a chosen method of biodeterioration control.

Responses to the questionnaire highlighted a degree of consensus as well as discrepancies between the two main specialist groups on the function of, and approach to microbiological investigations in the conservation of cultural heritage. The following is a brief summary of the questionnaire findings.

Objectives and approaches to microbiological assessment.

Seventy-five percent of respondents regularly encountered evidence of microbiological growth on cultural material, most commonly fungi and bacteria. The majority of respondents conceded that microbiological investigations should be prioritised based on the general condition of an object. The main objectives for undertaking investigations were dependant on whether respondents had previously undertaken investigations into the microflora of cultural material. For those who had, obtaining *information to aid in the remedial treatment of microbiological growth* was considered the main objective and for those who had not, the main objective would be *to investigate organisms associated with biodeterioration*.

When the presence of microbiological growth was detected, the common response of those questioned was to document areas where microbiological growth was present (*Chart 1*). Twenty-eight percent of respondents would carry out further investigations, the nature and extent of which would be dependant on available funding and the importance of microflora investigations within the scope of a conservation plan.

The majority of respondents expressed that ideally, all aspects of investigations,

from sampling through to laboratory based investigations and monitoring should involve both conservation and microbiological specialists. However, the level of involvement at any stage of an

were the non-invasive use of a swab and the invasive removal of fragments. For the purposes of cultivation just over half of the respondents selected their media in response to the organisms likely to

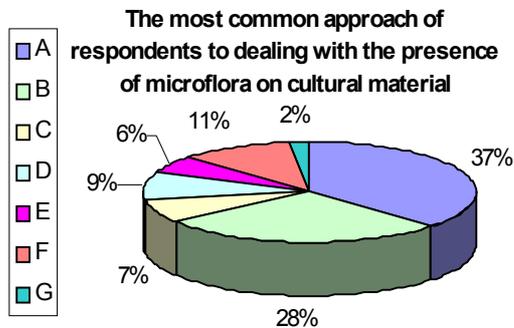


Chart 1: Shows the most common approach of respondents in dealing with the presence of microflora on cultural material; from the options given; A) document areas where growth is present, B) investigate microflora, C) treat in the absence of investigations, D) treat in response to investigations, E) not treat due to the absence of specialist knowledge, F) intervene passively, G) not treat due to the unlikely-hood of long-term control. (Question 2c)

investigation was dependant on expertise. The participation of the conservation specialist was felt to be *highly significant* in all aspects of observation, sampling and development of a treatment approach, with a lesser role in stages involving the identification and assessment of microbiological activity. The microbiological specialist was viewed as having a *significant* role in almost all stages of investigation.

Methodologies and techniques used in investigations.

To determine the methodologies and protocols employed by respondents in investigations, part four of the questionnaire was directed at respondents with experience in conducting investigations into the microflora of cultural heritage. For this group – the majority of whom were microbiological specialists – 48% indicated that evidence of microbiological growth was the most common reason for the initiation of an investigation. However 38% were likely to carry out microflora investigations as part of preliminary investigations. For the majority, microflora investigations involved the sampling, cultivation and identification of organisms in addition to the assessment of the impact of microbiological activity on a substrate. Forty-eight percent undertook further monitoring of microbiological activity.

In the collection of samples, respondents indicated that the two preferred methods

present. In the identification of species, 34% chose metabolic screening tests, while 41% chose molecular biological methods such as Polymerase Chain Reactions (PCR), Fatty Acid Profile Analysis (FAA) and Southern Blot Hybridisation. Techniques commonly used in the evaluation of microbiological activity such as measurements of Adenosine triphosphate (ATP) concentration and Dehydrogenases Activity (DHA) were also used. For many respondents these investigative tools had a significant role in the rapid quantification of microbial activity both pre and post intervention.

The answers and comments provided by respondents to this section provided informative insights and accounts of the range of microbiological, molecular biological and ultrastructural techniques being adapted and utilised in the examination of biodeterioration and its causative agents. With apparent access to a range of analytical tools and the expertise to interpret the results generated, this portion of the respondent community also felt that in response to initial objectives, information generated from investigations was *'highly relevant'*.

Questionnaire conclusions

In the concluding section of the questionnaire all respondents were asked to assess *'the effect of investigations into microflora on the chosen method of control'*. The majority felt that the method of control was *'dependent on the*

findings' with many respondents preferring to adopt a multi-faceted approach involving environmental control and modification of a proposed conservation treatment. Almost 20% of respondents incorporated biocidal treatments in the control of biodeterioration.

Some further thoughts

With previous remedial measures in treating biodeterioration with the use of biocides now shown to be ephemeral, current research focuses on developing passive methods of control. In response, investigations into biodeterioration have developed from the general characterisation and identification of species sampled to attempts to understand those involved in biodeterioration — the biochemistry of microbial aggression and the conditions under which it is likely to occur. Such information is of greater relevance to those specialists who are aiming to control biodeterioration of cultural material.

In addition, the realisation that many species are taken from 'extreme' environments such as stone monuments and wall paintings are often 'uncultivable' has resulted in a range of molecular biological techniques currently being refined and developed for application in conservation. These techniques are being used to compensate for the limitations of the more traditional methods of sampling and cultivation. Such techniques can provide quantitative and qualitative information on a given population with many approaches relying on the genetic and nutritional dispositions of the species under investigation.

Information on the emerging importance of such technology should ideally be passed on to conservation specialists who can then, in conjunction with the micro/molecular biology specialists make informed decisions as to their appropriateness and feasibility in microbiological assessment. In order to achieve this it is important that there is a degree of consensus among specialists

regarding the function and purpose of microbiological assessment of cultural material. Moreover, specific information on the approaches and techniques currently being employed and developed requires consolidation, so that model approaches can be reviewed, databases on aggressive organisms developed and guidelines for the use of molecular biological tools in conservation can be established.

PROTECTION AND PREVENTION OF THE IBERO AMERICAN CULTURAL HERITAGE FROM ENVIRONMENTAL BIODETERIORATION. THE RESEARCH NETWORK XV-E, CYTED.

Hector A. Videla

Department of Chemistry, College of Pure Sciences, University of La Plata, ARGENTINA. International Coordinator of Research Network XV-E (CYTED). Sub Program XV (Corrosion/Environmental Impact on Materials).

Microorganisms are strongly involved in chemo organotrophic biodeterioration processes that are notoriously enhanced by the high relative humidity and temperatures present in many of the areas where the cultural heritage of Latin America is located.

The Ibero American cultural heritage in Central and South America is mainly represented by three pre-Columbian civilizations : Aztec (in the high lands of the central valley of Mexico), Mayan (spread over the Mexican states of Yucatan, Campeche, Quintana Roo, Tabasco and Chiapas, Belize, Guatemala and Honduras) and Inca (Ecuador, Peru and North of Chile).

The Spanish Agency for International Cooperation (AECI) developed an active plan for the preservation and restoration of several buildings and monuments belonging to the Ibero American Cultural heritage during the late nineties. Further actions in this direction are taken within the Subprogram XV (Corrosion/Environmental impact on materials) of the Ibero American Program of Science and Technology of

Development (CYTED) through the Research Network XV-E (PRESERVAR) devoted to study the protection and prevention of the Ibero American cultural heritage from environmental biodeterioration. The research network actually involves experts from five different countries (Argentina, Brazil, Mexico, Portugal and Spain) and started its activities in February 2000.

CYTED Research Network XV-E (PRESERVAR)

Contents

The Ibero American cultural heritage covers a wide variety of archeological monuments and items deemed to have artistic or historic significance by both the local and the international societies. Due to the diversity of climates that can be found in such an extended geographical area (from the U.S./Mexican border at the North to the Antarctic at the South, plus the Iberian peninsula), several factors are involved in different extents, in relation to material decay. These factors are: a) biodeterioration processes; b) atmospheric corrosion or weathering of the materials exposed to open air; c) natural and anthropogenic pollution.

Goals

The main goals of the Research Network XV-E are:

- ◆ To assess the effects of the environmental biodeterioration on the Ibero American cultural heritage by identifying the microorganisms causing biodeterioration, their mechanisms, by classifying the bibliography, or publishing a data bank and a specialized manual.
- ◆ To facilitate the transfer of knowledge through seminars, papers to conferences, practical courses (both regional or international) and publications in general media. To divulge the research network activities in the internet.
- ◆ To develop prevention and protection strategies against the deleterious effects of the biodeterioration of the cultural property by complementing expertise among the research

network specialists and international cooperation programs as well (European Community Projects, Spanish Agency for International Cooperation, etc.).

Members

International coordinator: Prof. Hector A.Videla, University of La Plata. Argentina.

National delegates: ARGENTINA: Prof. Carlos Giudice (CIDEPIINT. La Plata), BRAZIL: Dr. Christine Claire Gaylarde (Federal University of Rio Grande do Sul. Porto Alegre. RS.), MEXICO: Dr. Lorenzo Martinez Gomez (Mexican Oil Institute. Mexico D.F.), PORTUGAL: Dr. Mario Ferreira (Technical University of Lisbon. Lisbon), SPAIN: Dr. Cesareo Saiz Jimenez (IRNAS-CSIC. Seville).

Dissemination Activities

Due to the characteristics of the subject, of high social impact, the Research Network XV-E has foreseen an active program of transfer of knowledge and information through:

- ◆ Workshops and round tables involving specialists from the Ibero American or the international scientific communities with the aim of synergizing efforts and to interchange technical and scientific information.
- ◆ To submit papers to national and international conferences on biodeterioration of materials and those related to the preservation of the cultural property.
- ◆ To organize seminars, sessions and forums in strategic places with relevant cultural property, either in the same sites or in their vicinity.
- ◆ To encourage regional actions between neighbouring countries in geographical areas such as the Iberian peninsula, Central America or the south of the American continent. This initiative will facilitate more frequent meetings at a lower cost.
- ◆ To publish books of proceedings of the most relevant conferences,

workshops or seminars organized by the Research Network XV-E and to edit a complete data bank including a list of specialists and cultural entities of Ibero America related with the preservation of the cultural heritage.

- ◆ To publish a specialized manual gathering information on the state of the art as well as the more recent contributions of the Research Network to the field.

Activities Developed During The First Year

The Research Network XV-E organized the "Jornadas sobre Biodeterioro de Monumentos Historicos de Iberoamerica" in the city of Morelia (honored as cultural property of the humankind by the UNESCO), state of Michoacan, Mexico (December 9-10, 1999). The sessions were supported by the Universidad Michoacana San Nicolas Hidalgo, the Mexican Academy for Material Sciences, the regional delegation of the INAH and the regional delegation of the CONACYT. The conferences presented during the first day of the event were: "Biodeterioration of Mayan archeological sites in the Yucatan peninsula, Mexico" (Dr. H.Videla), "Atmospheric corrosion of metals and biodeterioration of limestone in the Yucatan peninsula and the Caribbean area" (Dr. L. Maldonado), "Preservation of Morelia, cultural heritage of the mankind" (Prof. Ramirez Romero), "The role of environmental sulfur in the degradation of ignimbrites at the Morelia cathedral, Mexico"(Dr. L. Martínez Gómez).

During the second day of the meeting it was discussed several topics of interest between local and international specialists and the attendants, with the aim of planning future joint actions.

The first official meeting gathering all the international members of the research network was held in the city of Seville, Spain, on February 14 - 18, 2000, with the support of the CYTED, IRNAS-CSIC and the government of Andalucia. The proceedings of this meeting has been

published in Spanish in the second semester of the year 2000.

It was scheduled a research network contribution to the International Materials Research Congress, sponsored by the Mexican Academy of Material Science in Cancun, Quintana Roo (August 27-31, 2000) with papers presented in a special session related to the biodeterioration and preservation of the cultural heritage in Mexico.

Other activities of the RTXV-E in this conference were a plenary lecture given by Dr. H.A. Videla and a keynote lecture (Dr. C. Saiz-Jimenez)

A workshop on Biodeterioration of the Cultural Heritage was also held in the CINVESTAV, Merida, Yucatan (September 1-2, 2000) with the attendance of national and international specialists. A guided visit to the archeological sites of Chichen Itza and several Puuc monuments of the Yucatan peninsula was programed after this event.

The Research network XV-E has been also present in the forum of Subprogram XV CYTED at the 4th NACE Latin American Region Corrosion Congress (7th Ibero American Corrosion and Protection Congress) held in Cartagena de Indias, Colombia. September 17-22, 2000.

Preservar List

To facilitate a rapid and easy communication between national delegates and biodeterioration specialists the PRESERVAR list has been created in the web at the following mail address:

preservar@egroups.com

Further information on the PRESERVAR list can be found at the site www.egroups.com/group/preservar

As regards more information on the CYTED program the home page of RTX-E is available at the following URL: www.cytel.org.ar/preservar

Ph. D. Thesis Reports

**ANTIFUNGAL ACTIVITY OF
CYMBOPOGON NARDUS (L.) ESSENTIAL
OIL ON ASPERGILLUS NIGER.
EVALUATION OF A BIOREACTOR TO
STUDY THE INHIBITORY EFFECT OF
VOLATILE SUBSTANCES IN THE
VAPOUR PHASE, Ph. D Thesis, 2000,
University of Toulouse, France, 236p.**

**Virginia G. de Billerbeck,
Laboratory of Bacteriology, Virology and
Industrial Microbiology, Faculty of
Pharmacy, Toulouse, France.**

Micro-organisms are responsible of important biodeteriorations of cultural heritage. The main biodeteriorating micro-organisms are bacteria, micro-fungi and actinomycetes, but the most common are fungi because they show a greater tolerance to environmental conditions.

In order to find an alternative to the chemical treatments (i.e. ethylene oxide, quaternary ammonium compounds, formaldehyde) and physical treatments (i.e. gamma ray, UV ray, micro-waves) often recommended for disinfection, the antimicrobial properties of essential oils have been investigated in our Laboratory. Essential oils present several advantages: they possess a wide spectrum of action, they are active in the volatile phase and should not provoke damage to the objects and the environment in the practical conditions of use.

But the current methods in aqueous solutions described in the literature are not appropriate to test the fungicidal or fungistatic activity of essential oils vapours. The aim of this work is to develop an original model reactor to study the vapour phase inhibitory effect of essential oils as well as to observe the morphological modifications induced by the essential oil on fungal treated cells.

In order to simulate the actual conditions of use, assays have been carried in a bioreactor of 16 litres. The nutrient agar medium was replaced by inert germ-carriers (cotton threads) inoculated by

spores or mycelium of *A. niger*. The antifungal activity was determined on spores by CFU (Colonies Forming Units) counting and on mycelium, by measuring mycelium deshydrogenase activity which represented cells metabolic activity after treatment.

The validation of this model was performed with formaldehyde in controlled ambient conditions (temperature and relative humidity). The volatile phase of *Cymbopogon nardus* ("Ceylon Citronella") essential oil exhibited a fungistatic and reversible activity after 9 days of exposure at 21°C and 57% RH. This activity was strongest on mycelium stage than on latent spores. The second phenomenon observed was the inhibition of the mycelium sporulation, after the subculture of the treated inoculum (spores or mycelium). TEM observations of hyphae after the exposition to the essential oil volatile phase during 6 days showed that the plasmalemma was irregular and the cell wall appeared markedly thinner.

Thus, these findings indicate the possibility of employing essential oils in a preventive way, as atmospheric preservative agents, to limit the development and the dissemination of moulds in archives and museums reserves.

Forthcoming Activities

COALITION WORKSHOP

**DATABASES OF LIVING
MICROORGANISMS FROM STONE
MONUMENTS**

Ghent, March 9th 2001

**Het Pand
Onderbergen 1
B-9000 Gent**

Organised in the framework of
COALITION (EVK4-1999-00061) by :

Prof. Dr. Cesareo Saiz-Jimenez
Prof. Dr. ir. Jean Swings
Prof. Dr. Dr. h.c. Wolfgang E. Krumbein

Programme

9.00–10.00 Bacteria (Jeroen Heyrman and Peter Dawyndt)
10.00–10.30 Lichens (Antonio Gomez-Bolea)
10.30–11.00 Coffee break
11.00–12.00 Fungi (Katja Sterflinger)
12.00–13.30 Lunch
13.30–14.30 Algae and cyanobacteria (Gioia Lamenti)
14.30–15.00 Actinomycetes (Ingrid Groth and Peter Schumann)
15.00–15.30 Coffee break
15.30–17.30 Discussion of the Databank (Thomas Dornieden and Jeroen Heyrman)

Inscription

Maximum number of participants: 40
Deadline for inscription: 15 February 2001

Price for non-COALITION participants: 50 Euro

Inscriptions and further questions can be addressed to :

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Hotel reservation

You are kindly requested to arrange your own hotel reservation.

RELATED ACTIVITIES

FUNGI - A THREAT FOR PEOPLE AND CULTURAL HERITAGE THROUGH MICROORGANISMS

is the topic of a conference which will take place in Munich, June 21-23, 2001 within the framework of the MUTEK (Trade Fair for Museum Technology). A two-day workshop is planned in addition to the conference.

The damage of cultural heritage objects caused by microorganisms is a common problem which is dealt with by diverse

occupational groups. The purpose of this conference, organised by members of several conservation associations, is to sharpen the interdisciplinary view of this problem and to further develop methodologies for the identification and treatment of infestations.

The topics range from the diagnosis of damage caused to objects and the identification of fungi, bacteria and other microorganisms, to strategies for treatment and prevention. A detailed list of topics can be found in the Call for Papers.

Those who cannot attend the conference may find more information on microbial damage to cultural heritage objects on the site which is updated regularly: <http://www.fungi-info.de>

Organizer: Association of Restorers, Im Großacker 28, D-79252 Stegen, Germany. Phone +49 7661-61036, Fax +49 7661-62150, e-mail: AdR@t-online.de

COALITION web site

Our web page is already active. Reading on-line and downloading previous issues is there possible, and to obtain information on the Concerted Action or visit related sites. You can visit it at <http://www.geomic.uni-oldenburg.de/projekte/coalition>

Call for papers

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