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ACTINOMYCETES IN CAVES

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Parietal paintings are valuable works of art giving evidence of prehistoric life. In many cases these paintings were detected by chance after thousands of years as it happened upon the Altamira Cave in Spain and the Grotta dei Cervi in Italy. While the Altamira Cave (discovered in 1879) had been opened to tourists reaching a daily flow of up to 3000 visitors in the 1970's, the access to the Grotta dei Cervi (discovered in 1970) has been restricted only to scientists.

Within the frame of EC project ENVA-CT95-0104 microbial colonization in both caves was studied by conventional isolation and cultivation techniques. Sampling was done by touching the rock between the paintings with sterile cotton swabs and suspending the adherent bacteria in sterile 0.15 sodium phosphate buffer solution. Additionally contact plates with different culture media and pieces of rock or soil material were used for isolation of microorganisms from different sites in the caves.

Pure cultures of actinomycete isolates were classified by morphological, selected physiological and chemotaxonomic methods. On the basis of a polyphasic taxonomic approach in most of the cases a tentative genus affiliation was possible.

As the result of our study it was stated that actinomycetes were the most abundant microorganisms in the two caves (Groth et al. 1999, 1999a, 2001). The term actinomycetes refers to all Gram-positive bacteria with a high G+C content (> 55 mol %) in their DNA. Actinomycetes are morphologically diverse and comprise different mophological types; irregular rods, cocci and filamentous or mycelium forming organisms.

The cave isolates could be affiliated to 11 different families of the order *Actinomycetales,* class *Actinobacteria.* This fact indicates a high phylogenetic diversity which has been developed within these special biotopes (Table 1). Furthermore it was demonstrated by fatty acid profiles (MIDI system), utilization patterns of 95 different carbon sources (BIOLOG-System) combined with morphological studies that there was an equally high diversity at the species or strain levels within the isolates being affilated to genera.

The predominant actinomycete isolates from both caves belong to the genus *Streptomyces.* Members of this genus are known for their versatile metabolic activities. In dependence on environmental or cultural conditions in the laboratory actinomycetes are able to

produce a wealth of structural diverse chemical compounds including pigments and organic acids which may contribute to biodeterioration of works of art.

Table 1: Taxonomic diversity of actinomycetes from the Altamira Cave and Grotta dei Cervi

Family	Genus
Brevibacteriaceae	*Brevibacterium*
Dermabacteraceae	*Brachybacterium*
Gordoniaceae	*Gordonia*
Microbacteriaceae	*Agromyces* *Microbacterium*
Micrococcaceae	*Arthrobacter* *Micrococcus* *Rothia*
Micromonosporaceae	*Micromonospora*
Nocardiaceae	*Nocardia* *Rhodococcus*
Nocardioidaceae	*Nocardioides*
Nocardiopsaceae	*Nocardiopsis*
Pseudonocardiaceae	*Amycolatopsis* *Saccharothrix*
Streptomycetaceae	*Streptomyces*

Furthermore Cañaveras et al. (1999) suggested that actinomycetes may play a role in the formation of moonmilk deposits in hypersaline environments of some caves as actinomycetes (mainly streptomycetes) were isolated from hydromagnesite and needle fiber aragonite deposits in the Altamira Cave. This assumption was supported by the fact that numerous actinomycete isolates were able to produce either calcite or both calcite and vaterite crystals under special laboratory conditions (Groth et al. 2001).

It is now generally accepted that biodeterioration of outdoor monuments is not only caused by phototrophic microorganisms and fungi covering the surfaces with green or dark biofilms but is rather a concerted action of diverse procaryotic and eucaryotic microorganisms. Among the heterotrophic bacteria actinomycetes predominate in hypogean environments. They are able to colonize nearly all habitats and can adapt to very poor and highly saline environments, e.g. stalactites. By formation of dormant cells actinomycetes can survive unfavourable conditions.

Our studies in the two different caves revealed that mass growth of actinomycetes seemed to be controlled in an undisturbed environment, like Grotta dei Cervi, by natural factors (low temperature, high humidity and limited amount of nutrients). While microbial colonization in the Grotta dei Cervi did not result in visible damages of the paintings over thousands of years, the deteriorations observed in the caves of Altamira and Lascaux after their opening clearly demonstrate the negative effects of mass tourism within a relative short period.

To combine both access to works of art in underground environments and to preserve these early manifestations of prehistoric life, basic multidisciplinary studies are necessary. Concerning microbial colonization both noncultural (Rölleke et al. 1996) and cultural techniques should be combined. While molecular approaches based on detection and identification of DNA sequences encoding for 16S rRNA or 23S rRNA equally include culturable and nonculturable bacteria, classical isolation and cultivation techniques selectively support the growth of only a minor fraction of the really existing bacteria in a special biotope. However pure cultures of selected bacteria obtained by isolation procedures enable scientists to study the metabolic activities of these organisms and contribute to a better understanding of their role in biodecay processes. Furthermore living cultures are a prerequisite for the discovery and description of novel taxa from these interesting but up to now less studied environments. The occurrence of novel bacteria was confirmed by recent publications of two novel genera isolated from a Chinese cave (Groth et al. 1999, 2002).

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Forthcoming activities

QUESTIONNAIRE ON THE APPLICATION OF MOLECULAR BIOLOGICAL TECHNIQUES IN CONSERVATION

A questionnaire is being prepared by Caroline Kyi on behalf of COALITION to know the real impact on molecular tools into the conservation field and its feasibility in microbiological assessment. This will be distributed in April to the COALITION list and it is expected that we will obtain a high number of respondents. We ask you to collaborate with us in this task. The results will be presented in the Florence Advanced Course (8-9 November, 2002) and published in the Newsletter.

ADVANCED COURSE ON BIODETERIORATION OF THE CULTURAL HERITAGE

Organized by COALITION in collaboration with the Opificio delle Pietre Dure, Florence, Italy.

Florence, 8-9 November 2002

Addressed to biologists, conservators and restorers under 35 years old.

Cost of participation: 100 €, includes registration fee, printed material and coffe-lunch breaks.

Preliminary programme

November 8th
Session 1. Biodeterioration Processes: organic/inorganic substrates
Session 2. Diagnostic techniques (traditional and innovative)
Session 3. Intervention procedures: chemical (biocides) - Pysical/mechanical (laser, UV)
Session 4. Bioremediation

November 9th
Session 5. Biohazard in Restoration: Allergenic and pathogenic, toxicity.
Session 6. Health protection guidelines
Session 7. COALITION questionnaire.

Send your preliminary inscription form (to be distributed) to tiano@cscoa.fi.cnr.it

INTERNATIONAL CONFERENCE ON MOLECULAR BIOLOGY AND CULTURAL HERITAGE

An International Conference on Molecular Biology and Cultural Heritage will be organized about 2-8 March 2003 in Seville (Spain).

This International Conference is open to researchers, conservators, restorers and Cultural Heritage authorities. The data collected and reviewed by COALITION partners, during the project, will be disseminated in the Conference, and the invited lectures and contributions will be published in a monographic book.

Preliminary registration forms (to be distributed) should be sent to coalition@irnase.csic.es

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.

Send your contributions by e-mail to: coalition@irnase.csic.es